

Oleanane Saponins from Rhizome of *Anemone raddeana*

by Li Fan^a), Jincai Lu^{*a}), Beibei Xu^a), Song Gao^b), Hongfen Zhang^a), and Runxiang Liu^a)

^a) College of Traditional Chinese Medicine, Shenyang Pharmaceutical University, No. 103 Wenhua Road, Shenyang 110016, P. R. China

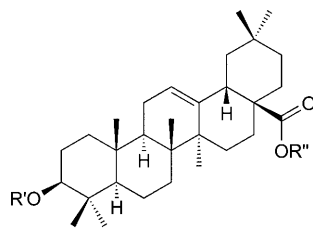
(phone: +8624-23986500; fax: +8624-23922065; e-mail: jincailu@yahoo.com.cn)

^b) Southwest Natural Product Center, Department of Arid Land Studied University of Arizona, 250 E. Valencia Road, Tucson, AZ 85710, USA
(e-mail: gaosong1999@yahoo.com.cn)

Two new oleanolic acid-type triterpenoid saponins, raddeanosides R₂₂ and R₂₃ (**1** and **2**, resp.), together with four known saponins were isolated from the rhizome of *Anemone raddeana* REGEL. The structures of the new compounds were elucidated as oleanolic acid 3-*O*- β -D-glucopyranosyl(1 \rightarrow 2)[β -D-glucopyranosyl(1 \rightarrow 4)]- α -L-arabinopyranoside (**1**) and oleanolic acid 3-*O*- α -L-arabinopyranosyl(1 \rightarrow 3)- α -L-rhamnopyranosyl(1 \rightarrow 2)[β -D-glucopyranosyl(1 \rightarrow 4)]- α -L-arabinopyranoside (**2**). The four known compounds were identified as oleanolic acid 3-*O*- α -L-arabinopyranoside (**3**), oleanolic acid 3-*O*- β -D-glucopyranosyl(1 \rightarrow 4)- α -L-arabinopyranoside (**4**), hederasaponin B (**5**), and hederacholchiside E (**6**) on the basis of chemical and spectral evidences. Compound **4** is reported for the first time from the *Anemone* genus, while the other three known compounds have been already found in this plant.

Introduction. – *Anemone raddeana* REGEL (Rununculaceae) is widely distributed in the northeast provinces of China, Russia (Far east), Japan, and Korea. Its rhizome is commonly used for curing rheumatism and neuralgia in Traditional Chinese Medicine [1]. More than 20 triterpenoid saponins, which revealed antitumor, antiinflammatory, demulcent, antieclampsia, or phenobarbital activities, have been reported from the title plant [2–6]. We have earlier observed that six of these reported saponins show inhibiting effects on the superoxide generation in human neutrophils [7]. Recently, we also have observed that other three saponins show these activities [8][9]. The present study was directed at investigating the remaining constituents of this herb medicine. The investigation resulted in the isolation and structural elucidation of two new triterpenoid saponins, **1** and **2**, and four known compounds, **3–6** (Fig. 1).

Results and Discussion. – Compound **1** was isolated as a white powder and showed positive results to the *Molish* and *Liebermann–Burchard* tests. The molecular formula was established as C₄₇H₇₆O₁₇ according to the HR-ESI-MS at *m/z* 935.4975 ([*M* + Na]⁺; calc. for C₄₇H₇₆NaO₁₇⁺, 935.4980). The IR spectrum showed absorptions of OH (3418 cm⁻¹), CO (1695 cm⁻¹), and C=C bonds (1650 cm⁻¹). Three anomeric H-atom signals at δ (H) 4.92 (*d*, *J* = 4.8, 1 H), 5.19 (*d*, *J* = 7.8, 1 H), and 5.12 (*d*, *J* = 7.8, 1 H), which were proved to be assigned to three anomeric C-atom signals at δ (C) 104.4, 105.1, and 105.9 in the HMQC experiment, were observed in the ¹H-NMR spectrum. After acid hydrolysis, the aglycone yielded from the organic layer was determined as



