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Triterpenoids from *Sanguisorba officinalis*

Xin Liu [a], Yanxing Cui [a], Qiang Yu [a,b], Biao Yu [a,*]

[a] *State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, PR China*
[b] *Shanghai Institute of Materia Medica, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, PR China*

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Abstract

Seven triterpenoids, i.e., 3β-[(α-L-arabinopyranosyl)oxy]-19β-hydroxyurs-12,20(30)-dien-28-oic acid (**1**), 3β-[(α-L-arabinopyranosyl)oxy]-urs-11,13(18)-dien-28-oic acid β-D-glucopyranosyl ester (**2**), 2α,3α,23-trihydroxyurs-12-en-24,28-dioic acid 28-β-D-glucopyranosyl ester (**3**), 3β-[(α-L-arabinopyranosyl)oxy]-urs-12,19(20)-dien-28-oic acid (**4**), 3β-[(α-L-arabinopyranosyl)oxy]-urs-12,19(29)-dien-28-oic acid (**5**), 3β-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyolean-12-en-28-oic acid (**6**), 2α,3β-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic cid (**7**), together with three known ones (**8**–**10**), were isolated from the roots of *Sanguisorba officinalis*. Their structures were determined by spectroscopic and chemical methods. Compounds **7** and **10** showed marginal inhibition activity against the growth of tumor cell lines.

Keywords: Triterpenoids; *Sanguisorba officinalis*; Rosaceae; Glycoside

1. Introduction

Sanguisorba officinalis (Rosaceae) is widely distributed in China. Its roots have been used as a traditional Chinese medicine for the treatment of hemostasis and inflammation (Jiangsu New Medical College, 1999). Triterpenoids, mainly 19α-hydroxyl ursolic acid (pomolic acid) derivatives, and 19α-hydroxyl oleanolic acid derivatives have been isolated from the roots (Cheng and Cao, 1992; Reher and Buděšínský, 1992; Jia et al., 1992; Mimaki et al., 2001; Liu et al., 2004). In a continuous search for bioactive constituents from the abundant saponin components (~3% weight of the dry roots) of *S. officinalis*, we isolated six new triterpene glycosides **1**–**6** and one new aromatic triterpene **7**, along with three known compounds 3β-[(α-L-arabinopyranosyl)oxy]-urs-12,18-dien-28-oic acid (**8**), 3β-[(α-L-arabinopyranosyl)oxy]-urs-12,18-dien-28-oic acid β-D-glucopyranosyl ester (**9**), and 3β-[(α-L-arabinopyranosyl)oxy]-urs-12,19(29)-dien-28-oic acid β-D-glucopyranosyl ester (**10**) (Mimaki et al., 2001; Zhang et al., 2002). All of these compounds were tested for their inhibition activity against the growth of tumor cell lines (e.g., human stomach tumor cells BGC and SGC) following conventional MTT method (Mosmann, 1983). Only compounds **7**and **10** displayed marginal inhibition activity, with IC_{50} values around 20 μM.

2. Results and discussion

2.1. Structure elucidation

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS, ^{13}C NMR (Table 1), and DEPT data indicate a molecular formula of $C_{35}H_{54}O_8$. Its ^{13}C NMR spectrum shows five signals assignable to a sugar moiety and 30 to the aglycone. After acid

* Corresponding author. Tel.: +86 21 54925131.
E-mail address: byu@mail.sioc.ac.cn (B. Yu).

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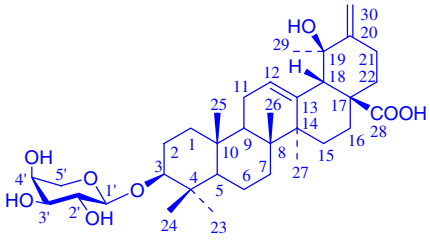
hydrolysis, L-arabinose was detected. Its ^{1}H NMR spectrum shows the presence of six tertiary methyl groups (δ 1.42, 1.37, 1.82, 1.10, 1.03, 0.92), one methine proton (δ 4.57, *s*) resembling H-18 of pomolic acid, one anomeric proton (δ 4.87, *d*, J = 7.0 Hz), one trisubstituted olefinic proton (δ 5.58, *br s*), and two exocyclic olefinic protons (δ 5.70, *s*; 5.63, *s*). Most NMR spectroscopic data of the aglycone moiety resemble those of 19α-hydroxy-20(30)-didehydroursolic acid (Nishimura et al., 1999). There



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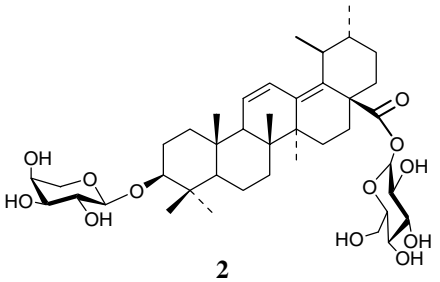

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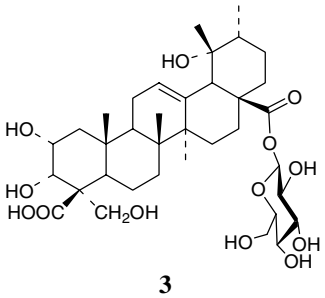

