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New triterpenoid saponins with strong α-glucosidase inhibitory activity from the roots of *Gypsophila oldhamiana*

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Abstract—Seven new triterpenoid saponins (**1**–**7**), have been isolated and elucidated from the roots of *Gypsophila oldhamiana* together with five known triterpenoid saponins (**8**–**12**). These saponins which could be classified into three series: 3-*O*-monoglucosides (**1**, **8**, **9**), 28-*O*-monoglucosides (**2**–**4**, **12**) and 3, 28-*O*-bidesmosides (**5**–**7**, **10**, **11**), have been evaluated for their α-glucosidase inhibition activity. As a result, the preliminary structure–activity relationships were discussed based on the position of sugar linkage attached to the aglycone, and 28-*O*-monoglucosides **2**–**4** and **12** showed significant inhibitory activities on α-glucosidase.

1. Introduction

Gypsophila oldhamiana (Miq.) (Caryophyllaceae) is a small perennial herb widely distributed in the north regions of China, its roots have been used as a substitute for the traditional Chinese medicine Yin-Chai-Hu (roots of *stellria dichotoma* var. *Lanceolata Bge*) to treat fever, consumptive disease, and infantile malnutrition syndrome. Its roots are also used as a remedy for diabetes in folk herb medicines of China.[1] In previous chemical study, saponins, sterols, and a cyclic peptide have been reported from *G. oldhamiana*.[2] Our continuing research on anti-diabetes activity constituents from the *n*-BuOH-soluble extracts of its roots resulted in the isolation of seven new triterpenoid saponins (**1**–**7**), together with five known triterpenoid saponins (**8**–**12**). In order to evaluate their anti-diabetes effects, all isolated saponins (**1**–**12**) have been screened as α-glucosidase inhibitors that can retard the uptake of dietary carbohydrates and thus suppress postprandial hyperglycemia.[3] In this paper, we report the structure elucidation of these seven new saponins as well as the strong α-glucosidase inhibitory activities of 28-*O*-monoglucosides **2**–**4** and **12**.

2. Results and discussion

The 70% EtOH extract of the roots of *G. oldhamiana* was partitioned by water and *n*-BuOH. The *n*-BuOH-soluble fraction, subjected to chromatographic purification over a silica gel column and repeated RP-C_{18} column, followed by HPLC purification, afforded seven new triterpenoid saponins (**1**–**7**) and five known saponins (**8**–**12**) (Scheme 1).[2b,4]

Compound **1** was obtained as an amorphous powder. The HRESIMS showed a pseudo molecular ion peak at *m*/*z* 985.4643 $[M-H]^-$ (calcd 985.4649) corresponding to molecular formula of $C_{48}H_{74}O_{21}$. The IR spectrum showed absorption bands at 3427 cm^{-1} (–OH), 1722 cm^{-1} (CO), and 1077 cm^{-1} (C–O–C). Acid hydrolysis of **1** with 2 M HCl afforded an aglycone identified as quillaic acid[5] (**1a**) by TLC comparison with an authentic sample, which was confirmed on the basis of the ^1H, ^{13}C NMR (Table 1), HMQC, and HMBC spectra of **1**. The sugars obtained from the saponin hydrolysates were identified as D-galactose and D-glucuronic acid (2:1) based on GC–MS analysis of their chiral derivatives. The assignments of the carbon signals of the sugar components (Table 2) were determined from TOCSY, HSQC, and HMBC spectra. The three sugar anomeric carbons were detected at δ_C 103.8, 104.2, and 105.0 in ^{13}C NMR spectrum, attached to protons at δ_H 4.84 (d, J = 7.5 Hz), 5.47 (d, J = 7.6 Hz), and 5.20 (d, J = 7.6 Hz), respectively, in the HSQC experiment. In addition, the ^{13}C NMR data of compound **1**

