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Three New Oleanane-Type Triterpene Saponins from *Gladiolus gandavensis*

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Three new oleanane-type triterpene saponins (**1**, **2** and **3**) were isolated from aerial parts of *Gladiolus gandavensis*, along with two known compounds (**4** and **5**). Their structures were elucidated as 29-*O*-(β-D-glucopyranosyl)-2*β*,3*β*-dihydroxyolean-12-en-28-oicacid(**1**), 3-*O*-(*β*-D-xylopyranosyl)-29-*O*-(*β*-D-glucopyranosyl)-12-en-28-oic acid (**2**), and 2*β*,3*β*,29-trihydroxyolean-12-en-28-oic acid 28-*O*-[*β*-D-glucopyranosyl(1→2)-(*α*-L-rhamnopyranosyl(1→6))-*β*-D-glucopyranosyl] ester (**3**), by spectroscopic methods, and by comparison with known analogues. These oleanane-type triterpene saponins glycosidated at C-29 were not obtained frequently.

Key Words: *Gladiolus gandavensis*, Triterpene saponins

Introduction

The plant *Gladiolus gandavensis* Van Houtte widely distributes in China. As a Chinese medicinal herb, the corm of this plant, named 'sou shan huang' is used for treatment of fever, boils**,** pharyngitis and parotitis in China.[1] The various chemical constituents were isolated from the corm of this plant.[2-4] To the best of our knowledge, there is no report on the chemical constituents of the aerial parts of *Gladiolus gandavensis*. Our chemical investigation on the aerial parts of this plant led to the isolation of three new oleanane-type triterpene saponins, 29-*O*-(*β*-D-glucopyranosyl)-2*β*,3*β*-dihydroxyolean-12-en-28-oic acid (**1**) 3-*O*-(*β*-D-xylopyranosyl)-29-*O*-(*β*-D-glucopyranosyl)-12-en-28-oic acid (**2**), and 2*β*,3*β*,29-trihydroxyolean-12-en-28-oic acid 28-*O*-[*β*-D-glucopyranosyl (1→2)-(*α*-L-rhamnopyranosyl(1→6))-*β*-D-glucopyranosyl] ester (**3**), together with two known triterpene saponins, 3-*O*-[*β*-galactopyranosyl-(1→4)-*β*-D-glucuronopyranosyl]-23-hydroxyolean-28-*O*-(*β*-D-glucopyranosyl) ester[5] (**4**), and 2*β*-hydroxyolean-3- *O*-(*β*-D-glucopyranosyl)-12-en-23, 28-dioic acid[4] (**5**) (Figure 1). In this paper, the isolation and structure elucidation of these three new saponins from the aerial parts of this plant were described. In addition, their cytotoxicities were also investigated.

Experimental Section

General experimental procedures. Melting point was measured on a Dresden HMK micromelting point apparatus and was uncorrected. The high resolution 1D and 2D NMR spectra (HMQC, HMBC, ^{1}H-^{1}H COSY and ROESY) were performed using Bruker DRX-500 MHz spectrometers. All chemical shifts (δ) are given in ppm, and TMS was used as an internal standard. MS spectra were measured on a VG Auto Spec-3000 mass spectrometer and Agilent G3250AA LC/MSD TOF spectro-

