FULL PAPER

Two New Triterpenoid Glycosides from the Roots of *Rosa cymosa* Tratt.

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Two new triterpenoid glycosides, 3α,19α,23α-trihydroxy-2-oxo-12-ursen-28-*O*-β-D-glucopyranoside (**1**) and 3α,19α,23α-trihydroxy-2-oxoolean-12-en-28-*O*-β-D-glucopyranoside (**2**) as well as three known compounds, 2α,3α,19α-trihydroxyolean-12-en-28-*O*-β-D-glucopyranoside (**3**), 2α,3α,19α,23-tetrahydroxy-12-ursen-28-*O*-β-D-glucopyranoside (**4**), and 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid (**5**) were isolated from 75% EtOH extract of *Rosa cymosa*. Their structures were elucidated by extensive spectroscopic methods. All the isolated compounds displayed moderate inhibitory activity against LPS-induced NO production in macrophages.

Keywords: *Rosa cymosa* Tratt., Triterpenoid glycosides, Anti-inflammatory activity, Ursane triterpenes, Oleanane triterpenes.

Introduction

Rosa cymosa Tratt. is a member of the genus *Rosa* in the *Rosaceae* family, and is mainly distributed in the south of China [1]. The roots of *R. cymosa* were traditionally used as folk medicine for the treatment of rheumatoid arthritis, contusions, diarrhea, and descensus uteri [2] [3]. Modern pharmacological studies indicated that the roots of *R. cymosa* displayed significant biological activities, such as anticoagulation [4][5], antibiosis [3], anti-inflammatory [6], and antioxidant [7]. Recent phytochemical studies suggested that the triterpenoid constituents were mainly responsible for the biological activities of this plant. With the aim of finding new bioactive triterpenoid agents, the 75% EtOH extraction of this plant were examined, and two new triterpenoid glycosides, named as 3α,19α,23α-trihydroxy-2-oxo-12-ursen-28-*O*-β-D-glucopyranoside (**1**) and 3α,19α,23α-trihydroxy-2-oxoolean-12-en-28-*O*-β-D-glucopyranoside (**2**) as well as three known compounds (*Fig. 1*) were obtained. In this article, we describe the isolation and structure determination of the isolated compounds, and their inhibitory activity against lipopolysaccharide (LPS)-induced NO production in macrophages.

Results and Discussion

Compound **1** was isolated as a white amorphous powder with $[\alpha]_D^{20}$ = +27.4 (*c* = 0.12, MeOH), exhibiting a *quasi*-molecular ion peak at *m*/*z* 687.3810 $[M + Na]^+$ (calc. for 687.3792) in the positive-ion mode. In conjunction with the analysis of ^{1}H- and ^{13}C-NMR (APT) (*Table 1*) spectra, the formula of compound **1** was deduced as $C_{36}H_{56}O_{11}$. Its IR spectrum showed the presence of OH group absorptions at 3573 – 3392 cm^{-1}, CO signal at 1689 cm^{-1}, and C=C signal at 1653 cm^{-1}. The ^{1}H-NMR spectrum **1** displayed the presence of six Me signals at δ(H) 0.72 (*s*), 0.90 (*s*), 1.00 (*d*, *J* = 6.6), 1.07 (*s*), 1.27 (*s*), 1.52 (*s*); one olefinic H-atom at δ(H) 5.41 (br. *s*, H–C(12)); one O-bearing CH signal at δ(H) 5.00 (*s*), two HO–CH_2 groups at δ(H) 3.93 (*d*, *J* = 10.8), 3.64 (*d*, *J* = 10.8), 4.30 (br. *d*, *J* = 12.0), 4.38 (br. *d*, *J* = 12.0); and one anomeric H-atom signal at δ(H) 6.18 (*d*, *J* = 8.4). The above data suggested that **1** was an ursane triterpenoid saponin derivative with a sugar moiety [8 – 11]. The ^{13}C-APT spectrum displayed 36 C-atom signals (*Table 1*) including six Me C-atom signals at δ(C) 14.2, 17.0, 17.4, 17.4, 24.8, 27.3; two olefinic C-atom signals at δ(C) 124.2 (C(12)), 139.8 (C(13)); two CO signals at δ(C) 177.3 (C(28)), 213.1 (C(2)); one sugar moiety C-atom signals at δ(C) 96.2, 74.4, 79.6, 71.7, 79.7, 62.8; and other three oxygenated C-atom signals at δ(C) 78.0, 65.3, 73.0. The assignment of