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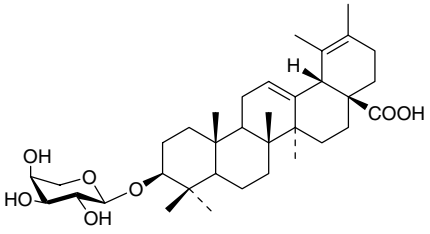

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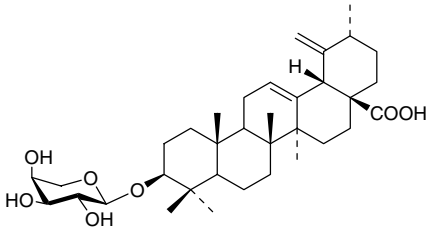

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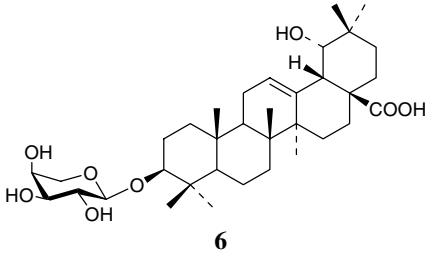

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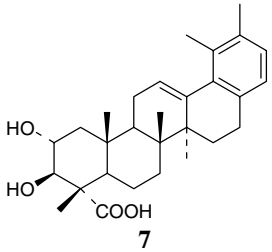


7

are three main differences between them is that the C-19 signal of **1** shifted to δ 81.5 vs. δ 73.0 of the reference compound, the C-18 of **1** shifted to δ 48.9 vs. δ 55.6 and the H-18 of **1** shifted to δ 4.57 vs. δ 3.06. A strong NOESY correlation was observed between the two tertiary methyl groups, Me-29 and Me-27, revealing that the C-29 hydroxy group of **1** takes β orientation other than α in the reference compound. The steric repulsion of C-19 hydroxy group causes high field shift of C-18 and downfield shifting of H-18. So the aglycone of **1** can be determined as 3β,19β-dihydroxyurs-12,20(30)-dien-28-oic acid, a new triterpene. Because HMBC correlation is observed between C-3 of the aglycone and H-1′ of the arabinopyranose, the structure of **1** is elucidated as 3β-[(α-L-arabinopyranosyl)oxy]-19β-hydroxyurs-12,20(30)-dien-28-oic acid.

Compound **2** was obtained as a white amorphous powder. Its UV absorption reveals the existence of conjugated unsaturated bonds. The HR-ESI-MS, ^{13}C NMR (Table 1), and DEPT data indicate a molecular formula of $C_{41}H_{64}O_{12}$. Its ^{13}C NMR spectrum shows 11 signals assignable to the sugar moiety and 30 to the aglycone. After acid hydrolysis, L-arabinose and D-glucose were

Table 1
^{13}C NMR spectroscopic assignment for compounds **1**–**7** in pyridine-d_5

C	1	2	3	4	5	6	7
1	38.9	38.9	43.9	39.2	39.0	38.9	48.7
2	26.8	27.1	67.9	26.9	26.8	26.9	68.8
3	88.8	89.2	71.5	88.8	88.9	89.0	81.1
4	39.7	40.2	55.1	39.7	39.7	39.8	55.0
5	56.2	55.9	45.7	56.2	56.2	56.3	52.6
6	18.6	19.0	21.1	18.6	18.6	18.9	21.3
7	33.6	33.2	34.0	34.4	33.6	33.6	34.1
8	39.8	41.4	40.9	39.7	39.8	40.3	40.4
9	48.2	55.3	47.6	48.3	48.2	50.0	48.6
10	37.2	37.2	39.5	37.0	37.2	37.5	38.6
11	23.9	126.6	24.8	23.8	24.0	24.4	23.9
12	128.5	127.9	128.9	127.4	128.3	123.7	125.5
13	137.9	135.1	139.6	138.6	138.3	145.1	139.1
14	43.1	43.1	42.6	43.9	43.0	42.4	44.4
15	29.2	26.3	29.5	28.8	29.3	29.5	32.6
16	26.3	33.4	26.5	24.1	26.3	28.6	31.4
17	49.0	47.8	49.0	47.1	49.7	46.3	138.8
18	48.9	138.8	54.8	50.8	52.6	45.1	139.4
19	81.5	35.3	73.0	129.2	154.1	81.5	133.9
20	150.3	28.4	42.5	123.9	37.9	36.0	135.4
21	31.7	25.6	27.0	28.8	31.1	29.5	127.9
22	32.3	32.6	38.0	33.7	38.1	33.9	123.6
23	28.4	28.5	68.0	28.4	28.4	29.2	138.2
24	17.1	16.9	179.0	17.1	17.1	17.1	13.7
25	15.8	18.9	15.4	16.2	15.8	15.7	18.1
26	17.4	17.6	17.8	18.1	17.3	17.7	17.1
27	26.3	21.8	24.8	22.3	26.4	25.2	27.6
28	179.8	177.1	177.4	180.0	179.9	181.2	–
29	24.0	17.0	27.4	17.5	110.1	28.5	17.0
30	116.3	20.0	17.1	20.6	19.7	25.2	21.0
3-*O*-ara							
1′	107.7	108.0		107.7	107.7	107.8	
2′	73.1	73.4		73.1	73.1	73.1	
3′	74.8	75.1		74.8	74.7	74.8	
4′	69.7	70.0		69.7	69.8	69.8	
5′	67.0	67.2		66.9	67.0	67.0	
28-*O*-glc							
1′		96.9	96.1				
2′		74.7	74.3				
3′		79.4	79.2				
4′		71.9	71.5				
5′		79.8	79.5				
6′		63.0	62.6				

