Chemistry of Natural Compounds, Vol. 48, No. 5, November, 2012 [Russian original No. 5, September–October, 2012]

A NEW TRITERPENOID WITH ANTIMICROBIAL ACTIVITY FROM *Anemone rivularis*

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UDC 547.918

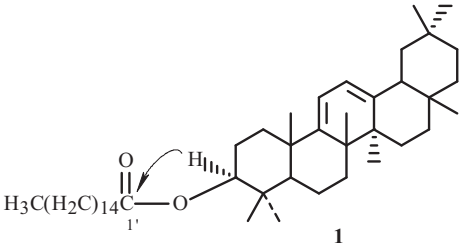
*A new triterpene ester **1** and six known triterpenoids were isolated from* Anemone rivularis *by repeated column chromatography. Their structures were identified as olean-9(11),12-dien-3-*O*-palmitate (**1**), lupeol (**2**), betulin (**3**), betulic acid (**4**), oleanolic acid (**5**), ursolic acid (**6**), and* β*-amyrin (**7**) by spectral analysis and comparison with the published data. In addition, compound **1** was evaluated* in vitro *for its antimicrobial activity. It was found to exhibit moderate activity against the Gram-positive bacteria* B. subtilis *and* S. aureus.

Keywords: *Anemone rivularis*, chemical constituents, triterpenoid.

Anemone rivularis is widespread in China. Its roots have been used as an antitumor agent in Chinese traditional medicine [1]. Although some triterpene glycosides were isolated from the genus *Anemone* [2–4], there have been few reports concerning the secondary metabolites of *A. rivularis*. In this paper, we describe the isolation of seven triterpenoids from *A. rivularis* as well as the identification of their structures by spectroscopic analysis and comparison with the published data. The antimicrobial activity of compound **1** is also reported.

Compound **1** was isolated as a white powder, mp 249–251°C. It showed a positive reaction with the Liebermann–Burchard reagent. The molecular formula of **1** was suggested as $C_{46}H_{78}O_2$ on the basis of HR-ESI-MS (found *m/z* 663.6112 $[M + H]^+$; calcd for $C_{46}H_{79}O_2$, 663.6080). The ^{1}H NMR spectrum of **1** was very similar to that of olean-9(11),12-dien-3β-ol [5] except for the chemical shift at δ 4.54 (1H, t like, J = 8.1 Hz, H-3) and additional signals including 28 protons at δ: 1.25–1.36, 1.63, 2.30 and another additional methyl signal at δ 0.86 (3H, t, J = 6.8 Hz, CH_3), which suggested that the proton of 3-OH is replaced by a long-chain fatty acid. The HMBC cross-peak of H-3 with C-1′ (δ 173.2) indicated that the long chain fatty acid was assigned to C-3.

Compound **1** was hydrolyzed with KOH [6]. The hydrolyzed alkaline solution was neutralizated with HCl and extracted with $CHCl_3$, and a white mixture was obtained. The mixture was chromatographed on Sephadex LH-20 with CH_2Cl_2–MeOH (3:1) to yield olean-9(11),12-dien-3β-ol (8.1 mg) [5] and a white fatty acid (5 mg). The EI-MS of the white fatty acid showed a molecular ion at *m/z* 256, corresponding to palmitic acid. Based on the above evidence, **1** was elucidated as olean-9(11),12-dien-3-*O*-palmitate.



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HMBC (H→C)

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TABLE 1. The Results of Antibacterial Activity of Compound **1**

Kind of Bacteria	**1**		Ciprofloxacin	Tetracycline
	Diameter of inhibition zone, mm	MIC values, μg·mL^{-1}	Diameter of inhibition zone, mm	MIC values, μg·mL^{-1}
B. subtilis	14.1	50	34.0	3
S. aureus	12.5	75	33.5	3
P. aeruginosa	7.5	> 100	30.0	25
E. coli	10.0	90	32.1	7
S. typhimurium	8.0	> 100	33.2	3

Compounds **2–7**, by comparison with the published data, were identified as lupeol (**2**) [7], betulin (**3**) [8], betulic acid (**4**), *β*-amyrin (**7**) [9], oleanolic acid (**5**), and ursolic acid (**6**) [10], respectively.

