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Saponins from Chinese Folk Medicine, "Zhu jie xiang fu," *Anemone raddeana* REGEL

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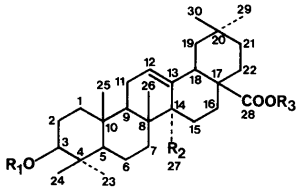
From the Chinese folk medicine "Zhu jie xian fu" (roots of *Anemone raddeana* REGEL, Ranunculaceae), two new oleanane-type glycosides, named raddeanosides R_8 (1) and R_9 (2), were isolated. The structures of 1 and 2 were determined as 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl 27-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, respectively.

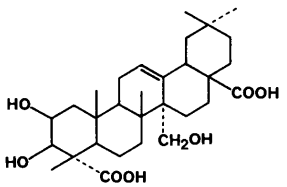
Keywords *Anemone raddeana*; Ranunculaceae; raddeanoside R_8; raddeanoside R_9; Zhu jie xiang fu; saponin; oleanane-type glycoside; Chinese folk medicine

The roots of *Anemone raddeana* REGEL (Ranunculaceae) (竹節香附, Chinese name; Zhu jie xian fu), a very important Chinese folk medicine, have been used to treat rheumatism, neuralgia, and so on.[1]

Isolation and structure elucidation of eight oleanane saponins from the roots of this plant were reported previously.[2] In a continuation of our chemical studies on the roots of this plant, we isolated two new saponins, named raddeanosides R_8 and R_9. The present paper deals with the isolation and structure elucidation of these compounds on the basis of spectral and chemical evidence. The extraction and separation were carried out as described in the experimental section.

Raddeanoside R_8 (**1**), a white powder, $[\alpha]_D$ −27.7°, showed absorption bands of hydroxyl and ester groups in the infrared (IR) spectrum and the pseudo molecular ion, *m/z* 1389 $(M+Na)^+$ in the positive fast atom bombardment mass spectrum (FAB-MS). From the MS and elementary analysis, the molecular formula was concluded to be $C_{65}H_{106}O_{30}$. On methanolysis, **1** afforded oleanolic acid (**5**), methyl D-glucoside, methyl L-arabinoside, and methyl L-rhamnoside. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, the signals due to the aglycone moiety were in good agreement with those of the 3,28-bisdesmoside of **5** and those due to the sugar moiety showed the presence of six monosaccharide units (anomeric carbon signals; δ 95.6, 101.7, 102.7, 104.8, 104.9, and 106.2). On selective cleavage of the ester-glycoside linkage with anhydrous LiI and 2,6-lutidine in anhydrous methanol,[3] **1** afforded **3**[2] and a methyl glycoside which was identified as an anomeric mixture of methyl *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-(α and β)-D-glucopyranoside (**8**) by comparison of its ^{13}C-NMR data with those of an authentic sample.[4] The above evidence led to the formulation of raddeanoside R_8 (**1**) as 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.





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	R_1	R_2	R_3
1	-ara^{2}glc^{2}rha	CH_3	-glc^{6}glc^{4}rha
2	-ara^{2}glc^{2}rha	CH_2OH	-glc^{2}glc^{4}rha
3	-ara^{2}glc^{2}rha	CH_3	H
4	-ara^{2}glc^{2}rha	CH_2OH	H
5	H	CH_3	H
6	-ara^{2}rha	CH_3	-glc^{6}glc^{4}rha