Keywords: *Gypsophila oldhamiana*; Caryophyllaceae; Triterpenoid saponins; α-Glucosidase inhibitors.
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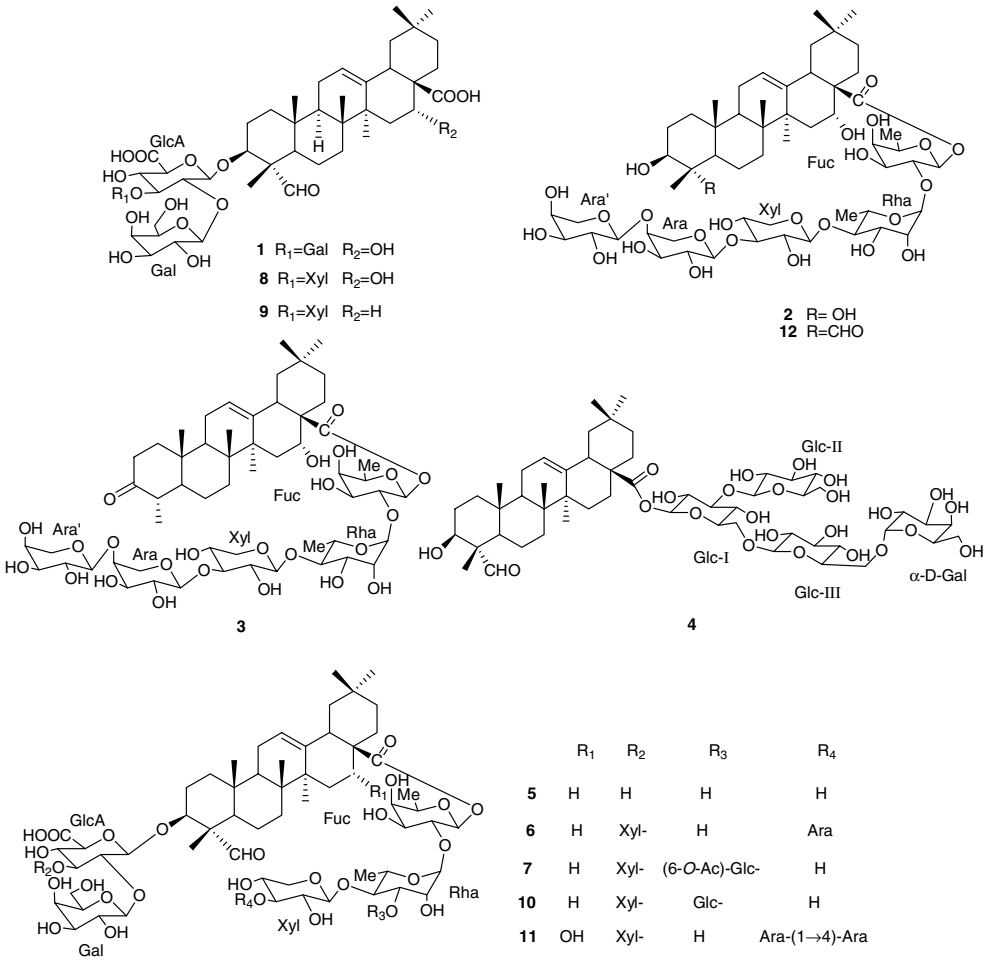


Scheme 1. Structures of triterpenoid saponins **1**–**12**.

indicated that the sugar residue was attached to C-3 of aglycone (δ_C 84.5), which was confirmed by cross-peak in the HMBC spectrum between δ_H 4.84 (H-1 of glucuronic acid) and δ_C 84.5 (C-3 of the aglycone). The linkages of the two other monosaccharides to glucuronic acid were established from the following HMBC correlations: H-1 (δ_H 5.47) of D-galactose with C-3 (δ_C 87.8) of D-glucuronic acid and H-1 (δ_H 5.20) of another D-galactose with C-2 (δ_C78.3) of D-glucuronic acid. The $^3J_{H1,H2}$ coupling constants (7.5–8.0 Hz) suggested β-anomeric configurations for three monosaccharide moieties. On the basis of these results, the structure of **1** was determined to be quillaic acid 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-galactopyranosyl-(1 → 3)]-β-D-glucuronopyranoside.

