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# OLEANOLIC ACID BASED BISGLYCOSIDES FROM *ANEMONE RADDEANA* REGEL

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**Key Word Index**—*Anemone raddeana*; Ranunculaceae; raddeanosides $R_{10}$ and $R_{11}$; triterpenoid saponins; oleanolic acid; structure elucidation.

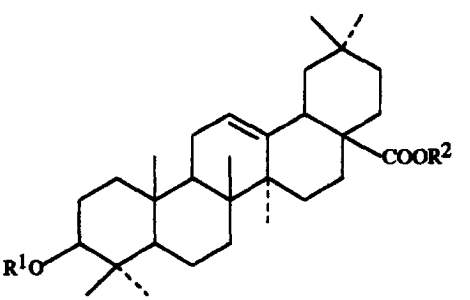
**Abstract**—From the roots of *Anemone raddeana*, two new triterpenoid bisglycosides, raddeanosides $R_{10}R_{11}$, have been characterized as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-1(1 → 2)-α-L-arabinopyranosyl] oleanolic acid 28-*O*-[α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside] and 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl] oleanolic acid 28-*O*-[β-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside]. 

## INTRODUCTION

The roots of *Anemone raddeana* Regel are used in Chinese folk medicine for curing rheumatism and neuralgia [1]. Eleven triterpenoid saponins have been reported from the roots of this plant [2–10]. This paper describes the isolation and characterization of two new oleanolic acid based bisglycosides from the methanolic extract of the roots of the title plant.

## RESULTS AND DISCUSSION

The water soluble saponin fractions were subjected to repeated CC on silica gel, affording saponins **1** and **2**. The yields were 0.0001 and 0.0006% of the dry plant. On mineral acid hydrolysis, both saponin **1** and **2** gave oleanolic acid (co-TLC) as the aglycone and D-glucose, L-rhamnose and L-arabinose (co-PC) as sugar components. A comparison of the $^{13}C$ NMR signals due to the aglycone moieties with those of reported saponins revealed that they were all bisdesmosides of 3,28-di-*O*-glycosides [11].




| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | −Ara(2 ← 1)Glc(2 ← 1)Rha | −Glc(6 ← 1)Glc(4 ← 1)Rha(3 ← 1)Glc |
| **2** | −Ara(2 ← 1)Glc(2 ← 1)Rha | −Glc(6 ← 1)Glc(4 ← 1)Rha(3 ← 1)Glc(6 ← 1) |
| **1a, 2a** | −Ara(2 ← 1)Glc(2 ← 1)Rha | |

On alkaline hydrolysis, saponins **1** and **2** gave the same prosapogenins **1a** and **2a**. The $^{13}C$ NMR data of **2a** was just the same as that of raddeanoside $R_3$ [2]. Compounds **1a** and **2a** were confirmed as raddeanoside $R_3$ by comparison with an authentic sample. Thus, **1a** and **2a** were identified as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl] oleanolic acid.

The EI mass spectrum of the acetate of saponin **1** showed a fragment ion at *m/z* 331 $[glc(Ac)_4]^+$, indicating a terminal D-glucose. The $^{13}C$ NMR spectrum

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due to sugar moieties of saponin **1** indicated the presence of seven monosaccharide units. By comparison with raddeanoside $R_3$ [10], the $^{13}C$ NMR data due to the six sugar moieties were the same. The other $^{13}C$ NMR data due to the sugar moieties were the same as α-D-glucopyranose [12, 13]. The glycosylation shift of the anomeric carbon of the α-D-glucose and 101.7 ppm, less than that of β-D-glucose (> 104 ppm), indicating the terminal sugar was α-D-glucose [14]. The $^1H$ NMR spectrum (δ 4.86, 1H, *d*, *J* = 3 Hz) of the sugar also supported this conclusion [15, 16], since the coupling constant of β-D-glucopyranose is about 7 Hz. According to the glycosylation shift reported in the literature [17], δ 83.6 indicated that the σ-L-rhamnopyranose was 3-*O*-glycosylated. Based on the above evidence, compound **1** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2) α-L-arabinopyranosyl] oleanolic acid 28-*O*-[α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside], which was named raddeanoside $R_{10}$.