The testing of antimicrobial activity of compound **1** was done *in vitro* using the Kirby-Bauer disc diffusion method [11]. The MIC values against bacterial strains were obtained using the broth macro-dilution method [12]. Both the Gram-positive (*Bacillus subtilis*, *Staphyloccocus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*) bacteria were grown in nutrient agar medium and incubated at 37°C for 48 h. Compound **1** was dissolved in dimethyl sulfoxide (DMSO-d_6). We found that compound **1** showed moderate activity against *B. subtilis* and *S. aureus*, and potent activity against *E. coli*, *S. typhimurium*, and *P. aeruginosa* (Table 1).

EXPERIMENTAL

Melting points were determined on a Yanaco MP-S3 melting point apparatus and are uncorrected. EI-MS spectra were measured with a VG-5050E mass spectrometer. HR-ESI-MS was performed on a QSTAR LCQ mass spectrometer. NMR spectra were recorded on a Bruker ARX-300 NMR spectrometer using TMS as an internal standard. Silica gel, 200–300 mesh, was from Qingdao Ocean Chemical Group Co. Ltd., P. R. China. TLC was performed on HSGF254 precoated silica gel plates, 10–40 μm, from Yantai Chemical Plant, Yantai, P. R. China. Sephadex LH-20 gel was from Pharmacia.

Olean-9(11),12-dien-3-*O*-palmitate (1). ^{1}H NMR (300 MHz, $CDCl_3$–CD_3OD, δ, ppm, J/Hz): 0.83 (3H, s), 0.86 (3H, t, J = 6.8, H-16′), 0.87 (3H, s), 0.91–0.92 (9H), 0.98 (3H, s), 1.08 (3H, s), 1.19 (3H, s), 1.25–1.26, 1.63 (26H, m, H-3′–15′), 2.30 (2H, m, H-2′), 4.54 (1H, t like, J = 8.1, H-3), 5.50 (1H, d, J = 5.7, H-12), 5.56 (1H, d, J = 5.7, H-11). ^{13}C NMR (75 MHz, $CDCl_3$–CD_3OD, δ, ppm): 37.1 (C-1), 23.5 (C-2), 80.4 (C-3), 37.8 (C-4), 51.2 (C-5), 18.2 (C-6), 32.0 (C-7), 38.1 (C-8), 153.8 (C-9), 40.7 (C-10), 115.9 (C-11), 120.6 (C-12), 147.2 (C-13), 42.6 (C-14), 25.7 (C-15), 27.2 (C-16), 32.2 (C-17), 45.6 (C-18), 46.8 (C-19), 31.2 (C-20), 34.6 (C-21), 36.9 (C-22), 28.6 (C-23), 16.7 (C-24), 20.1 (C-25), 21.0 (C-26), 25.4 (C-27), 28.2 (C-28), 23.6 (C-29), 33.2 (C-30), 14.1 (C-16′), 22.8 (C-15′), 25.4 (C-3′), 29.0–30.2 (C-4′–13′), 31.7 (C-14′), 34.5 (C-2′), 173.2 (C-1′).

The EI-MS (70 eV) data of palmitic acid in compound **1** after alkaline hydrolysis showed *m/z* (I_{rel}, %): 256 $[M]^+$ (100), 227 (7), 2132 (21), 185 (17), 157 (16), 129 (36).

ACKNOWLEDGMENT

This work was partially supported by the programs from the Analytical Detection Center, Shenyang Pharmaceutical University, and the Analytical Detection Center, Shanghai Second Military Medical University, the Analytical Detective Center, Yangzhou University. We are also grateful to Associate Professor Qingde Long, GuiYang Medical College, for identification of the plant.

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