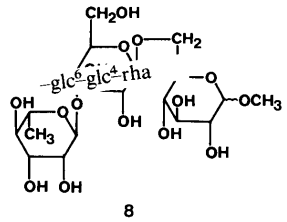

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Chart 1

Raddeanoside R_9 (**2**), a white powder, $[\alpha]_D$ −26.2°, showed absorption bands of hydroxyl and ester groups in the IR spectrum and the pseudo molecular ion, *m/z* 1405 $(M+Na)^+$ in the positive FAB-MS. From the MS and elementary analysis, the molecular formula was concluded to be $C_{65}H_{106}O_{31}$, which indicated that **2** contained one more oxygen atom than **1**. The ^{13}C-NMR spectrum of **2** showed the six anomeric carbon signals at δ 95.7, 101.7, 102.8, 104.8, 104.9, and 106.3. On methanolysis, **2** afforded methyl D-glucoside, methyl L-arabinoside, and methyl L-rhamnoside. In the ^{13}C-NMR spectrum of **2**, the carbon signals due to sugar moieties (Table II) were almost superimposable on those of **1**. The ^{13}C-NMR and proton (^{1}H)-NMR spectra of the aglycone moiety of **2** showed six methyl groups, which indicated that **2** had one less methyl group than **1**. These results indicated that the aglycone of **2** contained a hydroxymethyl group. The location of the hydroxyl group of **2** was determined by the following evidence. In a comparison of the ^{13}C-NMR spectrum of **2** with that of **1**, on going from **2** to **1**, the signals due to C-12 and C-14 of the aglycone moiety were moved downfield by *ca.* 5 ppm, and the signals due to C-13 and C-15 were moved upfield by *ca.* 5 ppm, while other signals remained almost unshifted. On the other hand, the carbon signals due to rings B—E of **2** were in good agreement with those of presenegin (**7**).[5] These results indicated that the hydroxyl group was located at the C-27 position and suggested that **2** could be formulated as 27-hydroxyraddeanoside-R_8. On selective cleavage of the ester-glycoside linkage, **2** afforded **4** as the prosapogenin and **8**. Further, the carbon signals due to the 3-*O*-glycosyl moiety of **2** were found to be almost superimposable on those **1**. These observations led to the formulation of raddeanoside R_9 (**2**) as 3-*O*-α-L-

Table I. ^{13}C-NMR Chemical Shifts of Aglycone Moieties in C_5D_5N

	5[6]	7[5]	3	1	2
C-1	38.9	45.0	38.8	38.9	38.9
C-2	28.2	71.6	26.5	26.5	26.6
C-3	78.0	75.8	88.6	88.7	88.7
C-4	39.4[a]	54.0	39.4[a]	39.5[a]	39.5
C-5	55.8	52.3	55.9	56.0	55.9
C-6	18.8	21.6	18.7	19.1	19.0
C-7	33.3	34.1	33.2[d]	33.1	33.9
C-8	39.8[a]	40.9	39.7[a]	39.9[a]	40.6
C-9	48.1	49.5	48.0	48.1	48.6
C-10	37.4	37.1	37.0	37.0	37.2
C-11	23.8	24.0[b]	23.6[b]	23.4[b]	23.8[b]
C-12	122.5	127.6	122.5	122.9	127.9
C-13	144.8	139.6	144.7	144.1	139.2
C-14	42.0	46.5	42.1	42.1	46.7
C-15	28.3	24.7[b]	28.3[c]	28.3[c]	24.0
C-16	23.8	24.1[b]	23.8[b]	23.8[b]	23.5[b]
C-17	46.7	48.1	46.6	46.3	47.9
C-18	42.0	41.8	41.9	41.7	41.5
C-19	46.7	45.5	46.4	47.0	45.4
C-20	31.0	31.0	30.9	30.7	30.7
C-21	34.3	33.5	34.2	33.1	33.0
C-22	33.3	33.2	33.1[d]	32.5	32.6
C-23	28.7	180.8	28.0[c]	28.0[c]	28.0
C-24	16.5	13.7	16.9	16.9	16.9
C-25	15.5	17.5	15.9	15.6	16.1
C-26	17.5	18.8	17.3	17.5	19.1
C-27	26.2	64.4	26.1	26.0	64.4
C-28	180.2	180.8	180.1	176.4	176.4
C-29	33.3	33.2	33.1[d]	33.1	33.0
C-30	23.8	23.2	23.7[b]	23.8[b]	23.8[b]

a—d) Assignments in each column may be reversed.

Table II. ^{13}C-NMR Chemical Shifts of Sugar Moieties in C_5D_5N

		3	6[6]	1	2
3-*O*-Sugar moieties					
Ara inner	1	104.8		104.9	104.9
	2	76.3		76.4	76.4
	3	72.4		72.5	72.5
	4	69.7		69.3[b]	69.3[b]
	5	64.4		64.2	64.2
Glc inner	1	106.2		106.2	106.3
	2	78.6[a]		78.6[a]	78.6[a]
	3	75.3		75.3	75.3
	4	71.2		71.3	71.4
	5	78.4[a]		78.4[a]	78.5[a]
	6	62.5		62.4	62.6
Rha terminal	1	101.7		101.7	101.7
	2	72.4		72.4	72.3
	3	72.2		72.2	72.3
	4	73.9		74.0[b]	74.0[b]
	5	69.7		69.8[c]	69.8[c]
	6	18.5		18.5	18.5
28-*O*-Sugar moieties					
Glc iner	1		95.6	95.6	95.7
	2		73.9	75.4	75.5
	3		78.2[a]	78.7[a]	78.8
	4		70.5	70.9	71.0
	5		76.4	78.0	78.1
	6		69.9	70.3[b]	70.3[b]
Glc outer	1		104.7	104.8	104.8
	2		75.4	76.5	76.6
	3		76.4	78.4	78.5
	4		78.6[a]	79.2[a]	79.3
	5		77.0	77.1	77.2
	6		61.3	61.3	61.4
Rha terminal	1		102.6	102.7	102.8
	2		72.5	72.6	72.8
	3		72.3	72.7	72.9
	4		73.9	73.9	74.1
	5		70.3	70.3[b]	70.3[b]
	6		18.5	18.4[c]	18.6[c]