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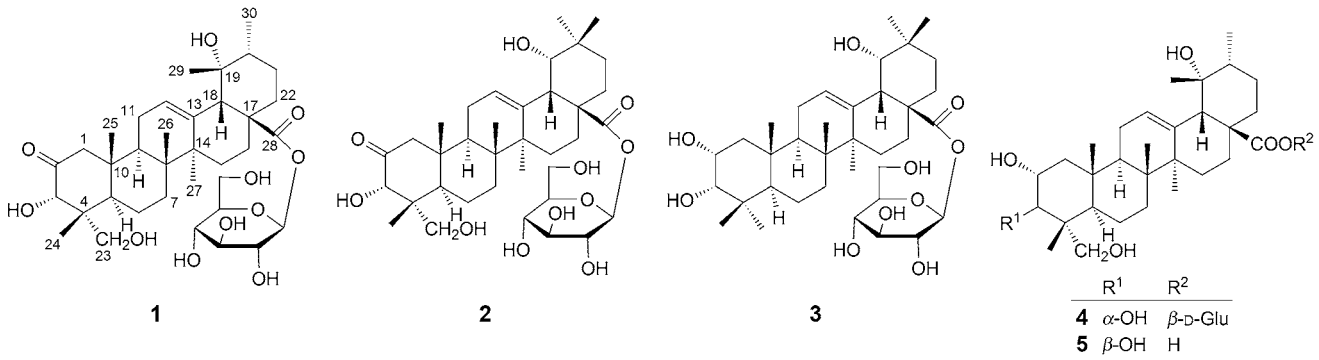

Fig. 1. The structures of compounds **1** – **5**

^{1}H-NMR and ^{13}C-APT spectroscopic data of **1** were based on the HSQC, HMBC, and ^{1}H,^{1}H-COSY spectrum. In fact, the above data of compound **1** were similar to those of the known compound 2α,3α,19α,23-tetrahydroxy-12-ursen-28-*O*-β-D-glucopyranoside (**4**) [12][13], except for the OH group at C(2) in compound **4** wherein it was O-bearing to CO group in compound **1**. In the HMBC spectrum (*Fig. 2*), the correlations from H–C(3) (δ(H) 5.00) to C(2) (δ(C) 213.1) and C(4) (δ(C) 49.0) confirmed the difference. The sugar moiety was located at C(28) on the basis of the correlation from the anomeric H-atom signal at δ(H) 6.18 (*d*, *J* = 8.4) to the CO signal at δ(C) 177.3 (C (28)). The type and absolute configuration of the sugar was identified as D-glucose on the basis of TLC method comparison with authentic monosaccharide ($CHCl_3$/MeOH/H_2O 3:2:0.2, visualization with EtOH/5%. H_2SO_4 spraying), followed by HPLC analysis. The NOESY spectrum was also employed to confirm the configuration of compound **1**. The NOE correlations of H–C(3) (δ(H) 5.00, *s*) with Me(24) (δ(H) 0.72, *s*) indicated the α-orientation of the OH group. Therefore, the structure of **1** was elucidated as 3α,19α,23α-trihydroxy-2-oxo-12-ursen-28-*O*-β-D-glucopyranoside.

Compound **2** was isolated as a white amorphous powder with $[\alpha]_D^{20}$ = +19.1 (*c* = 0.12, MeOH). Its molecular formula was assigned as $C_{36}H_{56}O_{11}$ on the basis of HR-ESI-MS at *m*/*z* 687.3802 $[M + Na]^+$ (calc. for 687.3790) in the positive-ion mode. Its IR spectrum showed the presence of OH group absorptions at 3623 – 3452 cm^{-1}, CO signal at 1699 cm^{-1}, and C=C bond at 1643 cm^{-1}. The ^{1}H and ^{13}C-NMR (APT) (*Table 1*) spectroscopic data were quite similar to those of **1**, except for the Me group at C (19) in compound **1** wherein it was transferred to C(20) in compound **2**. This difference was fully supported by the 2D-NMR spectra. In the HMBC spectrum, the correlations from the H-atom signal of an O-bearing group at δ (H) 3.55 (*d*, *J* = 4.8, H–C(19)) to δ(C) 45.0 (C(18)), 35.9 (C(20)), and Me signals at δ(H) 1.12 (*s*, Me(29)), 0.96 (*s*, Me(30)) to C(20) (δ(C) 35.9) indicated that Me(29) and Me(30) were both attached to C(20). Therefore, compound **2** was an oleanane triterpenoid saponin [6][9]. Acid hydrolysis of **2** afforded sugar moiety of D-glucose and identified by TLC and HPLC analysis. In the NOESY spectrum, the enhancement from H–C(3) (δ(H) 5.00) to Me(24) (δ(H) 0.81) and Me(24) to Me(25) (δ(H) 0.97) indicated that the OH group at C(3) was α-oriented. Thus, the structure of **2** was identified as 3α,19α,23α-trihydroxy-2-oxoolean-12-en-28-*O*-β-D-glucopyranoside.