R'	R''
1 Glc(1→2)[Glc(1→4)]Ara	H
2 Ara(1→3)Rha(1→2)[Glc(1→4)]Ara	H
3 Ara	H
4 Glc(1→4)Ara	H
5 Rha(1→2)Ara	Rha(1→4)Glc(1→6)Glc
6 Rha(1→2)[Glc(1→4)]Ara	Rha(1→4)Glc(1→6)Glc

Fig. 1. Structures of compounds from rhizome of *Anemone raddeana*

oleanolic acid by TLC. D-Glucose and L-arabinose were isolated from the H₂O layer by PTLC. The absolute configuration of each of the sugars were established from their $[\alpha]_D$ values. From the coupling constants of the anomeric signals, configuration was deduced to be α for arabinose and β for glucose. In the HMBC experiment, long-range correlations between the anomeric H-atom H–C(1'') ($\delta(\text{H})$ 5.19) and the C-atom C(4') ($\delta(\text{C})$ 77.2), H–C(1''') ($\delta(\text{H})$ 5.12) and C(2') ($\delta(\text{C})$ 80.9) indicated that the two glucoses were attached to position 4 and 2 of the arabinose, respectively (Fig. 2). Long-range correlations between the anomeric H-atom H–C(1') ($\delta(\text{H})$ 4.92) and C(3) ($\delta(\text{C})$ 88.9) suggested that the arabinose was linked to the aglycone at C(3). This was also in agreement with the fragment ions observed in the HR-ESI-MS spectrum at m/z 773.4290 ($[M - \text{Glc} + \text{Na}]^+$) and 480.1415 ($[M - \text{Glc} - \text{Glc} - \text{Ara} + \text{Na}]^+$). The ¹H- and ¹³C-NMR data of compound **1** were assigned on the basis of DEPT, ¹H,¹H-COSY, HMQC, and HMBC experiments (see Table I). Thus, the structure of **1** was established as oleanolic acid 3-*O*- β -D-glucopyranosyl(1→2)[β -D-glucopyranosyl(1→4)]- α -L-arabinopyranoside. This compound has not been reported previously, and was named raddeanoside R₂₂.

Compound **2** was obtained as a white powder with positive results to the *Molish* and *Liebermann–Burchard* tests. The HR-ESI-MS of **2** displayed a *pseudo*-molecular $[M + \text{Na}]^+$ ion at m/z 1051.5431, corresponding to the molecular formula C₅₂H₈₄O₂₀ (calc. for C₅₂H₈₄NaO₂₀⁺, 1051.5454), which was also confirmed by ¹H- and ¹³C-NMR analysis. The IR spectrum showed absorptions at 3421, 1695, and 1650 cm⁻¹ accounting for OH groups, a CO group, and a C=C bond. Anomeric H-atom signals in the ¹H-NMR spectrum at $\delta(\text{H})$ 4.71 (*d*, $J = 7.2$, 1 H), 6.33 (*s*, 1 H), 5.99 (*d*, $J = 3.6$, 1 H), and 5.14 (*d*, $J = 7.8$, 1 H), together with corresponding C-atom signals at $\delta(\text{C})$ 105.3, 101.5, 104.8, and 106.9 in the ¹³C-NMR spectrum suggested that **2** was a glycoside with four sugar units. Acid hydrolysis of **2** yielded the aglycone which was determined as oleanolic acid by TLC from the organic layer. In addition, D-glucose, L-rhamnose, and L-arabinose were obtained from the H₂O layer by PTLC and identified by TLC and $[\alpha]_D$ values. By comparing with the aglycone in the ¹³C-NMR spectrum, C(3) ($\delta(\text{C})$