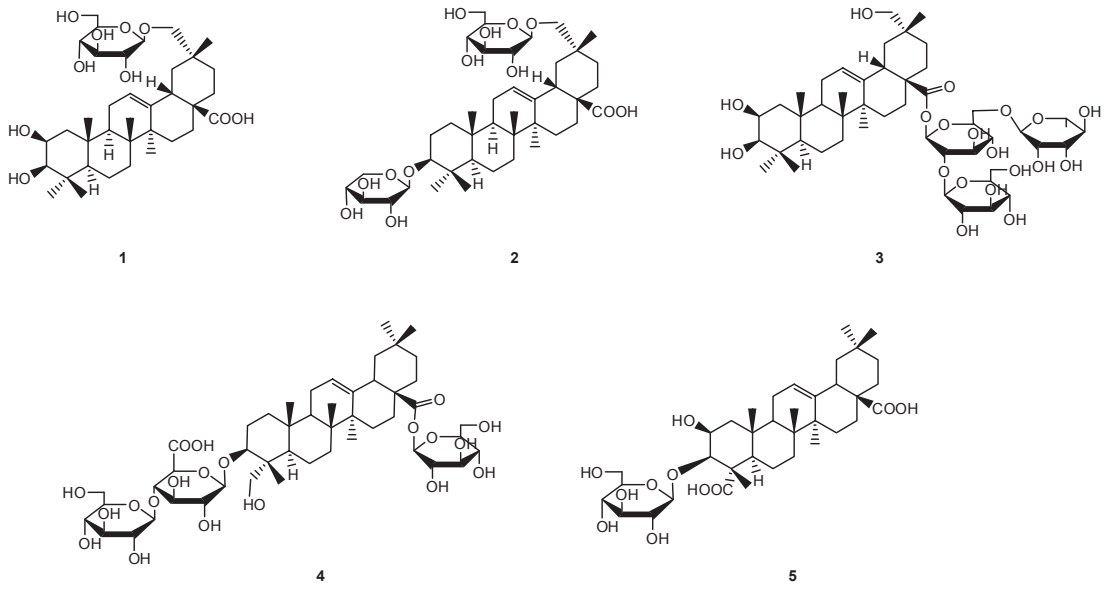

Figure 1. Structures of compounds **1**-**5**.

meter. IR was measured on a Nicolet FTIR 5DX spectrophotometer with KBr pellets. Specific rotation was obtained on a JASCO P-1020 digital polarimeter. Silica gel (200-300 mesh) was the product of the Qingdao Marine Chemical Ltd. Sephadex LH-20 was purchased from Amersham Biosciences. RP-18 was purchased from Merck (Merck, Darmstadt, Germany). GC analysis was performed using a GC-2010 instrument (Shimadzu).

Plant materials. The aerial part of *Gladiolus gandavensis* was collected in Kunming, Yunnan province, China, in February 2008, and authenticated by Prof. Shu-Gang Lu. A voucher specimen (2008-Ding-Tai-2) has been deposited in the Key Laboratory of Medicinal Chemistry for Nature Resource of Yunnan University.

Table 2. ^{1}H and ^{13}C NMR data of **3** in pyridine-d_5

Position	δ_H	δ_C	Position	δ_H	δ_C
1	2.29, m, 1.17, m	45.0	28	-	176.7
2	4.37, m	71.5	29	3.49, s	73.8
3	3.37, 3.7 Hz	79.0	30	1.06, s	19.8
4	-	40.2	C_{28}-trisaccharide		
5	0.86, m	56.1		(inner-Glc)	(inner-Glc)
6	1.60, m, 1.52, m	18.7	1′	6.17, d, 8.2 Hz	93.6
7	1.66, m, 1.50, m	33.4	2′	4.50, m	78.8
8	-	38.8	3′	4.26, m	78.4
9	1.61, m	48.7	4′	4.22, m	70.9
10	-	37.4	5′	4.01, m	77.9
11	2.23, m, 2.18, m	23.9	6′	4.50, m, 4.18, m	66.9
12	5.48, brs	122.8		(terminal-Glc)	(terminal-Glc)
13	-	144.7	1″	5.78, d, 7.7 Hz	104.7
14	-	42.3	2″	4.08, m	76.0
15	3.31, m, 1.21, m	28.9	3″	4.27, m	78.4
16	2.11, m, 2.01, m	24.0	4″	4.20, m	72.2
17	-	47.6	5″	4.00, m	79.0
18	3.30, m	41.3	6″	4.69, m, 4.42, m	63.2
19	2.11, m, 1.36, m	41.1		(terminal-Rha)	(terminal-Rha)
20	-	36.4	1‴	5.47, brs	102.2
21	1.72, m, 1.42, m	29.1	2‴	4.59, m	72.7
22	2.01, m, 1.90, m	31.8	3‴	4.48, m	72.8
23	1.18, s	30.3	4‴	4.25, m	74.1
24	1.29, s	18.2	5‴	4.87, m	69.8
25	1.47, s	16.8	6‴	1.59, d, 6.0 Hz	18.7
26	1.09, s	17.6			
27	1.25, s	26.3			