The three known compounds, 2α,3α,19α-trihydroxyolean-12-en-28-*O*-β-D-glucopyranoside (**3**) [14][15], 2α,3α,19α,23-tetrahydroxy-12-ursen-28-*O*-β-D-glucopyranoside (**4**) [5], and 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid (**5**) [13] were identified by comparing their ^{1}H- and ^{13}C-NMR data with the reported literatures.

All five compounds were studied for their anti-inflammatory activities on LPS-induced nitric oxide (NO) production in RAW 264.7. The results are presented in *Table 2*. It may be said that all the tested compounds showed moderate inhibitory activities against the production of NO with IC_{50} values between 3.24 and 9.28 μg/ml compared to aminoguanidine used as the positive control.

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Experimental Part

General

Optical rotations: *PerkinElmer 341* digital polarimeter (*PerkinElmer*, Norwalk, CT, USA). UV and IR spectra: *Shimadzu UV2550* and *FTIR-8400S* spectrometers (*Shimadzu*, Kyoto, Japan), respectively. NMR Spectra:

Table 1. ^{1}H- and ^{13}C-NMR Data (600 MHz and 150 MHz, resp., in (D_5)pyridine) for Compounds **1** – **2**

Position	**1**		**2**	
	δ(H) (*J* in Hz)	δ(C), type	δ(H) (*J* in Hz)	δ(C), type
1	2.42 (*d*, *J* = 12.0) 2.11 (*d*, *J* = 12.0)	54.1, CH_2	2.46 (*d*, *J* = 12.0) 2.19 (*d*, *J* = 12.0)	53.9, CH_2
2		213.1, C		213.1, C
3	5.00 (*s*)	78.0, CH	5.00 (*s*)	78.0, CH
4		49.0, C		42.6, C
5	2.07 – 2.10 (*m*)	47.7, CH	2.79 – 2.81 (*m*)	48.4, CH
6	1.24 – 1.27 (*m*) 1.47 – 1.49 (*m*)	19.2, CH_2	1.33 – 1.36 (*m*) 1.59 – 1.62 (*m*)	19.3, CH_2
7	1.36 – 1.38 (*m*) 1.57 – 1.61 (*m*)	33.2, CH_2	1.36 – 1.38 (*m*) 1.95 – 1.97 (*m*)	33.4, CH_2
8		42.6, C		41.0, C
9	1.99 – 2.01 (*m*)	46.9, CH	1.99 – 2.02 (*m*)	47.0, CH
10		38.0, C		36.8, C
11	1.86 – 1.89 (*m*)	24.4, CH_2		24.5, CH_2
12	5.41 (*t*, *J* = 2.4)	124.2, CH	5.47 (*t*, *J* = 2.4)	123.0, CH
13		139.8, C		144.9, C
14		43.9, C		44.1, C
15	2.30 – 2.34 (*m*) 1.08 – 1.12 (*m*)	29.6, CH_2	2.26 – 2.29 (*m*) 1.03 – 1.05 (*m*)	29.4, CH_2
16	2.94 – 2.97 (*m*) 1.92 – 1.95 (*m*)	26.4, CH_2	2.64 – 2.66 (*m*) 1.99 – 2.01 (*m*)	28.3, CH_2
17		50.4, C		50.4, C
18	2.81 (*s*)	54.8, CH	3.56 (br. *s*)	45.0, CH
19		73.0, C	3.55 (*d*, *J* = 4.8)	81.4, CH
20	1.18 – 1.21 (*m*)	42.5, CH		35.9, C
21	1.31 – 1.34 (*m*) 1.91 – 1.94 (*m*)	27.0, CH_2	1.33 – 1.35 (*m*) 2.21 – 2.24 (*m*)	29.3, CH_2
22	0.99 – 1.01 (*m*) 1.24 – 1.26 (*m*)	41.3, CH_2	1.00 – 1.02 (*m*) 1.33 – 1.36 (*m*)	32.9, CH_2
23	3.93 (*d*, *J* = 10.8) 3.64 (*d*, *J* = 10.8)	65.3, CH_2	4.02 (*d*, *J* = 10.8) 3.73 (*d*, *J* = 10.8)	65.3, CH_2
24	0.72 (*s*)	14.2, CH_3	0.81 (*s*)	14.1, CH_3
25	1.07 (*s*)	17.4, CH_3	0.97 (*s*)	17.2, CH_3
26	0.90 (*s*)	17.0, CH_3	1.12 (*s*)	17.6, CH_3
27	1.52 (*s*)	24.8, CH_3	1.55 (*s*)	25.0, CH_3
28		177.3, C		177.6, C
29	1.27 (*s*)	27.3, CH_3	1.12 (*s*)	29.1, CH_3
30	1.00 (*d*, *J* = 6.6)	17.4, CH_3	0.96 (*s*)	25.2, CH_3
1′	6.18 (*d*, *J* = 8.4)	96.2, CH	6.36 (*d*, *J* = 7.8)	96.3, CH
2′	4.11 – 4.14 (*m*)	74.4, CH	4.19 – 4.22 (*m*)	74.5, CH
3′	4.20 – 4.22 (*m*)	79.6, CH	4.28 – 4.30 (*m*)	79.3, CH
4′	4.24 – 4.27 (*m*)	71.7, CH	4.36 – 4.38 (*m*)	71.5, CH
5′	4.09 – 4.12 (*m*)	79.7, CH	4.18 – 4.20 (*m*)	79.6, CH
6′	4.30 (br. *d*, *J* = 12.0) 4.38 (br. *d*, *J* = 12.0)	62.8, CH_2	4.39 (br. *d*, *J* = 12.0) 4.44 (br. *d*, *J* = 12.0)	62.6, CH_2