Compounds **2** and **3**, respectively, displayed quasi-molecular ion peaks $[M-H]^-$ at *m*/*z* 1161.5688 and 1143.5560 in the negative HRESIMS, which was consistent with the molecular formula of $C_{56}H_{90}O_{25}$ and $C_{56}H_{88}O_{24}$, respectively. ^1H NMR and ^{13}C NMR spectra indicated that compounds **2** and **3** had the same sugar moieties (Table 2) but differed in the aglycone parts (Table 1). The aglycone part of **2** showed six methyl proton singlets (δ_H 0.92, 0.96, 1.00, 1.17, 1.38, and 1.70) and a triplet-like vinyl proton (δ_H 5.63). The characteristic t-like signal coupled with the two olefinic carbons (δ_C 122.5 and 144.6) indicated that the aglycone was of an olean-12-ene skeleton. Detailed analysis of the TOCSY, HMQC, HMBC, and ROESY data indicated that the aglycone in **2** possessed the same partial structure in the C, D, and E-rings as that of quillaic acid. Additionally, a salient downshift (+20.0) at C-4 (δ_C 76.0) and the absence of CHO indicated that a hydroxyl was located at C-4 to replace the CHO group of quillaic acid. The β-configuration of methyl group (24-Me) was evident from the ROESY spectrum which showed significant correlations through space interaction between 24-Me (δ_H 1.38) and 25-Me (δ_H 0.92). The above information suggested that the aglycone in **2** was segetalic acid, which was first isolated as an aglycone of a triterpenoid saponin from *Vaccaria segetalis* (Neck) Garcke (Caryophyllaceae).[6] Acid hydrolysis of **2** furnished a rearranged aglycone and identified as 3-keto, 16α-hydroxy, 24-noroleanolic acid[6] (**2a**), and the monosaccharide components were identified as D-fucose, L-rhamnose, D-xylose, and L-arabinose (1:1:1:2) based on GC–MS analysis of their chiral derivatives. The presence of five sugar moieties was further evidenced by the ^1H and ^{13}C NMR spectra which displayed five anomeric protons at δ 6.00 (d, J = 8.7 Hz), 6.51 (br s), 5.02 (d, J = 7.0 Hz), 5.12 (d, J = 7.0 Hz), and 4.98 (d, J = 7.3 Hz), and carbons at δ_C 94.7, 101.1, 106.6, 105.7, and 106.8, respectively. On the basis of the

Table 1. ^{13}C NMR data for aglycone moieties of compounds **1**–**7**

Carbon	1[a]	2[a]	3[b]	4[a]	5[a]	6[a]	7[a]
1	38.0	38.8	41.5	38.6	38.1	37.9	38.1
2	23.7	28.6	38.3	27.1	25.1	25.2	25.4
3	84.5	79.7	216.5	71.7	83.6	84.6	84.4
4	55.1	76.0	45.9	56.3	55.0	54.9	55.1
5	48.6	56.3	55.3	47.8	48.6	48.6	48.9
6	20.4	18.0	25.0	21.1	20.7	20.6	21.0
7	32.8	32.9	31.9	32.5	32.6	32.4	32.7
8	40.1	40.2	40.6	40.2	40.2	40.0	40.2
9	48.8	47.2	45.8	48.0	47.8	47.6	47.8
10	36.2	38.0	37.8	36.2	36.2	36.0	36.3
11	25.2	24.0	23.4	23.8	23.3	23.0	23.4
12	122.1	122.5	123.4	122.7	122.4	122.2	122.4
13	145.2	144.6	144.9	144.1	144.1	143.9	144.1
14	42.1	42.3	42.8	42.2	42.2	42.1	42.2
15	36.3	36.5	36.5	28.3	28.4	28.4	28.2
16	74.6	74.0	74.6	23.2	23.7	23.5	23.7
17	47.0	49.3	50.1	47.0	46.6	46.9	47.0
18	41.4	41.7	42.4	41.7	42.0	41.9	42.0
19	47.2	47.4	48.0	46.2	46.3	46.1	46.4
20	31.0	30.8	31.3	30.7	30.7	30.6	30.7
21	36.1	36.0	36.5	34.0	33.9	33.8	33.9
22	32.8	32.1	33.2	32.4	32.3	32.2	32.4
23	209.9	—	12.2	207.3	209.6	210.4	210.2
24	11.0	17.6	—	9.7	11.0	11.0	11.2
25	15.7	15.4	13.7	15.8	15.7	15.6	15.8
26	17.4	17.5	17.8	17.4	17.4	17.2	17.4
27	27.2	27.2	27.1	26.1	25.9	25.7	26.0
28	179.9	176.0	177.3	176.2	176.6	176.5	176.5
29	33.3	33.2	33.3	33.1	33.1	33.0	33.1
30	24.7	24.5	24.8	23.7	23.7	23.5	23.8