detected. Its ^{1}H NMR spectrum shows the presence of five tertiary methyl groups (δ 1.35, 1.11, 1.25, 1.03, 0.99), two secondary methyl groups (δ 1.08, *d*, J = 7.0 Hz; δ 0.86, *d*, J = 6.6 Hz) characteristic of ursane derivative, two anomeric protons (δ 6.39, *d*, J = 8.2 Hz; δ 4.88, 1H, *d*, J = 6.9 Hz), and two *cis* olefinic protons (δ 6.61, *dd*, J = 10.8,2.7; δ 5.81, *d*, J = 10.4). Additionally, its ^{13}C NMR spectrum shows seven methyl carbons, two anomeric carbons, four olefinic carbons (two disubstituted and two tetrasubstituted), as well as one carboxyl group. Having HMBC correlations with Me-25 and Me-26, the signal at δ 2.09 is assigned to H-9. ^{1}H–^{1}H COSY correlations were also observed between H-9 and the two *cis* olefinic protons. According to their coupling behavior they are assigned as H-11 and H-12, respectively. HMBC correlations were observed from H-12 and Me-27 to one of the two tetrasubstituted carbons, which is then assigned as C-13. HMBC correlations are also observed from H-22 and H-20 to the other tetrasubstituted carbon, which is assigned as C-18. Based on the evidences above, the aglycone of **2** is elucidated as 3β-hydroxyurs-11,13(18)-dien-28-oic acid, a new triterpene. Through analysis of the TOCSY and HMBC spectra, arabinopyranose is revealed to be attached to C-3 and glucopyranose to C-28. Compound **2** is determined to be 3β-[(α-L-arabinopyranosyl)oxy]-urs-11,13(18)-dien-28-oic acid β-D-glucopyranosyl ester.

Compound **3** was obtained as a white amorphous powder, where molecular formula was inferred as $C_{36}H_{56}O_{13}$ from HRESI-MS, DEPT and ^{13}C NMR (Table 1) data analysis. ^{13}C NMR spectrum shows six signals assignable to the sugar moiety and 30 to the aglycone. After acid hydrolysis, D-glucose was detected. Its ^{1}H NMR spectrum shows the presence of four tertiary methyl groups (δ 1.72, 1.48, 1.39, 1.35), one secondary methyl group (δ 1.15), one methine proton characteristic of H-18 of pomolic acid (δ 2.99, *s*), one trisubstituted olefinic proton (δ 5.64, *br s*), and one anomeric proton (δ 6.29, *d*, J = 8.0Hz). Additionally, its ^{13}C NMR spectra displayed five methyl carbons, two olefinic carbons, one characteristic methine carbon at δ 73.0, and one carboxyl carbon. HMBC correlations were observed between one broad singlet (δ 5.39) and six carbons. One methylene and one methine of the six signals are assigned to the C-1 and C-5, respectively, because they both show HMBC correlations with the Me-25 group. Accordingly, the proton signal at δ 5.39 is assigned as H-3. Because NOESY correlations are observed from Me-25 to H-2 and H-3, the C-2 and C-3 hydroxyl groups are determined to have α-configurations. The remaining hydroxylmethylene and carboxyl groups, which also show HMBC correlations with H-3, were then revealed substituted at C-4. Alkaline hydrolysis of **3** with 3% NaOH gave an aglycone (**3a**), whose ^{13}C NMR spectra are identical to those of 2α,3α,23-trihydroxyurs-12-en-24,28-dioic acid (Ohtani et al., 1990). Because an HMBC correlation is observed between C-28 and the anomeric proton of the glucose, the structure of **3** is determined to be 2α,3α,23-trihydroxyurs-12-en-24,28-dioic acid 28-β-D-glucopyranosyl ester.

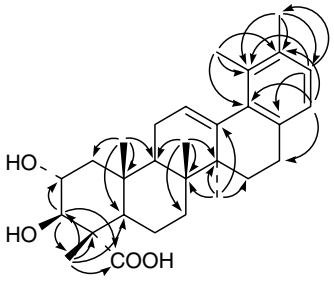
Compound **4** was obtained as a white amorphous powder. The HR-ESI-MS, ^{13}C NMR (Table 1), and DEPT data indicate a molecular formula of $C_{35}H_{54}O_7$. Its ^{13}C NMR spectrum shows five signals assignable to the sugar moiety and 30 to the aglycone. After acid hydrolysis, L-arabinose was detected. Its ^{1}H NMR spectrum shows the presence of seven tertiary methyl groups (δ 1.80, 1.73, 1.36, 1.27, 1.10, 1.04, 0.96), one methine proton (δ 3.72, *s*) resembling H-18 of pomolic acid, one anomeric proton (δ 4.86, *d*, J = 7.0Hz), and one trisubstituted

olefinic proton (δ 5.77, *br s*). Additionally, its ^{13}C NMR spectrum shows seven methyl carbons, one anomeric carbon, four olefinic carbons (one trisubstituted and three tetrasubstituted) as well as one carboxyl group. By analyzing the 2D NMR data, two tetrasubstituted olefinic carbons are then assigned as C-19 and C-20, respectively. By comparing with the reference, the aglycone of **4** is determined as 3β-hydroxyurs-12,19(20)-dien-28-oic acid (Mimaki et al., 2001). Because the C-3 of the aglycone shows an HMBC correlation with the anomeric proton of the arabinopyranose, the structure of **4** is elucidated as 3β-[(α-L-arabinopyranosyl)oxy]-urs-12,19(20)-dien-28-oic acid.