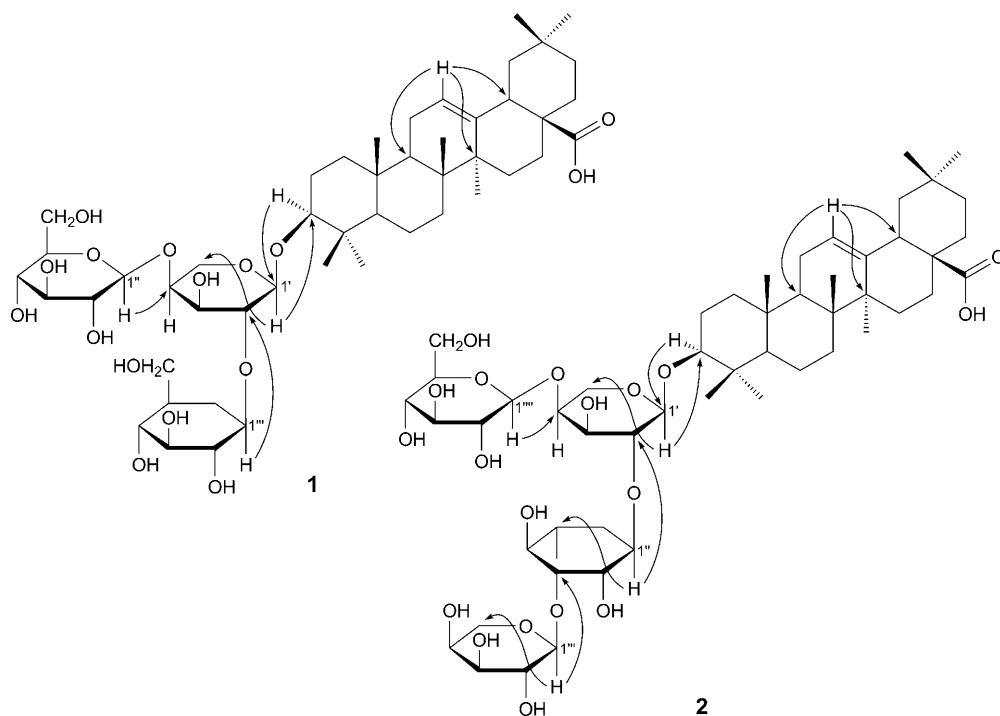


Fig. 2. Key HMBCs of compounds **1** and **2**

88.8) was shifted downfield, indicating that the sugar moiety was attached to this position. From the coupling constants of the anomeric signals, the α configuration of the rhamnose and arabinose residues, and the β configuration of the glucose unit were deduced. In the HMBC experiment, long-range correlations between the anomeric H-atom H-C(1'') ($\delta(\text{H})$ 6.33) and the C-atom C(2') ($\delta(\text{C})$ 75.9), H-C(1''') ($\delta(\text{H})$ 5.14) and C(4') ($\delta(\text{C})$ 80.6) indicated that the rhamnose and glucose were attached to position 2 and 4 of the arabinose, respectively (Fig. 2). Long-range correlations between the anomeric H-atom H-C(1') ($\delta(\text{H})$ 4.71) and C(3) ($\delta(\text{C})$ 88.8) suggested the arabinose linked the aglycone at C(3). Long-range correlations between the anomeric H-atom H-C(1''') ($\delta(\text{H})$ 5.99) and C(3'') ($\delta(\text{C})$ 81.2) indicated that the other arabinose unit was attached to position 3 of the rhamnose. Fragment ions observed in the HR-ESI-MS spectrum at m/z 889.4849 ($[M - \text{Glc} + \text{Na}]^+$), 773.4358 ($[M - \text{Glc} - \text{Ara} + \text{K}]^+$), 613.1872 ($[\text{Glc} + \text{Ara} + \text{Rha} + \text{Ara} + \text{Na}]^+$), and 595.1769 ($[\text{Glc} + \text{Ara} + \text{Rha} + \text{Ara} + \text{Na} - \text{H}_2\text{O}]^+$) also supported this deduction. The ^1H - and ^{13}C -NMR data of compound **2** were assigned on the basis of DEPT, ^1H , ^1H -COSY, HMQC, and HMBC experiments (see Table 2). On the basis of these results, the structure of **2** was established as oleanolic acid 3-*O*- α -L-arabinopyranosyl(1 \rightarrow 3)- α -L-rhamnopyranosyl(1 \rightarrow 2)[β -D-glucopyranosyl(1 \rightarrow 4)]- α -L-arabinopyranoside. This compound has not been reported previously and was named raddeanoside **R**₂₃.