Extraction and isolation. The air-dried and powered plant of *Gladiolus gandavensis* (4.5 kg) was extracted three times each with 20 L of 85% methanol (each 48 hours) at room temperature. The methanolic extract was decanted, filtered under vacuum, concentrated in a rotary evaporator. The resulting crude extract was partitioned successively with petroleum ether, EtOAc and *n*-BuOH to yield soluble fractions of petroleum ether extract (65 g), EtOAc extract (108 g) and *n*-BuOH extract (175 g), respectively. The *n*-BuOH extract was separated by a normal phase silica gel column chromatography (1:0→0:1, $CHCl_3$/CH_3OH) to give six fractions (Frs. I-VI). Fraction VI was purified by Sephadex LH-20 with methanol to afford compound **3** (15 mg) and **4** (7 mg). Fraction III was subjected to C_{18} reverse-phase chromatography (7:3→2:8, H_2O/CH_3OH) to give five fractions (Frs. III_1-III_5). Frs. III_1, Frs. III_3 and Frs. III_5 were subjected to Sephadex LH-20 with methanol to afford compound **2** (29 mg), **1** (35 mg) and **5** (11 mg), respectively.

Compound 1: White powder (MeOH), mp 218 - 220 °C; $[\alpha]_D^{20}$ +19.8 ° (*c* 0.14, MeOH); HRESIMS $[M-H]^-$ *m/z* (%) 649.3957 (Calcd. for $C_{36}H_{57}O_{10}$, 649.3952); FABMS: *m/z* (%) 651 $[M+H]^+$ (76), 489 $[M+H-Glc]^+$ (49), 471 $[M+H-Glc-H_2O]^+$ (100); IR (KBr) ν_{max} 3419 (OH), 1696 (C=O), 1633 (C=C), 1080 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data see Table 1.

Compound 2: White powder (MeOH); mp 205 - 208 °C; $[\alpha]_D^{20}$ +4.9 ° (c 1.29, MeOH); HRESIMS $[M-H]^-$ *m/z* 765.4431 (Calcd. for $C_{41}H_{65}O_{13}$, 765.4425); FABMS: *m/z* (%) 789$[M+Na]^+$ (7), 749 $[M+H-H_2O]^+$ (9), 617 $[M+H-Xyl-H_2O]^+$ (59), 455 $[M+H-Glc-Xyl-H_2O)]^+$ (91); IR (KBr) ν_{max} 3429 (OH), 1697 (C=O), 1641 (C=C), 1078 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data see Table 1.

Compound 3: White powder (MeOH); mp 230-233 °C; $[\alpha]_D^{20}$ −34.3 ° (c 1.04, MeOH); HRESIMS $[M-H]^-$ *m/z* 957.5064 (Calcd. 957.5059); IR (KBr) ν_{max} 3433 (OH), 1738 (C=O), 1633 (C=C), 1066 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data see Table 2.

Acid hydrolysis and determination of the absolute configuration of the sugars. A solution of **1** (10 mg) , **2** (10 mg) and **3** (10 mg) in 80% methanol-benzene (10 mL) were refluxed for 8 h with 10 mL of 1 M HCl, respectively. After cooling, the organic layer was evaporated under reduced pressure. The water soluble fraction was neutralized with Na_2CO_3, and sugars in the aqueous solution were identified by co-chromatography with authentic samples using TLC with solvent system (4:1:1, n-BuOH/CH_3COOH/H_2O). The chromatograms were sprayed with aniline hydrogen phthalate reagent, and their data (R_f) revealed the presence of Glc (R_f 0.10), Rha (R_f 0.13) and Xylose (R_f 0.16) units. Furthermore, the absolute configuration of these sugars was determinate by GC analysis, according to a method previously reported with some modification.[6] Briefly, solution of compounds **1-3** (1 mg) in 1 M HCl were each heated at 90 °C for 8.0 h. The aqueous layer was repeatedly evaporated with solution (H_2O/CH_3OH, 1:1) under reduced pressure to dryness to furnish a neutral residue. The residue was dissolved in pyridine (100 μL) and then mixed with a pyridine solution of D-