Compound **5** was obtained as a white amorphous powder. The HR-ESI-MS, ^{13}C NMR (Table 1), and DEPT data indicate a molecular formula of $C_{35}H_{54}O_7$. Its ^{13}C NMR spectrum shows five signals assignable to the sugar moiety and 30 to the aglycone. After acid hydrolysis, L-arabinose was detected. Its ^1H NMR spectrum shows the presence of five tertiary methyl groups (δ 1.40, 1.36, 1.10, 1.03, 0.94), one secondary methyl group (δ 1.20, 3H, *d*, J = 6.2 Hz), one methine proton (δ 4.06, *s*) resembling H-18 of pomolic acid, one anomeric proton (δ 4.86, *d*, J = 6.9 Hz), one trisubstituted olefinic proton (δ 5.65, *br s*), and two exocyclic olefinic protons (δ 5.29, *s*; 5.15, *s*). Strong HMBC correlations are observed from the two exocyclic olefinic protons to C-18 and C-20, indicating the two protons to be H_2-29. By comparing with the reference, the aglycone of **5** is determined as 3β-hydroxyurs-12,19(29)-dien-28-oic acid (Mimaki et al., 2001). Because the C-3 of the aglycone shows an HMBC correlation with the anomeric proton of the arabinopyranose, the structure of **5** is elucidated as 3β-[(α-L-arabinopyranosyl)oxy]-urs-12,19(29)-dien-28-oic acid.

Compound **6** was obtained as a white amorphous powder. The HR-ESI-MS, ^{13}C NMR (Table 1), and DEPT data indicate a molecular formula of $C_{30}H_{48}O_4$. Its ^{13}C NMR spectrum shows five signals assignable to the sugar moiety and 30 to the aglycone. Its ^1H NMR spectrum shows the presence of seven tertiary methyl groups (δ 1.35, 1.02, 0.96, 1.11, 1.74, 1.28, 1.20), one anomeric proton (δ 4.81, *d*, J = 7.0 Hz), and one trisubstituted olefinic proton (δ 5.64, *br s*). After acid hydrolysis, L-arabinose was detected. The genuine aglycone 3β,19α-dihydroxyolean-12-en-28-oic acid (**6a**) and the artificial one 3β,19β-dihydroxyolean-12-en-28-oic acid (**6b**) are obtained in a ratio of 1:19 (Mimaki et al., 2001; Aplin et al., 1971). The steric repulsion of the C-19β hydroxyl group causes high-field shifting of C-30 and down-field shifting of C-21 in **6b**. The configuration reversal of the hydroxyl group might be provided by an elimination-addition reaction. Because an HMBC correlation is observed between C-3 of aglycone and H-1′ of arabinopyranose, the structure of **6** is elucidated as 3β-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyolean-12-en-28-oic acid.

Compound **7** was obtained as a white amorphous powder. Its UV absorption reveals the existence of conjugated unsaturated bonds. The HR-ESI-MS, ^{13}C NMR (Table 1), and DEPT data indicate a molecular formula of $C_{29}H_{40}O_4$. Its ^1H NMR spectrum presents six tertiary methyl groups (δ 2.37, 2.33, 1.84, 1.26, 1.08, 0.91), two oxymethine protons (δ 4.40, *m*; 4.75, *d*, J = 9.5Hz), one trisubstituted olefinic proton (δ 5.65, *br s*), and two *o*-positioned aromatic protons (δ 7.10, *d*, J = 7.5 Hz; 6.98, *d*, J = 7.3 Hz). Additionally, its ^{13}C NMR spectrum shows six tertiary methyl groups, five quaternary olefinic carbons and three olefinic methines, two hydroxyl methines, and one carboxyl carbon. By analyzing the 2D NMR spectra, **7** is revealed to have a 28-nor-ursane skeleton. HMBC correlations are observed from Me-29 to quaternary C-18, C-19 and C-20, as well as from Me-30 to quaternary C-19, C-20 and C-21. One of the two aromatic protons shows HMBC correlations with quaternary C-17, C-19 and Me-30. The other shows HMBC correlations with quaternary C-18, C-20 and methylene C-16. The two aromatic protons are then assigned to be H-21 and H-22 of ring E which experiences hexa-dehydrolization, i.e., aromatization. Through analyzing HMQC, HMBC and ^1H–^1H COSY spectra, the two hydroxymethine protons are, respectively, assigned as H-2 and H-3. A strong NOESY correlation between H-2 and Me-25 indicates an α-orientation of the C-2 hydroxyl group. Consequently, the C-3 hydroxyl group must take β-orientation due to the multiplet and coupling constants of H-3 (*d*, J = 9.5 Hz). Carboxyl group shows an HMBC correlation with H-3, indicating that it is substituted at C-4. A strong NOESY correlation between Me-24 and Me-25 suggests that the carboxyl group takes α-orientation. Based on the above evidences, the structure of **7** is elucidated as 2α,3β-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic cid.