Together with **1** and **2**, four known compounds were isolated and identified as oleanolic acid 3-*O*- α -L-arabinopyranoside (**3**) [10], oleanolic acid 3-*O*- β -D-

Table 1. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) Data of Compound **1** (in (D_5) pyridine)

	$\delta(\text{C})^{\text{a) b)}$	$\delta(\text{H})^{\text{b) c)}$		$\delta(\text{C})^{\text{a) b)}$	$\delta(\text{H})^{\text{b) c)}$
1	38.6 (<i>t</i>)	1.40–1.43 (<i>m</i> , H_{ax}), 0.78–0.85 (<i>m</i> , H_{eq})	3-Ara-1'	104.4 (<i>d</i>)	4.92 (<i>d</i> , $J = 4.8$, 1 H)
2	26.4 (<i>t</i>)	1.70–1.78 (<i>m</i> , H_{ax}), 1.92–2.00 (<i>m</i> , H_{eq})	2'	80.9 (<i>d</i>)	4.56 (<i>t</i> , $J = 6.0, 7.2$, 1 H)
3	88.9 (<i>d</i>)	3.12 (<i>dd</i> , $J = 11.7, 4.5$, 1 H)	3'	72.5 (<i>d</i>)	4.43–4.47 (<i>m</i> , 1 H)
4	39.5 (<i>s</i>)		4'	77.2 (<i>d</i>)	4.49–4.53 (<i>m</i> , 1 H)
5	55.8 (<i>d</i>)	0.69 (<i>d</i> , $J = 12.0$, 1 H)	5'	63.7 (<i>t</i>)	4.34–4.42, 3.81 (<i>m</i> , d , $J = 10.2$, 2 H)
6	18.5 (<i>t</i>)	1.43–1.47 (<i>m</i> , H_{eq}), 1.22–1.27 (<i>m</i> , H_{ax})	Glc-1''	105.1 (<i>d</i>)	5.19 (<i>d</i> , $J = 7.8$, 1 H)
7	33.2 (<i>t</i>)	1.35–1.40 (<i>m</i> , H_{ax}), 1.22–1.27 (<i>m</i> , H_{eq})	2''	75.7 (<i>d</i>)	4.04 (<i>dd</i> , $J = 16.8, 8.4$, 1 H)
8	39.7 (<i>s</i>)		3''	78.4 (<i>d</i>)	4.20 (<i>t</i> , $J = 8.4, 9.0$, 1 H)
9	48.0 (<i>d</i>)	1.60 (<i>t</i> , $J = 9.0$, 1 H)	4''	71.4 (<i>d</i>)	4.26 (<i>t</i> , $J = 9.0$, 1 H)
10	37.0 (<i>s</i>)		5''	78.8 (<i>d</i>)	3.84–3.89 (<i>m</i> , 1 H)
11	23.8 (<i>t</i>)	1.89 (<i>dd</i> , $J = 8.4, 3, 2$ H)	6''	62.6 (<i>t</i>)	4.47–4.49, 4.34–4.42 (<i>m</i> , 2 H)
12	122.5 (<i>d</i>)	5.45 (<i>br. s</i> , 1 H)	Glc-1'''	105.9 (<i>d</i>)	5.12 (<i>d</i> , $J = 7.8$, 1 H)
13	144.9 (<i>s</i>)		2'''	76.2 (<i>d</i>)	4.06 (<i>dd</i> , $J = 16.8, 8.4$, 1 H)
14	42.2 (<i>s</i>)		3'''	78.2 (<i>d</i>)	4.16 (<i>t</i> , $J = 9.0$, 1 H)
15	28.3 (<i>t</i>)	2.12–2.19 (<i>m</i> , 2 H)	4'''	71.6 (<i>d</i>)	4.30 (<i>t</i> , $J = 9.0$, 1 H)
16	23.7 (<i>t</i>)	2.06–2.12 (<i>m</i> , 2 H)	5'''	78.1 (<i>d</i>)	3.73–3.78 (<i>m</i> , 1 H)
17	46.7 (<i>s</i>)		6'''	62.6 (<i>t</i>)	4.47–4.49, 4.34–4.42 (<i>2m</i> , 2 H)
18	42.0 (<i>d</i>)	3.30 (<i>dd</i> , $J = 13.8, 4.2$, 1 H)			
19	46.5 (<i>t</i>)	1.78–1.81 (<i>m</i> , H_{ax}), 1.27–1.30 (<i>m</i> , H_{eq})			
20	31.0 (<i>s</i>)				
21	34.2 (<i>t</i>)	1.43–1.47 (<i>m</i> , H_{ax}), 1.18–1.22 (<i>m</i> , H_{eq})			
22	33.2 (<i>t</i>)	1.81–1.84 (<i>m</i> , H_{ax}), 2.00–2.06 (<i>m</i> , H_{eq})			
23	28.2 (<i>q</i>)	1.18 (<i>s</i> , 3 H)			
24	16.8 (<i>q</i>)	1.01 (<i>s</i> , 3 H)			
25	15.5 (<i>q</i>)	0.82 (<i>s</i> , 3 H)			
26	17.4 (<i>q</i>)	0.98 (<i>s</i> , 3 H)			
27	26.2 (<i>q</i>)	1.26 (<i>s</i> , 3 H)			
28	180.2 (<i>s</i>)				
29	33.3 (<i>q</i>)	0.95 (<i>s</i> , 3 H)			
30	23.8 (<i>q</i>)	1.00 (<i>s</i> , 3 H)			