[a] Measured in pyridine-d_5.
[b] Measured in CD_3OD.

combined analysis of TOCSY, HMQC, HMBC, and ROESY spectra, all proton and carbon signals of these sugars were assigned (Table 2). The β-anomeric configurations for the D-fucose and D-xylose units, the α-anomeric configurations for two L-arabinose ($^4C_1$ configuration) units were determined from their $^3J_{H1,H2}$ coupling constants (7–8 Hz) and ^{13}C NMR data.[7] And the α-anomeric configuration of L-rhamnose was judged by its C_5 (δ_C 68.3).[8] The HMBC spectrum elucidated the sugar sequence as α-L-arabinopyranosyl-(1 → 4)-α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl as supported by the cross-peaks of H-1 (δ_H 6.51) of rhamnose with C-2 (δ_C 73.9) of fucose, and H-1 (δ_H 5.02) of xylose with C-4 (δ_C 84.8) of rhamnose, H-1 (δ_H 5.12) of inner arabinose with C-3 (δ_C 87.6) of xylose, and H-1 (δ_H 4.98) of terminal arabinose with C-4 (δ_C 78.5) of inner arabinose (Fig. 1). The same conclusion with regard to the sugar sequence was also drawn from the ROESY experiments (Fig. 1). The pentasaccharide moiety was attached to C-28 of segetalic acid by the observation of HMBC correlation between C-28 (δ_C 176.0) of aglycone and H-1 (δ_H 6.00) of D-fucose. Thus, the structure of **2** was determined as segetalic acid 28-*O*-α-L-arabinopyranosyl-(1 → 4)-α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)- β-D-fucopyranosyl ester. The aglycone part of **3**, unlike segelic acid, showed a doublet methyl group (δ_H 0.99, d, J = 6.5 Hz) and a ketone carbonyl carbon (δ_C 216.5) besides the characteristic information of the olean-12-ene skeleton including five angular methyl groups (δ_H 0.86, 0.88, 0.95, 1.18, and 1.38), and a triplet-like vinyl proton (δ_H 5.34) coupled with the two olefinic carbons (δ_C 123.4 and 144.9). Detailed analysis of the HMQC, HMBC, and ROESY data indicated that the aglycone in **3** was a rearranged product of segelic acid and identified as 3-keto, 16α-hydroxy, 24-noroleanolic acid (**2a**).[6] The α-configuration of methyl group (23-Me) was evident from the ROESY spectrum which showed significant correlation through space interaction between 23-Me (δ_H 0.99) and H-5 (δ_H 1.09, α-H), but no correlation between 23-Me with 25-Me (δ_H 1.18, β-Me). Based upon the above information, the structure of **3** was elucidated to be 3-keto, 16α-hydroxy, 24-noroleanolic acid 28-*O*-α-L-arabinopyranosyl-(1 → 4)-α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester.