Main HMBC correlations of **7**

2.2. Tumor cell growth inhibitory activity

All of these compounds were tested for their inhibition activity against the growth of tumor cell lines (e.g., human stomach tumor cells BGC and SGC) following the conventional MTT method. The SGC cell

line was found to be more sensitive to these compounds. Only compounds **7** and **10** showed marked inhibition activity at a concentration of about 20 μM. Then we treated SGC cells with **7** and **10** (at concentrations ranging from 0 to 50 μM) to generate and plot their dose-dependent curve. The EC_{50} of **7** and **10** on SGC cell were 10.2 and 18.3 μM, respectively.

3. Conclusion

Compound **1** and **2** represent new ursane type triterpene aglycone, respectively. Compound **3** has the aglycone reported only from the genus *Rubus*, while the aglycones of **4**, **5** and **6** have been reported previously from the title plant. **7** is a novel aromatized 28-nor-triterpene and to our knowledge it is the first report of a nor-triterpene or aromatized triterpene from the family of Rosaceae. The family Rosaceae is a rich source of pomolic acid and 19α-hydroxyl oleanolic acid derivatives. Although five new compounds (**1**, **2**, **4**, **5** and **7**) represent different triterpene aglycones, but all are pomolic acid derivatives. Consequently, it can be concluded that the content in triterpenoids of the title plant does not differ much from that found in other rosaceae species.

4. Experimental

4.1. General

Optical rotations were taken with a P-1020 (Jasco) apparatus. UV data were taken on a Cary 100 (Varian) instrument. IR data were obtained using a Perkin-Elmer 16 PC FT-IR spectrometer. ^1H, ^{13}C, and 2D NMR spectra were recorded in pyridine-d_5 on AVANCE-500 (Bruker) NMR spectrometer. Chemical shifts are expressed in δ (ppm) with reference to pyridine-d_5 ($\delta_{C\text{-}3}$ 135.6, $\delta_{H\text{-}3}$ 7.65). HR-ESI-MS were measured on a FTMS-7 instrument (Bruker Daltonics). GC experiments were carried out on a HP-1 TCD instrument (Hewlett-Packard), using HP-Chiral column ($30 \times 0.25 \times 1.0$, 20% permethylated β-cyclodextrin). The conditions selected for analysis were: front inlet 250 °C, column 80 °C → 230 °C, 5 °C/min. Open column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao, People's Republic of China) or octadecyl silica gel (ODS, 25-40 μm, Fuji) as stationary phases. TLC was conducted on Si gel GF_{254} plates. All chemical reagents (AR grade) were purchased from Shanghai Reagent Co. Ltd.

4.2. Plant material

The roots of *S. officinalis* were purchased from Nanjing Chinese Medicine Co. (China) and were authenticated by Dr. Qixin Yan, Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. SIOC-Bio-20030320) has been deposited in the State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, People's Republic of China.

4.3. Extraction and isolation

Dried roots of *S. officinalis* (10 kg) were extracted with EtOH (95%). The extract was concentrated, defatted with cyclohexane, and partitioned sequentially with $CHCl_3$ and *n*-BuOH. The *n*-BuOH layer was dried in vacuo to yield a triterpene-enriched fraction (460 g), which was then separated by silica gel column chromatography using $CHCl_3$–CH_3OH as solvent to yield seven fractions. Fraction 1 was further separated by silica gel column and reversed phase (C-18) silica gel column chromatography to yield **1** (23.6 mg), **4** (15.9 mg), **5** (22.5 mg), **6** (210 mg) and **7** (6.2 mg). Fraction 2 was further separated to yield **2** (16.4 mg). Fraction 6 was further separated to yield **3** (92 mg).

4.3.1. Compound *1*

A white amorphous powder, $C_{35}H_{54}O_8$ exact mass: 602.38; $[\alpha]_D^{28.2} + 30.8°$ (*c* 0.52, MeOH); IR ν_{max} (KBr) cm^{-1} 3441, 2939, 1712, 1639, 1455, 1389, 1086, 866, 781, 650; ^{13}C and ^1H NMR, see Tables 1 and 2; ESI-MS *m/z* 601 $[M - H]^+$, 469 $[M - ara]^+$; HR-ESI-MS *m/z* 625.3702 (calcd. for $C_{35}H_{54}O_8Na$, 625.3711).

4.3.2. Compound *2*

A white amorphous powder, $C_{41}H_{64}O_{12}$ exact mass: 748.44; $[\alpha]_D^{29.7} - 25.4°$ (*c* 0.26, MeOH); UV (MeOH) λ_{max} 209, 255 nm; IR ν_{max} (KBr) cm^{-1} 3442, 2926, 1684, 1422, 1208, 1141, 1075, 846, 801, 726; ^{13}C and ^1H NMR, see Tables 1 and 2; ESI-MS *m/z* 771 $[M + Na]^+$; HR-ESI-MS *m/z* 771.4277 (calcd. for $C_{41}H_{64}O_{12}Na$, 771.4290).