Table 1. ^{1}H and ^{13}C NMR data of **1** and **2** in pyridine-d_5

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	2.30, d,11.8 Hz 1.18, m	44.9	1.48, m 0.93, m	38.3
2	4.42, m	71.5	2.15, m ,1.85, m	25.3
3	3.43, d, 3.5 Hz	78.4	3.33, dd, 3.3, 9.4 Hz	88.7
4	-	39.9	-	39.8
5	0.97, m	56.0	0.78, m	55.9
6	1.61, m, 1.53, m	18.6	1.50,m, 1.25, m	18.6
7	1.53, m, 1.33, m	33.3	1.43,m, 1.25, m	33.5
8	-	38.8	-	39.6
9	1.62, m	48.5	1.65, m	48.0
10	-	37.4	-	37.0
11	1.97, m	23.9	2.05, m, 1.85, m	23.8
12	5.45, brs	122.7	5.41, brs	122.7
13	-	144.8	-	144.7
14	-	42.3	-	42.1
15	2.17, m, 1.20, m	28.3	2.11, m, 1.21, m	28.3
16	2.07,1.90	23.9	2.05, m, 1.85, m	23.8
17	-	47.1	-	47.0
18	3.35, m	41.2	3.33, m	41.2
19	2.01, m,1.42, m	41.3	2.01, m, 1.42, m	41.3
20	-	35.7	-	35.8
21	1.72, m,1.40, m	30.0	1.80, m, 1.40, m	30.0
22	2.04, m,1.82, m	32.4	2.03, m, 1.85, m	32.5
23	1.25, s	30.3	1.26, s	28.3
24	1.35, s	18.2	0.93, s	17.0
25	1.49, s	16.6	0.82, s	15.5
26	1.05, s	17.5	0.97, s	17.4
27	1.25, s	26.3	1.24, s	26.2
28	-	180.4	-	180.2
29	3.91, d, 9.2 Hz 3.40, d, 9.2 Hz	81.6	3.93, d, 9.1 Hz 3.42, d, 9.1 Hz	81.6
30	1.18, s	19.8	1.18,s	19.8
C_{29}-sugar	(Glc)	(Glc)	(Glc)	(Glc)
1′	4.83, d, 7.7 Hz	105.5	4.85, d, 7.8 Hz	105.5
2′	4.07, m	75.4	4.12, m	75.4
3′	4.26, m	78.6	4.27, m	78.7
4′	4.24, m	71.7	4.25, m	71.7
5′	3.98, m	78.7	4.07, m	78.7
6′	4.57, m, 4.41, m	62.9	4.58, m, 4.42, m	62.9
C_3-sugar			(Xyl)	(Xyl)
1′			4.76, d, 7.0 Hz	107.6
2′			4.44, m	73.0
3′			4.20, m	74.7
4′			4.31, m	69.6
5′			4.31, m, 3.84, m	66.9

cysteine methyl ester hydrochloride (0.08 M, 150 μL). After warming at 60 °C for 1.5 h, the reaction mixture was dried in vacuo. Then the residue was trimethylsilylated with 1-trimethylsilylimidazole (100 μL) for 2.0 h. The mixture was partitioned between *n*-hexane and H_2O (300 μL, each), and *n*-hexane layer was analyzed by GC under the following conditions: capillary column, DM-5 (30 m × 0.25 mm × 0.25 μm, Dikma); injection temperature, 250 °C; initial temperature 160 °C, then raised to 280 °C at 5 °C/min, final temperature maintained for 10 min; carrier, He gas; detection, FID; detector temperature, 280 °C. From the hydrolysate of compounds **1-3**, D-glucose, D-xylose and L-rhamnose were confirmed by comparison of the retention times of their derivatives with those of authentic sugars derivatized in a similar way, which showed retention times of 19.61, 19.20, and 15.68 min, respectively.

