Chemistry of Natural Compounds, Vol. 49, No. 2, May, 2013 [Russian original No. 2, March–April, 2013]

A NEW TRITERPENE GLYCOSIDE FROM *Anemone raddeana*

Feng-Zhi Ren,* Xue-Xia Zhang, Shu-Hong Chen, Li-Hong Li, and Xiao-Xun Cheng

UDC 547.918

*A new triterpene glycoside, 27-hydroxyoleanolic acid-3-*O*-α-L-arabinopyranoyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl ester, was isolated from the rhizomes of* Anemone raddeana*. The structure was identified by physichemical properties and spectral analysis, especially 2D NMR techinques.*

Keywords: *Anemone raddeana*, triterpene glycoside, NMR.

The dried rhizomes of *Anemone raddeana* Regel (Ranunculaceae), known as *Liang Tou Jian* in Chinese traditional medicine, have been used for the treatment of rheumatism, spasm of limbs, arthralgia, carbuncle ulcers, etc. [1]. The crude saponins obtained from the plant exhibited antitumor, analgesic, and calming activities [2, 3]. In order to search for the active constituents from this plant, we carried out extensive studies on the chemical constituents of the rhizomes of *Anemone raddeana*. A new compound, 27-hydroxyoleanolic acid-3-*O*-*α*-L-arabinopyranoyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside-28-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl ester (**1**), was isolated and identified.

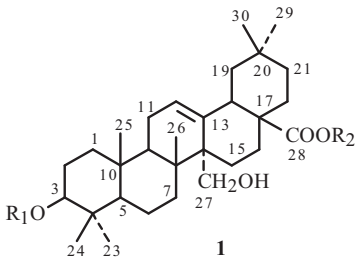
Compound **1** was assigned the molecular formula $C_{64}H_{104}O_{30}$ on the basis of HR-FAB-MS (*m/z* 1353.6676 $[M + H]^+$) and ^{13}C NMR spectrum data (Table 1). Glucose, arabinose, and rhamnose were detected in the acid hydrolysate compared with the corresponding authentic samples. In the ^1H NMR spectrum, six signals for the anomeric protons of the sugar moieties were observed at 4.79 (d, J = 6.0 Hz), 4.99 (d, J = 8.0 Hz), 5.85 (br.s), 5.94 (d, J = 4.5 Hz), 6.29 (br.s), and 6.24 (d, J = 8.0 Hz). The ^{13}C NMR spectrum contained six signals due to the anomeric carbons of sugar moieties at δ 95.7, 101.4, 102.8, 104.9, 104.8, and 105.4, and thirty signals due to the aglycone carbons. Therefore, compound **1** was identified as a triterpene glycoside with six sugar moieties. Compared to the ^{13}C NMR data of oleanolic acid, the C-27 signal was observed at δ 64.4, and the H-27 (3.75/4.02) correlated with C-27 (δ 64.5) in the HMQC spectrum and showed long-range correlations with C-8 and C-15 in the HMBC spectrum. In the ^{13}C NMR spectra , the C-28 signal was observed at a higher field, and the C-3 and C-14 signals were found at a lower field. Therefore, the aglycone of compound **1** was 27-hydroxyoleanolic acid, and sugar chains are linked to the C-3 and C-28 positions on the aglycone.

The sugar sequence and glycosidic linkage positions were established as follows. On alkaline hydrolysis, glucose and rhamnose were detected. The ESI-MS spectrum showed fragment ions at *m/z* 881 $[M - Rha - Glc - Glc - H]^-$. These indicated the presence of three monosaccharide units at the C-28 position. In the HMBC spectrum, correlation peaks were observed between signals at δ 6.24 (H-1 of Glc-1)/176.6 (C-28 of Agl), 4.99 (H-1 of Glc-2)/69.2 (C-6 of Glc-1), and 5.85 (H-1 of Rha)/78.8 (C-4 of Glc-2). The above results indicated that the sugar chain at the C-28 position was Rha-(1→4)-Glc(1→6)-Glc [4]. The sugar chain at the C-3 position contained arabinose, and rhamnose. In the HMBC spectrum of compound **1**, correlation peaks were observed between signals at δ 4.79 (H-1 of Ara)/88.8 (C-3 of Agl), 6.29 (H_1 of Rha)/75.2 (C-2 of Ara), δ 5.94 (H-1 of Ara)/81.3 (C-3 of Rha). The results indicate that the sugar sequence at the C-3 position was Ara-(1→3)-Rha-(1→2)-Ara [5]. Based on these finding, compound **1** was elucidated as 27-hydroxyoleanolic acid-3-*O*-*α*-L-arabinopyranoyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside-28-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl ester.