a—c) Assignments in each column may be reversed.

rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl 27-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Experimental

The IR spectra were recorded with a Hitachi 290-30 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL GX-400 (^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz) spectrometer. Chemical shifts are given on the δ-scale (ppm downfield from tetramethylsilane as an internal standard). FAB-MS were run on a JEOL JMS-DX-303 mass spectrometer. Optical rotations were determined on a JASCO DIP-4 digital polarimeter. For gas liquid chromatography (GLC), a Hitachi 163 apparatus was used. For column chromatography, silica gel (BW-820 MH, Fuji Davison) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used.

Plant Material The medicinal herb was obtained from at the Lanzhou Chinese Medicinal Plant (in China) and identified by Gansu Province Medicinal Inspection Office.

Extraction Dried and powdered roots of the plant of *Anemone raddeana* Regel (3.0 kg) were extracted with 50 l of ethanol at room temperature for 72 h, and after removal of the solvent by evaporation, the syrupy extract was dispersed in water and filtered. The filtrate was extracted with *n*-butanol, and after removal of the solvent, gave the butanol extract (15.0 g).

Isolation The butanol extract (15.0 g) was chromatographed on silica gel. Elution with $CHCl_3$–MeOH–H_2O (10 : 1 : 0.01) gave a mixture fraction (1.0 g), which was separated by preparative thin-layer chromatography (preparative TLC) developed with $CHCl_3$–MeOH–H_2O (10 : 3 : 0.2) to give a mixture of **1** and **2** (0.4 g). The mixture was separated by high-performance liquid chromatography (HPLC) [column, Capcell pak ODS (Shiseido), 4.6 mm i.d. × 25 cm; solvent, CH_3OH–H_2O (2 : 5); flow rate, 1 ml/min; detection, UV 234 nm] to give **1** (100 mg) and **2** (17 mg).

Raddeanoside R_8 (**1**): A white powder, $[\alpha]_D^{20}$ −27.7° (*c* = 1.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3450, 1720, 1150—1000. FAB-MS *m/z*: 1389 [$(M^+ + Na)^+$], 1225, 1109, 919, 873, 741, 671, 603, 493, 439, 311, 279, 221. *Anal.* Calcd for $C_{65}H_{106}O_{30} \cdot 3H_2O$: C, 54.92; H, 7.94. Found: C, 55.06; H, 8.24. ^{1}H-NMR δ (pyridine-d_5): 0.90 (6H, s), 0.91 (3H, s), 1.08 (3H, s), 1.09 (3H, s), 1.17 (3H, s), 1.25 (3H, s), 1.60 (3H, d, *J* = 6 Hz, Me of rhamnoside), 1.67 (3H, d, *J* = 6 Hz, Me of rhamnoside), 4.78 (1H, d, *J* = 6 Hz, anomeric proton), 4.97 (1H, d, *J* = 7 Hz, anomeric proton), 5.11 (1H, d, *J* = 8 Hz, anomeric proton), 5.41 (1H, br t, 12-H), 5.80 (1H, s, anomeric proton of rhamnoside), 6.08 (1H, s, anomeric proton of rhamnoside), 6.20 (1H, d, *J* = 8 Hz, anomeric proton). ^{13}C-NMR data are given in Tables I and II.