Compound **4** was isolated as an amorphous powder, with the molecular formula $C_{54}H_{86}O_{24}$, as determined from data of the negative-ion HRESIMS (*m*/*z* 1117.5406 $[M-H]^-$). On acid hydrolysis, **4** afforded gypsogenin[6] (**4a**) as the aglycone, and D-glucose and D-galactose in the ratio of 3:1 as component sugars. The ^1H and ^{13}C NMR chemical shift assignments of sugar moieties (Table 2) were accomplished by a combination of TOCSY, HMQC, and HMBC experiments. The β-anomeric configurations for the glucose were determined from their $^3J_{H1,H2}$ coupling constants (7.8–8.2 Hz), while the α-anomeric configuration for the galactose was determined from its small $^3J_{H1,H2}$ coupling constant (3.5 Hz) and a direct comparison of the NMR data with those of the literature data.[9] The tetrasaccharide moiety attached to C-28 was established by the following HMBC correlations: H-1 of galactose (δ_H 5.46) with C-6 of glucose III (δ_C 68.4), H-1 of glucose III (δ_H 4.90) with C-6 of glucose I (δ_C 69.3), H-1 of glucose II (δ_H 5.24) with C-3 of glucose I (δ_C 88.5), H-1 of glucose glucose I (δ_H 6.19) with C-28 of the aglycone (δ_C 176.2). Thus, the structure of **4** was determined to be gypsogenin 28-*O*-α-D-galactopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl ester.

Compound **5** was obtained as an amorphous powder. It revealed an $[M-H]^-$ ion peak at *m*/*z* 1231 in the negative ESIMS. The molecular formula of $C_{59}H_{92}O_{27}$ was confirmed by HRESIMS. On acid hydrolysis, **5** afforded gypsogenin[6] (**4a**) as the aglycone, and D-xylose, D-galactose, D-fucose, D-glucoronic acid, and L-rhamnose in the ratio of 1:1:1:1:1 as component sugars. The chemical shifts of δ_C 83.6 (C-3) and 176.6 (C-28) revealed that compound **5** was a bisdesmosidic glycoside. The anomeric proton signals at δ_H 6.41 (s), 6.00 (d, J = 8.2 Hz), 5.21 (d, J = 7.5 Hz), 5.00 (d, J = 7.2 Hz), and 4.90 (d, J = 7.5 Hz) gave correlations with anomeric carbon signals at δc 101.3, 94.7, 106.3, 107.7, and 103.5 in HMQC spectrum, respectively. The ^1H and ^{13}C NMR chemical shift assignments (Table 3) were accomplished by a combination of TOCSY, HMQC, and HMBC experiments. The linkage of the sugar units at C-3 of the aglycone was established from the following HMBC correlations: H-1 of galactose (δ_H 5.21) with C-2 of glu-