Results and Discussions

Compound **1** was obtained as white powder. Its molecular formula was determined to be $C_{36}H_{58}O_{10}$ by negative HRESIMS (*m/z* 649.3957 $[M-H]^-$, calcd. 649.3952), together with ^{13}C and ^{1}H NMR data. Its IR spectrum exhibited absorptions at ν_{max} 3419 (OH), 1696 (C=O), 1633 (C=C) and 1080 (*O*-glycosidic linkage) cm^{-1}. The ^{1}H NMR spectrum of compound **1** clearly showed the presence of six methyls [δ 1.05, 1.18, 1.25, 1.25, 1.35, 1.49 (3H each, all s, H-26, 30, 23, 27, 24, 25)], one methylene and two methines bearing an oxygen atom, respectively [δ 3.91, 3.40 (1H each, both d, J = 9.2 Hz, H-29), 3.43 (1H, d, J = 3.5 Hz, H-3), 4.42 (1H, m, H-2)], one olefin [δ 5.45 (1H, brs, H-12)] and a glucopyranosyl unit. The coupling constant of the anomeric proton [δ 4.83 (1H, d, J = 7.7 Hz, H-1′)] discovered a β configuration of glucopyranosyl unit (Table 1). The ^{13}C NMR data showed the presence of 36 signals, of which 30 signals were assigned to a triterpene of oleanane skeleton containing a double bond between C-12 (δ 122.7) and C-13 (δ 144.8).[7] Another six signals at δ 105.5, 75.4, 78.6, 71.7, 78.7, 62.9, indicating the presence of one glucose unit, which were further confirmed by its positive FABMS quasi-molecular-ion and fragmentation peaks at *m/z* 651 $[M+H]^+$, 489 $[M+H-Glc]^+$ and 471 $[M+H-Glc-H_2O]^+$.

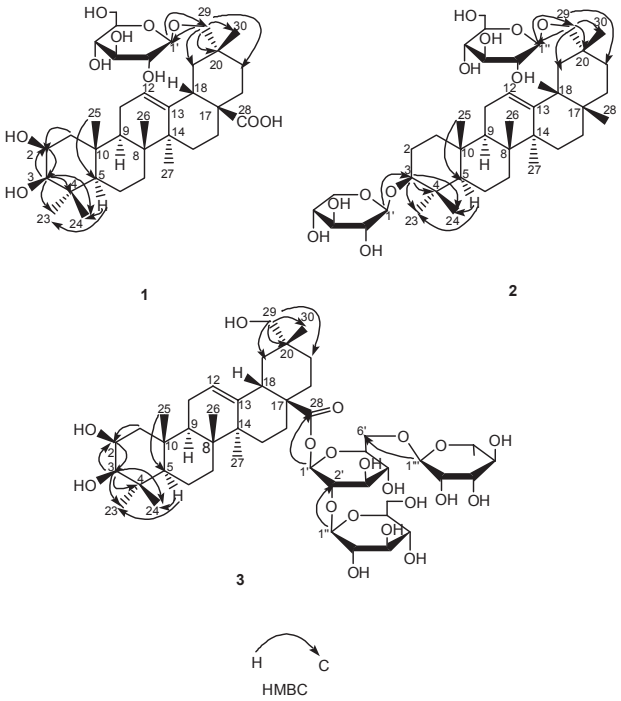

Figure 2. Key HMBC correlations of compound **1**, **2** and **3**.

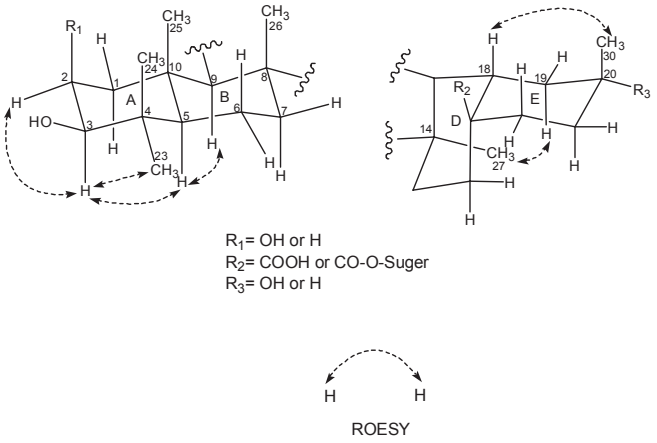

Figure 3. Key ROESY correlations of skeleton of compound **1**, **2** and **3**.