^{a)} Multiplicity determined by DEPT experiments. ^{b)} Signals were assigned by HMQC, HMBC, and ^1H , ^1H -COSY experiments, chemical shifts (δ) in ppm relative to TMS. ^{c)} Coupling constants (J in Hz) are given in parentheses.

glucopyranosyl(1 \rightarrow 4)- α -L-arabinopyranoside (**4**) [11], hederasaponin B (**5**) [12], and hederacholchiside E (**6**) [13] by chemical evidences and spectral data comparison with literature values. Compound **4** is reported for the first time from the *Anemone* genus, while the other three known compounds have been already found in this plant.

Table 2. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) Data of Compound **2** (in (D₅)pyridine)

	$\delta(\text{C})^{\text{a) b)}$	$\delta(\text{H})^{\text{b) c)}$		$\delta(\text{C})^{\text{a) b)}$	$\delta(\text{H})^{\text{b) c)}$
1	38.9 (<i>t</i>)	1.43–1.48 (<i>m</i> , H _{ax}), 0.85–0.92 (<i>m</i> , H _{eq})	3-Ara-1'	105.3 (<i>d</i>)	4.71 (<i>d</i> , <i>J</i> = 7.2, 1 H)
2	26.8 (<i>t</i>)	1.81–1.85 (<i>m</i> , H _{ax}), 2.04–2.09 (<i>m</i> , H _{eq})	2'	75.9 (<i>d</i>)	4.52–4.55 (<i>m</i> , 1 H)
3	88.8 (<i>d</i>)	3.20 (<i>dd</i> , <i>J</i> = 11.7, 3.9, 1 H)	3'	75.2 (<i>d</i>)	4.20–4.23 (<i>m</i> , 1 H)
4	39.6 (<i>s</i>)		4'	80.6 (<i>d</i>)	4.18–4.20 (<i>m</i> , 1 H)
5	56.1 (<i>d</i>)	0.75 (<i>d</i> , <i>J</i> = 12.0, 1 H)	5'	65.6 (<i>t</i>)	4.35–4.37, 3.73 (<i>m</i> , <i>d</i> , <i>J</i> = 12, 2 H)
6	18.5 (<i>t</i>)	1.39–1.43 (<i>m</i> , H _{ax}), 1.21–1.26 (<i>m</i> , H _{eq})	Rha-1''	101.5 (<i>d</i>)	6.33 (<i>s</i> , 1 H)
7	33.2 (<i>t</i>)	1.39–1.43 (<i>m</i> , H _{eq}), 1.21–1.26 (<i>m</i> , H _{ax})	2''	72.0 (<i>d</i>)	4.91 (<i>s</i> , 1 H)
8	39.7 (<i>s</i>)		3''	81.2 (<i>d</i>)	4.74 (<i>dd</i> , <i>J</i> = 9.6, 2.4, 1 H)