The EI mass spectrum of the acetate of saponin **2** showed a fragment ion at *m/z* 331 $[glu(Ac)_4]^+$, indicating a terminal D-glucose. The $^{13}C$ NMR spectrum due to the sugar moieties of saponin **2** indicated the presence of eight monosaccharide units. By comparison with saponin **1**, the $^{13}C$ NMR data due to the seven sugar moieites were the same. The other $^{13}C$ NMR data due to sugar moieites were the same as β-D-glucopyranose. The glycosylation shift (δ 68.7) revealed that C-6 of the α-D-glucopyranose was 6-*O*-glycosylated. Based on above studies, **2** was characterized as 3-*O*-[α-Lrhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-arabinopyranosyl] oleanolic acid 28-*O*-[β-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside], which was named raddeanoside $R_{11}$.

## EXPERIMENTAL

$^1H$ NMR and $^{13}C$ NMR spectra were recorded at 400 MHz in pyridine-$d_5$ using TMS as int. standard. EIMS were measured at 70 eV accelerating voltage after acetylation. Optical rotations were measured with a PE-241 polarimeter at 18° in pyridine.

*Plant material.* The roots of *A. raddeana* were bought from Changcun (China) and identified by Prof. S.C. Xiao.

*Extraction and isolation.* Dry roots (8.2 kg) were extracted with MeOH. The extracts were homogenized in $H_2O$ and extracted with *n*-BuOH. The $H_2O$ part was poured into a D101 resin column and washed with $H_2O$ first, then washed with MeOH. The methanolic fr. was concd to dryness (wt. 42 g) and fractionated by repeated CC (silica gel, $CHCl_3$–MeOH, 2:1, $H_2O$ saturated), affording **1** (8 mg) and **2** (50 mg) as a colourless amorphous powder.

Compound **1**, $[\alpha]_D^{18}$ (pyridine, *c* 0.4) −270°. EIMS, *m/z* (rel.int.): 331 $[glc(Ac)_4]^+$ (4.2). $^1H$ NMR (300 MHz, ppm): δ 6.20 (1H, *d*, *J* = 8.0 Hz, Ara-H-1), 6.13 (1H, *d*, *J* = 4.8 Hz, Rha-H-1), 5.82 (1H, *d*, *J* = 4.6 Hz, Rha-1), 5.05 (1H, *d*, *J* = 7 Hz, Glc-H-1), 4.97 (1H, *d*, *J* = 8 Hz, β-Glc-H-1), 4.89 (1H, *d*, *J* = 7 Hz, β-Glc-H-1), 4.86 (1H, *d*, *J* = 3 Hz, α-Glc-H-1), 1.82 (3H, *d*, *J* = 6 Hz, $CH_3$ of rhamnose), 1.69 (3H, *d*, *J* = 6 Hz, $CH_3$ of rhamnose), 1.23 (3H, *s*, $CH_3$), 1.15

Table 1. $^{13}C$ NMR spectral data of the saponin sugar moieties (in pyridine-$d_5$, ppm)

| | 1 | 2 | 2a |
|---|---|---|---|
| $R^1$-Ara-1 | 105.2 | 104.7 | 105.6 |
| 2 | 77.1[a] | 76.6[a] | 76.8 |
| 3 | 72.7[b] | 72.2[b] | 72.9 |
| 4 | 68.4 | 69.3 | 69.8 |
| 5 | 64.4 | 63.9 | 63.8 |
| Glc-1 | 106.1 | 105.8 | 106.6 |
| 2 | 78.6[c] | 78.2[c] | 79.0 |
| 3 | 76.1[d] | 75.6[d] | 76.0 |
| 4 | 72.0[b] | 71.5[b] | 71.7 |
| 5 | 78.4[c] | 78.2[c] | 78.8 |
| 6 | 62.5 | 62.2 | 62.0 |
| Rha-1 | 101.9 | 101.4 | 102.1 |
| 2 | 72.2[b] | 72.0[b] | 72.6 |
| 3 | 72.0[b] | 71.7[b] | 72.3 |
| 4 | 73.9 | 73.4 | 74.1 |
| 5 | 70.3 | 69.8 | 69.8 |
| 6 | 18.5 | 18.0 | 19.1 |
| $R^2$-Glc-1 | 95.6 | 95.1 | |
| 2 | 75.4 | 75.8[d] | |
| 3 | 78.1[c] | 77.6[c] | |
| 4 | 71.2 | 70.7[e] | |
| 5 | 77.3[a] | 77.4[c] | |
| 6 | 69.9 | 69.4 | |
| glc-1 | 104.9 | 104.4 | |
| 2 | 76.3[d] | 76.6[a] | |
| 3 | 78.4[c] | 77.9[c] | |
| 4 | 79.5 | 79.0 | |
| 5 | 77.1[a] | 76.9[a] | |
| 6 | 61.3 | 60.8 | |
| Rha-1 | 102.6 | 102.0 | |
| 2 | 72.5[b] | 71.9[b] | |
| 3 | 84.5 | 84.0 | |
| 4 | 73.9 | 73.4 | |
| 5 | 70.7 | 70.2[b] | |
| 6 | 18.5 | 18.0 | |
| Glc-1 | 101.7 | 101.2 | |
| 2 | 72.5[b] | 72.2[b] | |
| 3 | 75.4 | 74.9 | |
| 4 | 71.2 | 70.7[e] | |
| 5 | 73.9 | 73.4 | |
| 6 | 61.3 | 68.7 | |
| Glc-1 | | 105.6 | |
| 2 | | 74.9 | |
| 3 | | 77.9[c] | |
| 4 | | 72.0[b] | |
| 5 | | 76.6[a] | |
| 6 | | 60.8 | |