Table 2. ^{13}C and ^{1}H NMR data for sugar moieties of **1**–**4**

	1			**2**		**3**			**4**	
	δ_C	δ_H		δ_C	δ_H	δ_C	δ_H		δ_C	δ_H
3-O–			28-O–					28-O–		
GlcA			Fuc					GlcI		
1	103.7	4.84 (d, 7.5)	1	94.7	6.00 (d, 8.7)	95.2	5.32 (d, 8.2)	1	95.0	6.19 (d, 8.2)
2	78.3	4.26 (dd, 9.2, 7.5)	2	73.9	4.67 (dd, 9.2, 8.7)	74.5	3.82 (dd, 8.5, 8.2)	2	72.8	4.12 (m)
3	87.8	4.21 (t, 9.2)	3	76.8	4.15 (dd, 9.2, 3.2)	76.6	3.69 (m)	3	88.5	4.22 (t, 8.2)
4	71.6	4.48 (t, 9.2)	4	73.2	3.90 (d, 3.2)	73.6	3.57 (m)	4	69.1	4.22 (t, 8.2)
5	77.2	4.47 (d, 9.3)	5	72.6	3.89 (br d, 6.5)	72.7	3.68 (br d, 6.4)	5	77.6	4.04 (m)
6	171.7		6	16.9	1.46 (d, 6.5)	16.5	1.23 (d, 6.4)	6	69.3	4.25 (d,11.0, 4.0)
Gal			Rha							4.62 (br d, 11.0)
1	104.4	5.47 (d, 7.6)	1	101.1	6.51 (s)	101.2	5.40 (d, 1.5)	GlcII		
2	73.7	4.47 (dd, 9.7, 7.6)	2	71.9	4.78 (br d, 3.0)	71.9	3.84 (br s)	1	105.8	5.24 (d, 7.8)
3	75.6	4.12 (dd, 9.7, 3.2)	3	72.4	4.66 (dd, 9.4, 3.0)	72.3	3.94 (br d, 8.5)	2	75.4	4.00 (t, 7.8)
4	70.1	4.55 (br d, 3.3)	4	84.8	4.30 (t, 9.4)	84.6	3.54 (t, 8.5)	3	78.3	4.11 (m)
5	76.6	3.92(dd, 7.7, 4.0)	5	68.1	4.42 (dd, 9.4, 6.4)	68.7	3.80 (dd, 8.5, 6.2)	4	71.7	4.13 (m)
6	61.8	4.52 (dd, 11.3, 4.0)	6	18.4	1.58 (d, 6.4)	18.3	1.33 (d, 6.2)	5	78.5	3.97 (m)
		4.41(dd,11.3, 7.7)	Xyl					6	62.5	4.22 (br d, 11.8, 4.2)
Gal			1	106.6	5.02 (d, 7.1)	106.7	4.55 (d, 7.8)			4.50 (br d, 11.8)
1	105.2	5.20 (d, 7.6)	2	75.4	3.95 (dd, 9.0)	75.2	3.41 (dd, 10.7, 7.8)	GlcIII		
2	73.0	4.47 (dd, 9.8, 7.6)	3	87.6	3.94 (m)	87.2	3.50 (t, 10.7)	1	105.4	4.90 (d, 7.8)
3	77.3	4.10 (dd, 9.8, 3.4)	4	68.9	3.94 (m)	69.7	3.57 (m)	2	74.9	3.95 (t, 8.0)
4	70.2	4.45 (br d, 3.4)	5	66.9	4.08(dd, 11.5, 5.1)	66.9	3.94 (dd, 12.0, 5.3)	3	78.2	4.14 (m)
5	75.4	4.10 (dd, 7.8,4.1)			3.41 (d, 5.1)		3.24 (*d*, 5.3)	4	71.7	4.06 (m)
6	62.0	4.46 (m)	Ara					5	76.2	3.90 (m)
		4.30 (dd, 11.9, 4.1)	1	105.7	5.12 (d, 7.0)	105.3	4.49 (d, 7.0)	6	68.4	4.28 (dd, 11.5, 4.4)
			2	73.2	4.42 (dd, 8.7, 7.0)	73.0	3.70 (dd, 8.5, 7.0)			4.45 (br d, 11.5)
			3	73.4	4.16 (dd, 8.7, 3.1)	74.3	3.84 (br d, 8.5)	Gal		
			4	78.5	4.25 (m)	79.4	3.90 (m)	1	100.6	5.46 (d, 3.5)
			5	66.2	4.44 (dd, 11.0, 5.0)	66.8	4.20 (dd, 11.5, 5.2)	2	70.6	4.63 (dd, 9.6, 3.5)
					3.47 (d, 5.0)		3.61 (d, 5.3)	3	71.7	4.51 (dd, 9.6, 3.2)
			Ara					4	71.1	4.55 (m)
			1	106.8	4.98 (d, 7.3)	106.9	4.38 (d, 7.2)	5	72.5	4.61 (m)
			2	72.9	4.43 (dd, 9.6, 7.3)	72.8	3.86 (dd, 9.5, 7.2)	6	62.7	4.39 (2H, m)
			3	74.5	4.06 (dd, 9.6, 3.2)	74.1	3.80 (br d, 9.5)			
			4	69.6	4.33 (m)	69.8	3.80 (m)			
			5	67.2	3.68 (dd, 11.1, 5.0)	67.2	3.54 (dd, 11.0, 5.1)			
					4.17 (d, 5.0)		3.88 (d, 5.1)			