4.3.3. Compound *3*

A white amorphous powder, $C_{36}H_{56}O_{13}$ exact mass: 696.37; $[\alpha]_D^{23.0} + 34.1$ (*c* 2.7, MeOH); IR ν_{max} (KBr) cm^{-1} 3423, 2933, 1720, 1656, 1459, 1381, 1228, 1073, 1049, 932, 773, 689; ^{13}C and ^1H NMR, see Tables 1 and 2; ESI-MS *m/z* 719.3 $[M + Na]^+$; HR-ESI-MS *m/z* 719.3604 (calcd. for $C_{36}H_{56}O_{13}Na$, 719.3613).

4.3.4. Compound *4*

A white amorphous powder, $C_{35}H_{54}O_7$ exact mass: 586.39; $[\alpha]_D^{30.4} - 0.32°$ (*c* 0.30, MeOH); IR ν_{max} (KBr) cm^{-1} 3451, 2925, 1688, 1441, 1209, 1141, 1086, 998, 844, 802, 725; ^{13}C and ^1H NMR, see Tables 1 and 2; ESI-MS *m/z* 609 $[M + Na]^+$, 437 $[M - ara]^+$; HR-ESI-MS *m/z* 609.3760 (calcd. for $C_{35}H_{54}O_7Na$, 609.3762).

Table 2
^{1}H NMR assignment for compounds **1**–**7** in pyridine-d_5

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1α	1.02 (1H)	1.10 (1H)	1.95 (1H)	0.99 (1H)	1.05 (1H)	1.03 (1H)	1.67 (1H)
1β	1.56 (1H)	1.95 (1H, *br d*, J = 13.1 Hz)	2.19 (1H)	1.66 (1H)	1.61 (1H)	1.60 (1H)	2.54 (1H)
2α	2.26(1H)	2.34 (1H)		2.27 (1H)	2.28 (1H)	2.22 (1H)	
2β	1.96 (1H)	2.05 (1H)	5.14 (1H, *br d*, J = 10.9 Hz)	1.98 (1H)	1.97 (1H)	1.95 (1H)	4.40 (1H, *m*)
3	3.46 (1H, *dd*, J = 11.8, 4.3 Hz)	3.47 (1H, *dd*, J = 11.4, 4.2 Hz)	5.39 (1H, *br s*)	3.44 (1H, *dd*, J = 12.0, 4.2 Hz)	3.46 (1H, *dd*, J = 11.4, 3.7 Hz)	3.41 (1H, *dd*, J = 11.4, 3.8 Hz)	4.75 (1H, *d*, J = 9.5 Hz)
5	0.92 (1H)	0.92 (1H)	2.26 (1H)	0.89 (1H)	0.92 (1H)	0.92 (1H, *d*, J = 11.7)	2.33 (1H)
6α	1.58 (1H)	1.58 (1H)	2.38 (1H)	1.55 (1H)	1.57 (1H)	1.60 (1H)	1.92 (1H)
6β	1.37 (1H)	1.43 (1H)	2.10 (1H)	1.34 (1H)	1.38 (1H)	1.42 (1H)	1.77 (1H)
7α	1.42 (1H)	1.30 (1H)	1.82 (1H)	1.47 (1H)	1.41 (1H)	1.42 (1H)	1.62 (1H)
7β	1.59 (1H)	1.56 (1H)	2.11 (1H)	1.62 (1H)	1.58 (1H)	1.60 (1H)	1.76 (1H)
9	1.83 (1H)	2.09 (1H)	2.22 (1H)	1.60 (1H)	1.81–1.88 (1H, *m*)	1.91 (1H)	2.04 (1H)
11	1.92–2.08 (2H)	6.61 (1H, *dd*, J = 10.8, 2.7)	2.19–2.34 (2H)	1.91–2.02 (2H)	1.98–2.11 (2H)	2.03–2.15 (2H)	2.19–2.33 (2H)
12	5.58 (1H, *br s*)	5.81 (1H, *d*, J = 10.4)	5.64 (1H, *br s*)	5.77 (1H, *br s*)	5.65 (1H, *br s*)	5.64 (1H, *br s*)	5.65 (1H, *br s*)
15α	2.32 (1H)	2.18 (1H)	2.49–2.59 (1H, *m*)	2.33 (1H)	2.31 (1H)	2.20 (1H)	2.20 (1H)
15β	1.30 (1H)	1.09 (1H)	1.33 (1H)	1.36 (1H)	1.26 (1H)	1.35 (1H)	0.92 (1H)
16α	2.08 (2H)	1.78 (1H, *t* like, $J \approx 12.6$ Hz)	2.08 (1H)	2.16 (2H)	1.98 (2H)	2.20 (1H)	2.48 (2H)
16β		2.25 (1H, *br d*, J = 13.6 Hz)	3.09–3.19 (1H, *m*)			2.85–2.94 (1H, *m*)	
18	4.57 (1H, *s*)		2.99 (1H, *s*)	3.72 (1H, *s*)	4.06 (1H, *s*)	3.70 (1H)	
19		2.87–2.96 (1H, *m*)				3.70 (1H)	
20		2.41 (1H, *m*)	1.44 (1H)		2.09 (1H)		
21α	1.94 (1H)	1.31 (1H)	1.31 (1H)	1.93 (1H)	1.46 (1H)[a]	1.25 (1H)	7.10 (1H, *d*, J = 7.5 Hz)
21β	2.40 (1H)	2.23 (1H)	2.06 (1H)	2.41 (1H)	1.63 (1H)[a]	2.23 (1H)	
22α	2.79–2.90 (1H, *m*)	1.56 (1H)	2.11 (1H)	2.08 (2H)	2.18 (1H)[b]	2.25 (1H)	6.98 (1H, *d*, J = 7.3 Hz)
22β	2.00 (1H)	2.51–2.59 (1H, *m*)	1.90 (1H)		2.11 (1H)[b]		
23a	1.37 (3H, *s*)	1.35 (3H, *s*)	4.66 (1H, *d*, J = 9.7 Hz)	1.36 (3H, *s*)	1.36 (3H, *s*)	1.35 (3H, *s*)	
23b			4.80 (1H, *d*, J = 9.7 Hz)				
24	1.03 (3H, *s*)	1.03 (3H, *s*)		1.04 (3H, *s*)	1.03 (3H, *s*)	1.02 (3H, *s*)	1.84 (3H, *s*)