[a–e] Assignment in any column may be reversed.

(3H, *s*, $CH_3$), 1.10 (3H, *s*, $CH_3$), 1.07 (3H, *s*, $CH_3$), 0.87 (9H, *s*, each 3H, $CH_3$).

Compound **2**, $[\alpha]_D^{18}$ (pyridine, *c* 1.4) −830°. EIMS, *m/z* (rel.int.): 331 $[glc(Ac)_4]^+$ (2.2). $^1H$ NMR (300 MHz, ppm): $\delta$ 6.20 (1H, *d*, $J$ = 8.0 Hz, Ara-H-1), 6.13 (1H, *d*, $J$ = 4.8 Hz, Rha-H-1), 5.89 (1H, *d*, $J$ = 4.6 Hz, Rha-1), 5.39 (1H, *br*, 12-H), 5.31 (1H, *d*, $J$ = 7.0 Hz, $\beta$-Glc-H-1), 5.16 (1H, *d*, $J$ = 7.0 Hz, $\beta$-D-Glc-H-1), 5.09 (1H, *d*, $J$ = 7 Hz, $\beta$-Glc-H-1), 4.96 (1H, *d*, $J$ = 8 Hz, $\beta$-Glc-H-1), 4.94 (1H, *d*, $J$ = 3 Hz, $\alpha$-Glc-H-1), 1.81 (3H, *d*, $J$ = 6 Hz, $CH_3$ of rhamnose), 1.67 (3H, *d*, $J$ = 6 Hz, $CH_3$ of rhamnose), 1.23 (3H, *s*, $CH_3$), 1.15 (3H, *s*, $CH_3$), 1.09 (3H, *s*, $CH_3$), 1.07 (3H, *s*, $CH_3$), 0.89 (3H, *s*, $CH_3$), 0.87 (3H, *s*, $CH_3$), 0.86 (3H, *s*, $CH_3$).

*Acid hydrolysis of* **1** *and* **2**. Compound **1** (3 mg) and compound **2** (3 mg) were separately hydrolysed in $H_2O$ (1 ml) with 6 N HCl for 5 hr at 90°. The reaction mixt. was diluted with $H_2O$ and extracted with $CHCl_3$. Sapogenins were detected in the $CHCl_3$ layer by TLC. The water layer (containing all the sugars) was concd to dryness by high vacuum, then subjected to PC analysis with authentic sugars. $EtOAcC_5H_5N$–$H_2O$ (2:1:2) (upper layer) was used as solvent. Aniline-phthalate was used for detection of sugars on the paper.

*Alkaline saponification of saponins* **1** *and* **2**. Compound **1** (2 mg) was heated for 8 hr with 23–25% $NH_3 \cdot H_2O$ (5 ml) at 90°. The mixt. was concd to dryness by high vacuum to afford raddeanoside $R_3$ (co high performance TLC). Compound **2** (25 mg) was heated for 8 hr with 23–25% $NH_3 \cdot H_2O$ (5 ml) at 90°. The mixt. was concd to dryness by high vacuum and purified by CC (3 g silica gel, $CHCl_3$–MeOH, 3:1); 15 mg **2a** was obtained.

*Acetylation of saponins* **1** *and* **2**. Compound **1** (3 mg) and **2** (3 mg) were separately dissolved in pyridine (0.5 ml, dried with KOH) and $(AcO)_2O$ (0.5 ml). They were heated at 70° for 1 hr and left for 24 hr, then the solvent was removed and the product subjected to EIMS analysis.

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