9	48.0 (<i>d</i>)	1.62 (<i>t</i> , <i>J</i> = 9.0, 1 H)	4''	72.9 (<i>d</i>)	4.45 (<i>t</i> , <i>J</i> = 9.6, 1 H)
10	37.0 (<i>s</i>)		5''	69.9 (<i>d</i>)	4.67–4.73 (<i>m</i> , 1 H)
11	23.7 (<i>t</i>)	1.85–1.91 (<i>m</i> , 2 H)	6''	18.6 (<i>q</i>)	1.58 (<i>d</i> , <i>J</i> = 6.0, 3 H)
12	122.5 (<i>d</i>)	5.45 (<i>br. s</i> , 1 H)	Ara-1'''	104.8 (<i>d</i>)	5.99 (<i>d</i> , <i>J</i> = 3.6, 1 H)
13	144.9 (<i>s</i>)		2'''	72.8 (<i>d</i>)	4.31–4.35 (<i>m</i> , 1 H)
14	42.2 (<i>s</i>)		3'''	70.4 (<i>d</i>)	4.14–4.18 (<i>m</i> , 1 H)
15	28.3 (<i>t</i>)	1.17–1.21 (<i>m</i> , 2 H)	4'''	68.7 (<i>d</i>)	4.48–4.52 (<i>m</i> , 1 H)
16	23.7 (<i>t</i>)	1.92–1.99 (<i>m</i> , 2 H)	5'''	65.3 (<i>t</i>)	4.14–4.18, 4.35–4.37 (<i>2m</i> , 2 H)
17	46.7 (<i>s</i>)		Glc-1''''	106.9 (<i>d</i>)	5.14 (<i>d</i> , <i>J</i> = 7.8, 1 H)
18	42.0 (<i>d</i>)	3.29 (<i>d</i> , <i>J</i> = 10.2, 1 H)	2''''	75.5 (<i>d</i>)	4.03 (<i>t</i> , <i>J</i> = 8.4, 1 H)
19	46.5 (<i>t</i>)	1.76–1.81 (<i>m</i> , H _{ax}), 1.26–1.33 (<i>m</i> , H _{eq})	3''''	78.6 (<i>d</i>)	4.18–4.20 (<i>m</i> , 1 H)
20	31.0 (<i>s</i>)		4''''	71.2 (<i>d</i>)	4.26 (<i>t</i> , <i>J</i> = 9.0, 1 H)
21	34.2 (<i>t</i>)	1.43–1.48 (<i>m</i> , H _{ax}), 1.17–1.21 (<i>m</i> , H _{eq})	5''''	78.9 (<i>d</i>)	3.88–3.93 (<i>m</i> , 1 H)
22	33.3 (<i>t</i>)	1.76–1.81 (<i>m</i> , H _{ax}), 2.00–2.04 (<i>m</i> , H _{eq})	6''''	62.5 (<i>t</i>)	4.48–4.52, 4.37–4.42 (<i>m</i> , 2 H)
23	28.2 (<i>q</i>)	1.30 (<i>s</i> , 3 H)			
24	17.2 (<i>q</i>)	1.15 (<i>s</i> , 3 H)			
25	15.6 (<i>q</i>)	0.81 (<i>s</i> , 3 H)			
26	17.4 (<i>q</i>)	0.98 (<i>s</i> , 3 H)			
27	26.2 (<i>q</i>)	1.28 (<i>s</i> , 3 H)			
28	180.3 (<i>s</i>)				
29	33.3 (<i>q</i>)	0.94 (<i>s</i> , 3 H)			
30	23.8 (<i>q</i>)	1.00 (<i>s</i> , 3 H)			