25	0.92 (3H, *s*)	0.99 (3H, *s*)	1.39 (3H, *s*)	0.96 (3H, *s*)	0.94 (3H, *s*)	0.96 (3H, *s*)	1.26 (3H, *s*)
26	1.10 (3H, *s*)	1.25 (3H, *s*)	1.35 (3H, *s*)	1.10 (3H, *s*)	1.10 (3H, *s*)	1.11 (3H, *s*)	1.08 (3H, *s*)
27	1.42 (3H, *s*)	1.11 (3H, *s*)	1.72 (3H, *s*)	1.27 (3H, *s*)	1.40 (3H, *s*)	1.74 (3H, *s*)	0.91 (3H, *s*)
28							
29a	1.82 (3H, *s*)	1.08 (3H, *d*, $J = 7.0$ Hz)	1.48 (3H, *s*)	1.80 (3H, *s*)	5.15 (*trans*, 1H, *s*)	1.28 (3H, *s*)	2.37 (3H, *s*)
29b					5.29 (*cis*, 1H, *s*)		
30a	5.63 (*trans*, 1H, *s*)	0.86 (3H, *d*, $J = 6.6$ Hz)	1.17 (3H, *s*)	1.73 (3H, *s*)	1.20 (3H, *d*, $J = 6.2$ Hz)	1.20 (3H, *s*)	2.33 (3H, *s*)
30b	5.70 (*cis*, 1H, *s*)						
3-*O*-ara							
1′	4.87 (1H, *d*, $J = 7.0$ Hz)	4.88 (1H, *d*, $J = 6.9$ Hz)		4.86 (1H, *d*, $J = 7.0$ Hz)	4.86 (1H, *d*, $J = 6.9$ Hz)	4.81 (1H, *d*, $J = 7.0$ Hz)	
2′	4.51 (1H, *t* like, $J \approx 7.8$ Hz)	4.54 (1H)		4.53 (1H, *t* like, $J \approx 7.6$ Hz)	4.51 (1H, *t*, $J = 7.8$ Hz)	4.48 (1H, *t* like, $J \approx 7.8$ Hz)	
3′	4.24 (1H, *dd*, $J = 8.5$, 2.8 Hz)	4.24 (1H)		4.25 (1H, *dd*, $J = 9.0$, 2.9 Hz)	4.25 (1H, *dd*, $J = 8.7$, 2.6 Hz)	4.24 (1H, *dd*, $J = 8.6$, 2.8 Hz)	
4′	4.40 (1H)	4.43 (1H)		4.42 (1H)	4.42 (1H)	4.40 (1H)	
5′	4.39 (1H)	4.41 (1H)		4.40 (1H)	4.39 (1H)	4.39 (1H)	
	3.91 (1H, *d*, $J = 10.8$ Hz)	3.92 (1H, *d*, $J = 11.0$ Hz)		3.91 (1H, *d*, $J = 10.9$ Hz)	3.90 (1H, *d*, $J = 12.0$ Hz)	3.91 (1H, *d*, $J = 10.8$ Hz)	
28-*O*-glc							
1′		6.39 (1H, *d*, $J = 8.2$ Hz)	6.29 (1H, *d*, $J = 8.0$ Hz)				
2′		4.24 (1H)	4.25 (1H, *t*, $J = 8.4$ Hz)				
3′		4.33 (1H, *t*, $J = 8.8$ Hz)	4.34 (1H, *t*, $J = 8.7$ Hz)				
4′		3.37 (1H, *t*, $J = 8.9$ Hz)	4.38 (1H, *t*, $J = 9.3$ Hz)				
5′		4.07–4.13 (1H, *m*)	4.05–4.12 (1H, *m*)				
6a′		4.43 (1H)	4.43 (1H, *br d*, $J = 12.0$ Hz),				
6b′		4.51 (1H)	4.50 (1H, *br dd*, $J = 4.4$, 12.0 Hz)				

[a,b]: chemical shifts exchangeable.