Bruker AV 600 NMR spectrometer (chemical shift values are presented as δ values with TMS as the internal standard; *Bruker*, Billerica, MA, USA). HR-ESI-MS: *LTQ-Obitrap XL* spectrometer (*Thermo Fisher Scientific*, Boston, MA, USA). Silica gel (SiO_2; 100 – 200 and 300 – 400 mesh, *Qingdao Marine Chemical Plant*, Qingdao, P. R. China) was used for column chromatography. Precoated SiO_2 *GF254* plates (*Zhi Fu Huang Wu Pilot Plant of Silica Gel Development*, Yantai, P. R. China) were used for TLC analysis. All solvents used were of analytical grade (*Beijing Chemical Works*, Beijing, P. R. China).

Plant Material

The roots of *Rosa cymosa* were provided by *Guilin Sanjin Pharmaceutical Company Limited*, collected from Guilin, Guangxi Province, P. R. China and identified by Prof. *Bin Dai*, Guangxi Research Institute of Minority Medicine. A

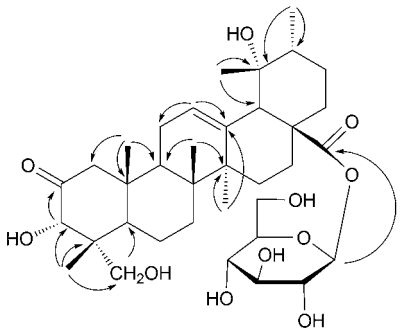

Fig. 2. Key HMBC correlations of compound **1**

Table 2. Inhibitory Activity of Compounds **1** – **5** on LPS-Induced NO Production in Raw 264.7 Macrophages

Compounds	IC_{50} [μg/ml][a])
1	9.28 ± 0.43
2	7.96 ± 0.32
3	3.42 ± 0.61
4	2.21 ± 0.55
5	3.24 ± 0.63
Aminoguanidine[b])	0.94 ± 0.20

[a]) Value present mean ± SD of triplicate experiments. [b]) Positive control substance.

voucher specimen (NO. 02132181) was deposited with the Guangxi Botanical Garden of Medical Plant.