^{a)} Multiplicity determined by DEPT experiments. ^{b)} Signals were assigned by HMQC, HMBC, and ^1H , ^1H -COSY experiments, chemical shifts (δ) in ppm relative to TMS. ^{c)} Coupling constants (*J* in Hz) are given in parentheses.

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Experimental Part

General. Reagents used were of anal. grade and purchased from *Yuwang Group Co., Ltd.* (Shandong, P. R. China). The authentic sugars were bought from *Aldrich*. TLC: silica gel *GF*₂₅₄ (*Qingdao*

Marine Chemical Company, P. R. China). CC: silica gel (SiO₂; 160–200 mesh; Qingdao Marine Chemical Company, P. R. China); Rp C-18 (25–40 μm) silica gel (Fuji Silysica Chemical Ltd.). Prep. HPLC: SSI instrument, consisting of a series III pump and a Model 500 UV detector; a YMC-Pack ODS-A (5 μm) stainless column (10 × 100 mm) was employed; the detection wavelength was 205 nm and the flow rate was 2 ml/min. M.p.: Yanaco-53 micromelting point apparatus; uncorrected. Optical rotations: Perkin-Elmer 241 MC automatic digital polarimeter. IR Spectra: Bruker IFS-55 IR spectrometer, with KBr disks. NMR Spectra: Bruker 300 and 600 FT-NMR spectrometers; TMS was used as internal standard. MS: Bruker Daltonic micrOTOF mass spectrometer.

Plant Material. Rhizomes of *Anemone raddeana* were collected from Liaoning Province of China, in May 2008, and they were identified by Prof. Qishi Sun (Department of Medicinal Plant, College of Traditional Chinese Medicine of Shenyang Pharmaceutical University). A voucher specimen (No. 001875) was deposited with the Herbarium of Shenyang Pharmaceutical University.

Extraction and Isolation. Air-dried and powdered rhizome of *A. raddeana* (4.0 kg) were refluxed with EtOH (75 %, 101 × 3 times, 2 h every time). The combined alcohol extracts were concentrated under reduced pressure to yield a dark brown residue (520 g), which was suspended in 10 l H₂O and then extracted successively with petroleum ether (10 l), CHCl₃ (10 l), AcOEt (10 l), and BuOH (15 l). The BuOH-soluble part (210 g) was chromatographed on 2.0 kg of SiO₂ column (Ø12 × 150 cm), eluting with AcOEt (4 l), AcOEt/MeOH (10:1, 8 l; 5:1, 12 l; 3:1, 14 l) and AcOEt/MeOH/H₂O (30:10:5 upper layer 16 l) to obtain 106 fractions. The fractions were combined into eight major fractions (B1–B8) based on TLC behaviors. The fraction B5 (19 g) was further subjected to SiO₂ CC (Ø5 × 100 cm) eluting with CH₂Cl₂/MeOH (10:1; 5:1; 3:1) to obtain 142 sub-fractions, and the sub-fractions were combined into 15 major sub-fractions (B5a–B5o). A crude mixture of compounds **1** and **2** (ca. 200 mg) was obtained from B5i by Rp C-18 SiO₂ CC (Ø2 × 70 cm, 30 g), eluting with MeOH/H₂O (6:4). Compounds **1** (*raddeanoside* R₂₂, 64 mg) and **2** (*raddeanoside* R₂₃, 30 mg) were isolated in pure form by prep. HPLC (MeOH/H₂O 7.5:2.5 t_R 113.81 min for **1**, 125.86 min for **2**). The fraction B1 (9.2 g) was further subjected to SiO₂ CC (Ø5 × 100 cm) eluting with AcOEt/MeOH/H₂O (30:1:0.1; 20:1:0.1; 15:1:0.1) to obtain compounds **3** (15 mg) and **4** (31 mg). The fraction B6 (12.7 g) was further subjected to Rp C-18 SiO₂ CC (Ø5 × 100 cm, 200 g) eluting with MeOH/H₂O (4:6, 5 l, 50 ml each) to obtain eight sub-fractions (B6a–B6h). Compound **5** (*hederasaponin B*, 26 mg, t_R: 37.67 min) was obtained from Fr. B6b by prep. HPLC with 35% MeOH. The fraction B8 (22.6 g) was further subjected to SiO₂ CC (Ø5 × 100 cm) eluting with CHCl₃/MeOH/H₂O (65:40:10) to obtain compound **6** (*hederacolchiside E*, 220 mg).