4.3.5. Compound 5

A white amorphous powder, $C_{35}H_{54}O_7$ exact mass: 586.39; $[\alpha]_D^{28.2} + 18.4°$ (*c* 0.38, MeOH); IR ν_{max} (KBr) cm^{-1} 3427, 2939, 1694, 1446, 1206, 1140, 1070, 1002, 843, 801, 725; ^{13}C and ^{1}H NMR, see Tables 1 and 2; ESI-MS *m/z* 609 $[M + Na]^+$, 437 $[M - ara]^+$; HR-ESI-MS *m/z* 609.3777 (calcd. for $C_{35}H_{54}O_7Na$, 609.3762).

4.3.6. Compound 6

A white amorphous powder, $C_{35}H_{56}O_8$ exact mass: 604.4; $[\alpha]_D^{28.4} + 24.7°$ (*c* 0.64, MeOH); IR ν_{max} (KBr) cm^{-1} 3450, 2943, 1702, 1459, 1389, 1073, 860, 781, 651; ^{13}C and ^{1}H NMR, see Tables 1 and 2; ESI-MS *m/z* 627 $[M + Na]^+$, 455 $[M - ara]^+$; HR-ESI-MS *m/z* 627.3862 (calcd. for $C_{35}H_{56}O_8Na$, 627.3867).

4.3.7. Compound 7

A white amorphous powder, $C_{29}H_{40}O_4$ exact mass: 452.29; $[\alpha]_D^{28.6} + 19.8°$ (*c* 0.21, MeOH); UV (MeOH) λ_{max} 214, 243 nm; IR ν_{max} (KBr) cm^{-1} 3430, 2930, 1683, 1454, 1395, 1207, 1143, 1050, 844, 804; ^{13}C and ^{1}H NMR, see Tables 1 and 2; ESI-MS *m/z* 475 $[M + Na]^+$; HR-ESI-MS *m/z* 475.2833 (calcd. for $C_{29}H_{40}O_4Na$, 475.2819).

4.4. Alkaline hydrolysis of 3

A 3% NaOH solution (10 mL) of **3** (55 mg) was heated at 50 °C for 24 h, and was then neutralized with 3 M HCl. The solution was extracted with water-saturated *n*-BuOH. The *n*-BuOH layer was concentrated and then subjected to silica gel chromatography for separation, affording 2α,3α,23-trihydroxyl-urs-12-en-24,28-dioic acid (**3a**, 10 mg).

4.5. Acid hydrolysis of 1–5

Compound **1** (11 mg) was heated at 90 °C under reflux in 10 mL of 1 M HCl (MeOH–H_2O, 3:1) for 3 h. After removal of the solvent, the residue was partitioned between *n*-BuOH and H_2O. The H_2O layer was neutralized with Dowex (HCO_3^-), and then filtered. The filtrate was evaporated down to 2 mL, then treated with $NaBH_4$ (20 mg) at room temp. for 3 h. Excessive $NaBH_4$ was removed with 30% AcOH. After evaporation at 60 °C and washing with 0.1% HCl in MeOH repeatedly until the BO_3^{3-} was removed, the reaction mixture was heated to dryness at 105 °C for 15 min, followed by the addition of pyridine (dry, 0.5 ml) and Ac_2O (0.3 ml). The mixture was incubated in a water bath at 100 °C for 1 h, and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated for GC analysis. The monosaccharide was identified as L-arabinose.

Following a similar procedure, the monosaccharide residues were identified as L-arabinose and D-glucose for **2**, D-glucose for **3**, L-arabinose for **4** and **5**.

4.6. Acid hydrolysis of 6

Compound **6** (76 mg) was heated under reflux at 90 °C in 25 mL of 1 M HCl (MeOH–H_2O, 3:1) for 20 h. After removal of the solvent, the residue was partitioned between *n*-BuOH and H_2O. L-Arabinose was detected in the H_2O layer. In the *n*-BuOH layer two triterpenes were identified as 3β,19α-dihydroxyolean-12-en-28-oic acid (**6a**) and 3β,19β-dihydroxyolean-12-en-28-oic acid (**6b**). The ratio between them was measured by HPLC as 1:19. The eluent selected for analyzing was AcCN–0.5% TFA (55:45) with the flowing rate of 1 mL/min. Wavelength selected for detection was 210 nm.

4.7. MTT colorimetric assay

Compounds were prepared as 10 mM top stocks, dissolved in DMSO, and stored at 4 °C, protected from light. Human-derived cell lines (human stomach tumor cells BGC and SGC) were routinely cultivated at 37 °C in an atmosphere of 5% CO_2 in DMEM medium supplemented with 10% fetal calf serum and subcultured twice weekly to maintain continuous logarithmic growth. Cells were seeded into 96-well microtiter plates at a density of 5000 per well and allowed 24 h to adhere before drugs were introduced. Serial drug dilutions were prepared in medium immediately prior to each assay. At the time of drug addition (Parallel triplicate wells were set) and following 48 h of exposure, MTT was added to each well and reduced by viable cells to an insoluble formazan product. Well contents were aspirated and formazan solubilized by addition of DMSO. Absorbance was read on a systems plate reader at 550 nm as a measure of cell viability. Thus, cell growth or drug toxicity was determined.

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