Extraction and Isolation

The dried root of *Rosa cymosa* (10.0 kg) were extracted three times with 75% EtOH. The 75% EtOH extraction was concentrated under reduced pressure and the residue was dissolved in H_2O. The mixture was extracted using petroleum ether (PE) and AcOEt, respectively. The H_2O-soluble fraction was subjected to *D-101* macroreticular resin eluted with H_2O, EtOH/H_2O (35:65), EtOH/H_2O (55:45), EtOH/H_2O (75:25), and EtOH/H_2O (95:5), respectively. The EtOH/H_2O (75:25) fraction (40.0 g) was subject to column chromatography on SiO_2 eluting with CH_2Cl_2/MeOH gradient (40:1; 20:1; 10:1; 5:1, 0:1 *v*/*v*), to yield five fractions (*Frs. I-V*). The *Fr. II* (2.1 g) was separated using SiO_2 CC eluting with CH_2Cl_2/MeOH (80:1; 60:1; 40:1; 20:1; 10:1; 5:1, *v*/*v*) to give six subfractions, II_{1-6}. Subfraction II_3 was prepared by HPLC using MeOH/H_2O (65:35, *v*/*v*) on *YMC-Pack ODS-A* column to give compounds **3** (20.0 mg, t_R = 20.5 min) and **5** (23.0 mg, t_R = 33.1 min). Subfraction II_4 was prepared by HPLC using MeOH/H_2O (65:35, *v*/*v*) on *YMC-Pack ODS-A* column to give compound **1** (10.0 mg, t_R = 21.6 min). The Fr. *III* (3.4 g) was subjected to SiO_2 CC eluting with CH_2Cl_2/MeOH (60:1; 40:1; 20:1; 10:1, *v*/*v*) to afford four subfractions III_{1-4}. Subfractions III_2 and III_3 were separated by prep. HPLC using MeOH/H_2O (58:42) and (65:35) on *YMC-Pack ODS-A* column, respectively, and yielded compounds **2** (10 mg, t_R = 19.7 min) and **4** (10 mg, t_R = 14.2 min).

3α,19α,23α-Trihydroxy-2-oxo-12-ursen-28-*O*-*β*-D-glucopyranoside (= **1-*O*-[(3α)-3,19,23-trihydroxy-2,28-dioxours-12-en-28-yl]-*β*-D-glucopyranose**; **1**). White amorphous powder. $[\alpha]_D^{20}$ = +27.4 (*c* = 0.12, MeOH); UV (MeOH): 208 (4.01). IR (KBr): 3573-3392, 1712, 1689, 1653. ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): see *Table 1*. HR-ESI-MS: 687.3810 ([*M* + Na]$^+$, $C_{36}H_{56}NaO_{11}^+$; calc. 687.3720).

3α,19α,23α-Trihydroxy-2-oxoolean-12-en-28-*O*-*β*-D-glucopyranoside (= **1-*O*-[(3α,19α)-3,19,23-Trihydroxy-2,28-dioxo-olean-12-en-28-yl]-*β*-D-glucopyranose**; **2**). White amorphous powder. $[\alpha]_D^{20}$ = +19.1 (*c* = 0.12, MeOH). UV: (MeOH): 208 (4.01). IR (KBr): 3623-3452, 1723, 1699, 1643. ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): see *Table 1*. HR-ESI-MS: 687.3802 ([*M* + Na]$^+$, $C_{36}H_{56}NaO_{11}^+$; calc. 687.3720).

Acid Hydrolysis of **1** – **2**

Compounds 1 – 2 (each 2.0 mg) were treated with 1M HCl (1 ml) and heated under reflux for 3 h, respectively. After extraction with CH_2Cl_2 (3 ml × 3), the H_2O-soluble layer was evaporated to dryness and subjected to the HPLC analysis under the following conditions, respectively: HPLC column, *Purospher STAR NH2*, 5 mm × 250 mm (*Merck*, Darmstadt, Germany); detection, optical rotation (*Chiralyser-MP* (*IBZ Messtechnik GMBH*, Hannover, Germany)); mobile phase, MeCN/H_2O (68:32, *v*/*v*); and flow rate 1.0 ml/min. D-Glucose from **1** – **2** presented in the aq. phase was carried out by comparison of its retention time and optical rotation with that of authentic samples, t_R 24.7 min (positive).

Assay for Inhibitory Ability Against LPS-Induced NO Production in RAW 264.7 Macrophages

RAW 264.7 macrophages were seeded in 24-well plates (10^5 cells/well). The cells were coincubated with drugs and LPS 1 μg/ml) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage supernates with *Griess* reagent. Aliquots of supernates (100 μl) were incubated, in-sequence, with 50 μl of 1% sulfanilamide and 50 μl of 0.1% naphthylethylenediamine in 2.5% phosphoric acid soln. The absorbance was recorded on a microplate reader at a wavelength of 570 nm.

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