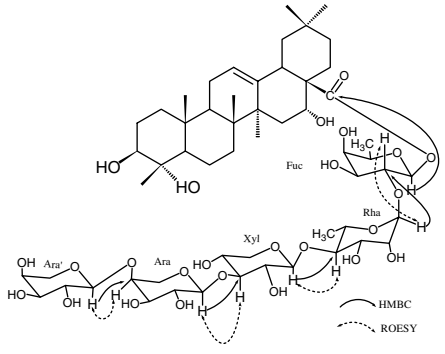


Figure 1. Selected HMBC and ROESY correlations for **2**.

curonic acid (δ_C 82.2), H-1 of glucuronic acid (δ_H 4.90) with C-3 of the aglycone (δ_C 83.6). Similarly, the sugar chain at C-28 was established from the following HMBC correlations: H-1 of rhamnose (δ_H 6.41) with C-2 of fucose (δ_C 74.7), H-1 of xylose (δ_H 5.00) with C-4 of rhamnose (δ_C 85.5), and H-1 of fucose (δ_H 6.00) with C-28 of the aglycone (δ_C 176.6). From the above evidences, the structure of **5** was elucidated as 3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl gypsogenin 28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside.

Compound **6** had a molecular formula of $C_{69}H_{107}O_{35}$ as established by HRESIMS. Acid hydrolysis of **6** afforded gypsogenin[6] (**4a**) and monosaccharides D-fucose, L-rhamnose, D-xylose, D-galactose, L-arabinose, and D-glucuronic acid (1:1:2:1:1:1). The chemical shifts of δ_C 84.6 (C-3) and 176.5 (C-28) revealed that compound **6** was a bisdesmosidic glycoside. The anomeric proton signals at δ_H 6.44 (s), 6.01 (d, J = 8.2 Hz), 5.55 (d, J = 7.7 Hz), 5.34 (d, J = 7.7 Hz), 5.19 (d, J = 7.1 Hz), 4.95 (d, J = 7.6 Hz), and 4.86 (d, J = 7.5 Hz) gave correlations with anomeric carbon signals at δc 101.2, 94.7, 103.9, 104.9, 105.4, 107.0, and 104.0 in HMQC spectrum, respectively. The ^{1}H and ^{13}C NMR chemical shift assignments (Table 3) were accomplished by a combination of TOCSY, HMQC, and HMBC experiments. The linkage of the sugar units at C-3 of the aglycone was established from the following HMBC correlations: H-1 of galactose (δ_H 5.55) with C-2 of glucuronic acid (δ_C 78.2), H-1 of xylose (δ_H 5.34) with C-3 of glucuronic acid (δ_C 78.2), and H-1 of glucuronic acid (δ_H 4.86) with