Raddeanoside R_9 (**2**): A white powder, $[\alpha]_D^{20}$ −26.2° (*c* = 1.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1150—1000. FAB-MS *m/z*: 1405 [$(M+Na)^+$], 1125, 1049, 889, 765, 671, 493, 459, 311, 279, 221. *Anal.* Calcd for $C_{65}H_{105}O_{31} \cdot 5/2H_2O$: C, 54.65; H, 7.85. Found: C, 54.80; H, 7.94. ^{1}H-NMR δ (pyridine-d_5): 0.83 (3H, s), 0.88 (3H, s), 0.90 (3H, s), 1.10 (3H, s), 1.11 (6H, s), 4.75 (1H, d, *J* = 6 Hz, anomeric proton), 5.01 (1H, d, *J* = 7 Hz, anomeric proton), 5.16 (1H, d, *J* = 8 Hz, anomeric proton), 5.78 (1H, br t, 12-H), 5.88 (1H, s, anomeric proton of rhamnoside), 6.18 (1H, s, anomeric proton of rhamnoside), 6.28 (1H, d, *J* = 8 Hz, anomeric proton). ^{13}C-NMR data are give in Tables I and II.

Methanolysis of 1 and 2 A solution of **1** (15 mg) in 10% HCl–MeOH (2 ml) was heated under reflux on a water bath for 5 h. The reaction solution was poured into H_2O and passed through Diaion HP-20 to give water and MeOH eluates. The MeOH eluate was dried and the aglycone from its identified by TLC behavior and IR spectrum in comparison with those of authentic oleanolic acid. The water eluate was neutraized with anion-exchange resin (Amberlite IRA-402), evaporated and dried on P_2O_5 to give methyl sugars, which were identified as methyl L-arabinoside, methyl L-rhamnoside, and methyl D-glucoside (as the trimethylsilyl derivatives) by GLC [2% SE-30 on Chromosorb W (60—80 mesh), column

temperature, 150 °C].

By the same method, **2** (5 mg) was hydrolyzed to an unknown aglycone (trace) and a mixture of methyl L-arabinoside, methyl L-rhamnoside, and methyl D-glucoside.

Selective Cleavage of Ester–Glycoside Linkage of 1 and 2[3] A solution of **1** (40 mg), anhydrous LiI (40 mg) and 2,6-lutidine (3 ml) in anhydrous MeOH was refluxed for 16 h. The reaction mixture was deionized with Amberlite MB-3 resin and concentrated to dryness. The residue was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (10 : 5 : 1→6 : 4 : 1)] to give **3** (8 mg) and a methyl oligoglycoside (**8**) (8 mg); the latter was identified as methyl α-L-rhamnosyl(1→4)-*O*-β-D-glucopyranosyl-(1→6)-(α and β)-D-glucopyranoside by comparison of the ^{13}C-NMR spectrum with that of an authentic sample.[4] Compound **3**: A white powder, $[\alpha]_D^{20}$ −2.7° [*c* = 0.8, CH_3OH, lit.[2] $[\alpha]_D^{25}$ −8° (*c* = 0.8, CH_3OH)]. IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1630, 1100—1000. FAB-MS *m/z*: 919 $(M+Na)^+$. ^{1}H-NMR δ (pyridine-d_5): 0.85 (3H, s), 0.97 (3H, s), 1.01 (3H, s), 1.02 (3H, s), 1.12 (3H, s), 1.20 (3H, s), 1.31 (3H, s), 1.67 (1H, d, *J* = 7 Hz, Me of rhamnoside), 4.79 (1H, d, *J* = 6 Hz, anomeric proton), 5.17 (1H, d, *J* = 8 Hz, anomeric proton), 5.49 (1H, t, *J* = 3 Hz, 12-H), 6.21 (1H, s, anomeric proton of rhamnoside). ^{13}C-NMR data are given in Tables I and II. Compound **3** was identified by comparison of the ^{1}H-NMR and ^{13}C-NMR spectra.[2]

By the same method, **2** (9 mg) afforded **4** (2 mg) and **8** (2 mg); the latter was identified by comparison of the ^{13}C-NMR spectrum with that of an authentic sample.[3] Compound **4**: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1620, 1150—1000. FAB-MS *m/z*: 935 $(M+Na)^+$. ^{1}H-NMR δ (pyridine-d_5): 0.85 (3H, s), 0.88 (3H, s), 0.95 (3H, s), 1.03 (3H, s), 1.10 (3H, s), 1.13 (3H, s), 1.66 (1H, d, *J* = 7 Hz, Me of rhamnoside), 4.74 (1H, d, *J* = 6 Hz, anomeric proton), 5.17 (1H, d, *J* = 8 Hz, anomeric proton), 5.86 (1H, br t, 12-H), 6.22 (1H, s, anomeric proton of rhamnoside).

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