In its HMBC spectrum (Figure 2), the anomeric proton of the glucopyranosyl (H-1′) showed a correlation with C-29 (δ 81.6). In addition, H-29 [δ 3.91, 3.40 (1H each, both d, J = 9.2 Hz)] exhibited a key correlation with anomeric carbon of the glucopyranosyl (δ 105.5, C-1′). The correlations from H-29 to C-19 (δ 41.3), C-20 (δ 35.7), C-21 (δ 30.0) and C-30 (δ 19.8), were also observed. Thus, the glucose unit should be located at C-29 through an ether linkage to C-1′. On the other hand, the HMBC correlations for H-1/C-2, H-2/C-3, H-3/C-2, H-3/C-4, H-3/C-23 and H-3/C-24, indicated that two OH groups linked at C-2 and C-3, respectively.

The relative configuration of **1** was established by ROESY experiments (Figure 3). The NOE correlations [H-3/CH_3-23, H-3/H-5, H-5/H-9, H-9/CH_3-27] showed H-3 was situated on the α-side of the triterpene. The NOE correlation from H-2 to H-3 indicated that H-2 is α configuration as H-3, which was confirmed by the coupling constant of H-3 (d, J = 3.5 Hz). In addition, the NOE correlation for H-18/H-30 and the ^{13}C NMR data of C-29 (δ 81.6) and C-30 (δ 19.8) suggested that the conformation of the E ring of **1** was a chair conformation as shown in Figure 1.

Based on the results obtained from the spectral studies and extensive review of the related literature,[8,9] the structure of compound **1** was determined to be 2β,3β-dihydroxyolean- 29-*O*-(β-D-glucopyranosyl)-12-en-28-oic acid.

Compound **2** was obtained as white powder. Its negative HRESIMS showed a quasi-molecular ion at *m*/*z* 765.4431 $[M-H]^-$ (calcd. 765.4425), which is consistent with a molecular formula of $C_{41}H_{66}O_{13}$. Acid hydrolysis of **2** afforded D-glucose and D-xylose, which were identified by TLC and GC comparison with the authentic samples. This result was further confirmed by its positive FABMS quasi-molecular-ion and fragmentation peaks at *m*/*z* 789$[M+Na]^+$, 749$[M+H-H_2O]^+$, 617 $[M+H-Xyl-H_2O]^+$ and 455 $[M+H-Glc-Xyl-H_2O]^+$. The ^{1}H NMR spectra displayed six methyls [δ 0.82, 0.93, 0.97, 1.18, 1.24, 1.26 (3H each, all s, H-25, 24, 26, 30, 27, 23)], one oxygenated methylene [δ 3.93, 3.42 (1H each, both d, J = 9.1 Hz, H-29)], one oxygenated methine [3.33(1H, dd, J = 3.3, 9.4 Hz, H-3)] and one olefin [δ 5.41 (1H, brs, H-12)]. Meanwhile, the ^{1}H NMR data suggested the presence of two sugar residues with two anomeric proton signals at δ 4.76 (1H, d, J = 7.0 Hz, H-1′) and δ 4.85 (1H, d, J = 7.8 Hz, H-1″). These data displayed a β configuration at the anomeric position of the two sugar residues.