Acid Hydrolysis of Compounds 1 and 2. An aq. soln. (3 ml) of each sample (8 mg) was refluxed with 2M HCl (3 ml) for 4 h. After neutralization with aq. NaHCO₃ soln., the mixture was extracted with CHCl₃ (3 × 6 ml). The org. phase was evaporated and subjected to PTLC using CHCl₃/MeOH (9:1) as eluent to yield the aglycone. The H₂O layer was concentrated and submitted to PTLC (AcOEt/MeOH/H₂O 7:3:0.4) to yield the sugars which were identified by TLC (AcOEt/MeOH/H₂O 7:3:0.4) with authentic samples and [α]_D [14] as following: D-glucose [α]_D²⁵ = +39.6 (c = 0.12, H₂O), L-arabinose [α]_D²⁵ = +97.2 (c = 0.10, H₂O), L-rhamnose [α]_D²⁵ = +6.2 (c = 0.10, H₂O). Spots were detected by spraying with EtOH/H₂SO₄/anisaldehyde 17:2:1, followed by heating.

Raddeanoside R₂₂ (= (3β,18α)-3-((4-O-(β-D-Glucopyranosyl)-2-O-[(1R,2R,3S,4R,5R)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexyl]-α-L-arabinopyranosyl]oxy)olean-12-en-28-oic Acid; **1**). White powder. M.p. 285–286°. [α]_D²⁵ = +18.8 (c = 1.00, MeOH). IR (KBr): 3418, 2945, 1695, 1650, 1075, 643. ¹H- and ¹³C-NMR: Table 1. HR-ESI-MS: 935.4775 ([M + Na]⁺), 773.4290 ([M – Glc + Na]⁺), 480.1415 ([M – Glc – Glc – Ara + Na]⁺).

Raddeanoside R₂₃ (= (3β,18α)-3-((2-O-[(1R,2S,3R,4S,5R)-3-(α-L-Arabinopyranosyloxy)-2,4-dihydroxy-5-methylcyclohexyl]-4-O-(β-D-glucopyranosyl)-α-L-arabinopyranosyl]oxy)olean-12-en-28-oic Acid; **2**). White powder. M.p. 248–250°. [α]_D²⁵ = +124 (c = 0.50, MeOH). IR (KBr): 3421, 2941, 1695, 1650, 1071, 642. ¹H- and ¹³C-NMR: Table 2. HR-ESI-MS: 1051.5431 ([M + Na]⁺), 889.4849 ([M – Glc + Na]⁺), 773.4358 ([M – Glc – Ara + K]⁺), 613.1872 ([Glc + Ara + Rha + Ara + Na]⁺), 595.1769 ([Glc + Ara + Rha + Ara + Na – H₂O]⁺).

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