New Drug Research and Development Center of North China Pharmaceutical Group Corporation, National Microbial Medicine Engineering and Research Center, 050015, Shijiazhuang, P. R. China, e-mail: rfzhhj@sohu.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 2, March–April, 2013, pp. 232–233. Original article submitted January 7, 2012.

TABLE 1. ^{13}C NMR Data of Compound **1** (125 MHz, C_5D_5N, δ, ppm)

C atom	δ_C	C atom	δ_C	C atom	δ_C	C atom	δ_C
1	38.9	17	46.9		75.1		78.1
2	26.7	18	41.4		69.6		70.9
3	88.8	19	45.3		66.1		78.1
4	39.6	20	30.8	Rha	101.4		69.2
5	55.9	21	33.9		72.1	Glc-2	104.9
6	18.6	22	32.5		81.3		75.4
7	33.6	23	28.1		72.9		76.5
8	40.6	24	17.2		69.6		78.8
9	48.6	25	16.2		18.6		77.2
10	37.2	26	18.9	Ara-2	104.8		61.3
11	23.4	27	64.4		72.8	Rha	102.8
12	127.9	28	176.6		68.8		72.8
13	139.2	29	33.1		69.9		72.6
14	48.0	30	23.8		65.3		74.0
15	24.4	3-Ara-1	105.4	28-Glc-1	95.7		70.3
16	23.8		75.2		73.9		18.5



1

R_1 = Ara-(1→3)-Rha-(1→2)-Ara-
R_2 = Rha-(1→4)-Glc-(1→6)-Glc-

EXPERIMENTAL

General Comments. ^1H NMR and ^{13}C NMR spectra were measured on a Varian INOVA 500 spectrometer at 500 and 125 MHz, respectively, with TMS as an internal standard; ESI-MS was performed on a ZMD Micromass spectrometer; HR-FAB-MS data were recorded on a Bruker Daltonics Apex II mass spectrometer. Melting points were recorded on an X-6 melting point tester. TLC was carried out on precoated silica gel 60 F_{254} plates (Merck). Column chromatography was conducted using silica gel (300–400 mesh, Qingdao Marine Chemical Co., China), Sephadex LH-20 (Sigma), a Waters 996 high-performance liquid chromatograph with a Venusil XBP C-18 (250 mm × 4.6 mm column, 10 μm) column, and a Waters 600-2487 PrepLC system with a Phenomenex 250 × 21.2 mm column.

Plant Material. The rhizomes of *A. raddeana* were purchased from Lerentang Pharmacy of Shijiazhuang and identified by Yan-Zhe Zhang.

Extraction and Isolation. The air-dried rhizomes of *A. raddeana* (4.5 kg) were refluxed twice with 80% ethanol, and the extract was concentrated under vacuum to afford a viscous residue (580 g). The residue was suspended in water (5 L), then extracted with petroleum ether, EtOAc and *n*-BuOH. The *n*-BuOH soluble part was subjected to column chromatography on AB-8 resin with a successive elution system of H_2O, 20% EtOH, 40% EtOH, 60% EtOH, 80% EtOH, and EtOH. The 40% EtOH portion was subjected to silica gel column and Pre-HPLC to afford compound **1**.

Compound 1. White amorphous powder, mp 260–262°C. Negative ESI-MS *m/z*: 1351 $[M - H]^-$, 881 [M – Rha – Glc – Glc – H]$^-$. Positive HR-FAB-MS *m/z* 1353.6676 $[M + H]^+$. ^1H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 0.81, 0.87, 0.89, 1.08, 1.13, 1.23 (3H, s, CH_3), 1.52 (3H, d, J = 6.0, Rha-CH_3), 1.69 (3H, d, J = 6.0, Rha-CH_3), 3.13 (1H, m, H-3), 3.75, 4.02 (each 1H, d, J = 12, 27-H), 4.79 (1H, d, J = 6.0, 3-Ara-H_1), 4.99 (1H, d, J = 8.0, 28-Glc′-H_1), 5.74 (1H, m, H-12); 5.85 (1H, br.s, 28-Rha-H_1), 5.94 (1H, d, J = 4.5, 3-Ara′-H_1), 6.29 (1H, br.s, 3-Rha-H_1), 6.24 (1H, d, J = 8.0, 28-Glc-H_1).

REFERENCES

1. *Pharmacopoeia Committee of P. R. China*, Vol. **I**, 2010.
2. E. X. Zhang and F. E. Wu, *Chin. J. Biochem. Pharm*., **63**, 61 (1993).
3. Y. Gao, P. Guo, S. F. Bai, and D. Y. Liu, *Mod. Pharm. Clin*., **24**, 91 (2009).
4. V. I. Grishkovets, E. A. Sobolev, A. S. Shashkov, and V. Ya. Chirva, *Chem. Nat. Compd*., **36**, 501 (2000).
5. R. P. Thapliyal and R. P. Bahuguna, *Phytochemistry*, **33**, 671 (1993).