Analysis of the ^{13}C NMR spectrum of **2** (Table 1) revealed that the structure of **2** was similar to that of **1**, but differed from **1** in terms of addition of a xylose group (107.6, 73.0, 74.7, 69.6, 66.9) at C-3 (δ 88.7) and the absence of a OH group at C-2 (δ 25.3) (Figure 1). On comparison of the report,[10] the glycosylation shifts was observed at C-3 (+10.7 ppm), this led to the conclusion that the xylose unit was connected to OH-3β. The result was confirmed by the HMBC correlation (Figure 2) from H-1′ (δ 4.76, d, J = 7.0 Hz) to C-3. In its ROESY spectrum (Figure 3), the NOE correlations [H-2/H-3, H-3/CH_3-23, H-3/H-5, H-9/H-5, H-9/H-27] suggested H-3 was located at the α -side of this triterpene. The conformation of the E ring of **2** showed the same chair conformation as that of **1**, because of their similar NOE correlations and NMR data. Therefore, the structure of **2** was elucidated as 3-(β-D-xylopyranosyloxy)-29-(β-D-glucopyranosyloxy)-12-en -28-oic acid.

Compound **3** was obtained as white powder, The negative HRESIMS showed a quasi-molecular ion at *m*/*z* 957.5064 $[M-H]^-$ (Calcd. 957.5059), consistent with the molecular formula of $C_{48}H_{78}O_{19}$. Hydrolysis of the compound **3** led to the identification of two glucoses and a rhamnose, and D-configuration for glucose and L for rhamnose were determined by GC analysis.

The NMR data (Table 2) revealed that compound **3** differed from **1** in the terms of a OH group at C-29 and one trisaccharide chain at C-28 (Figure 1). Comparison with **3** and **1**, C-29 (δ 73.8) of compound **3** was shifted to the higher-field, indicating a un-glycosylated hydroxyl at C-29 of compound **3**.[11] The HMBC correlations (Figure 2) from H-29 (δ 3.49, s) to C-19 (δ 41.1), C-20 (δ 36.4), C-21 (δ 29.1) and C-30 (δ 19.8) confirmed this result.

The ^{1}H NMR spectrum of **3** showed three anomeric proton signals at δ 6.17 (1H, d, J = 8.2 Hz, H-1′), δ 5.78 (1H, d, J = 7.7 Hz, H-1″), and δ 5.47 (1H, brs, H-1‴). The β-configuration of the two glucopyranosyl units and an α-configuration for the rhamnopyranose were suggested by their coupling constants. On the basis of the chemical shifts of H-1′ and C-1′ (δ 93.6), A glucopyranosyl unit should be located at C-28 (δ 176.7) through an ester linkage,[12] which was confirmed by the HMBC correlation between the H-1′ and C-28. The chemical shifts of C-2′ (δ 78.8) and C-6′ (δ 66.9) was at the relative lower-field implied this inner-glucopyranosyl unit might be glycosidated at C-2′ and C-6′.[13-14] The absence of any ^{13}C NMR glycosidation shift for an α-rhamnopyranosyl unit and the other β-glucopyranosyl unit suggested that these sugars were terminal units. The HMBC correlations (Figure 2) for H-1‴/C-2′ and H-1‴/C-6′, indicated that the other glucopyranosyl and the rhamnopyranosyl were located at C-2′ and C-6′ of the inner glucopyranosyl, respectively. Finally, the structure of **3** was elucidated as 2β,3β,29-trihydroxyolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl (1→2)-(α-L-rhamnopyranosyl (1→6))-β-D-glucopyranosyl] ester.

The oleanane-type triterpene saponins glycosidated at C-29 were not obtained frequently. Compounds **1**, **2** and **3** were evaluated *in vitro* against four tumor cell lines [liver carcinoma (HepG2), promyelocytic leukaemia (HL-60), ovarian carcino-

ma (Skov-3), epidermoid carcinoma (A431) purchased from the Institute of Cell Biology in Shanghai, Chinese Academy of Science] by microculture tetrazolium (MTT) assay.[15] All the experiments were done in triplicate. The weak inhibition of cell growth, achieving corresponding 50.0% in HL-60 cell lines at a concentration of 0.51 mmol/L, was observed for **1**. As for **2** and **3**, the similar result were also found for the HL-60 cell lines with the inhibition values of 50.0% at concentration of 0.50 mmol/L and 0.31 mmol/L, respectively. Compound **1**, **2** and **3** did not show the obvious inhibition of cell growth against HepG2, Skov-3 and A431 cell lines.

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