Table 3. ^{13}C and ^{1}H NMR data for sugar moieties of **5**–**7**

	5		**6**		**7**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-O-Sugars						
GlcA						
1	103.5	4.90 (d, 7.5)	104.0	4.86 (d, 7.5)	104.0	4.89 (d, 7.5)
2	82.2	4.23 (dd, 9.2, 7.5)	78.2	4.30 (dd, 9.1, 7.5)	78.6	4.32 (t, 7.5)
3	77.6	4.28 (t, 9.2)	85.8	4.21 (t, 9.1)	85.9	4.22 (dd, 9.5, 7.5)
4	70.8	4.22 (t, 9.5)	71.2	4.43 (t, 9.3)	70.8	4.48 (t, 9.5)
5	77.2	4.51 (d, 9.7)	77.2	4.47 (d, 9.3)	76.3	4.48 (d, 9.5)
6	172.3		171.7		170.9	
Gal						
1	106.3	5.21 (d, 7.5)	103.9	5.55 (d, 7.7)	104.2	5.50 (d, 7.6)
2	72.5	4.53 (dd, 9.5, 2.7)	73.5	4.44 (dd, 9.6, 7.7)	73.7	4.45 (t, 8.0)
3	74.0	4.11 (dd, 8.5, 3.3)	75.4	4.13 (dd, 9.6, 3.2)	75.2	4.13 (dd, 8.2, 3.0)
4	70.2	4.56 (d, 3.3)	70.0	4.55 (d, 3.2)	70.2	4.52 (d, 3.0)
5	74.8	3.96 (m)	76.4	3.96 (m)	76.7	4.00 (m)
6	62.1	4.52 (2H, m)	61.3	4.50 (m), 4.38 (m)	61.7	4.40 (2H, m)
Xyl						
1			104.9	5.34 (d, 7.7)	104.9	5.26 (d, 7.7)
2			75.2	3.92 (dd, 7.7, 8.3)	75.3	3.90 (m)
3			78.5	4.08 (t, 8.3)	78.6	4.12 (t, 8.4)
4			70.7	4.07 (m)	70.8	4.09 (m)
5			67.2	3.64 (dd, 12.0, 10.2)	67.3	3.63 (dd, 11.4, 10.0)
				4.22 (dd, 12.0, 5.4)		4.20 (br d, 11.4, 5.1)
28-O-Sugars						
Fuc						
1	94.7	6.00 (d, 8.2)	94.7	6.01 (d, 8.2)	94.8	5.99 (d, 8.2)
2	74.7	4.67 (t, 8.2)	73.8	4.64 (t, 8.2)	74.1	4.63 (t, 8.2)
3	76.7	4.15 (dd, 8.2, 4.0)	76.7	4.13 (dd, 8.4, 2.7)	75.1	4.15 (dd, 8.2, 3.3)
4	73.2	3.93 (d, 4.0)	73.0	3.90 (d, 3.0)	73.1	3.96 (d, 3.3)
5	72.2	3.88 (m)	72.2	3.84 (m)	72.4	3.90 (dd, 6.1, 3.3)
6	16.9	1.46 (d, 6.3)	16.8	1.45 (d, 6.3)	16.9	1.48 (d, 6.1)
Rha						
1	101.3	6.41 (s)	101.2	6.44 (s)	101.3	6.30 (s)
2	71.8	4.82 (br s)	71.5	4.82 (br s)	71.3	5.00 (br s)
3	72.9	4.67 (br d, 8.5)	72.5	4.59 (dd, 8.8, 2.3)	82.7	4.65 (br d, 8.3)
4	85.5	4.30 (t, 8.5)	85.6	4.25 (t, 8.8)	78.8	4.48 (t, 8.3)
5	68.2	4.47 (dd, 8.5, 6.2)	67.8	4.38 (dd, 8.8, 6.1)	68.2	4.47 (dd, 8.3, 5.5)
6	18.5	1.69 (d, 6.2)	18.4	1.65 (d, 6.1)	18.9	1.67 (d, 5.5)
Xyl						
1	107.7	5.00 (d, 7.2)	107.0	4.95 (d, 7.6)	105.3	5.34 (d, 7.8)
2	76.3	4.04 (m)	75.2	3.92 (t, 8.9)	75.9	3.89 (dd, 10.7, 7.8)
3	78.7	4.02 (m)	86.4	3.95 (m)	79.5	4.01 (t, 10.7)
4	68.7	4.10 (dd, 10.2, 8.5)	68.7	4.01 (dd, 9.9, 8.6,)	71.2	4.14 (m)
5	66.8	4.25 (dd, 11.3, 5.3)	66.8	3.45 (t, 11.1)	67.1	3.33 (t, 11.0)
		3.50 (t, 11.2)		4.14 (dd, 11.1, 5.4)		4.13 (dd, 11.0, 5.4)
			Ara		Glc	
1			105.4	5.19 (d, 7.1)	105.1	5.23 (d, 7.7)
2			72.6	4.51 (dd, 8.6, 7.1)	75.1	3.90 (t, 7.8)
3			74.4	4.15 (dd, 8.5, 3.0)	78.7	4.10 (t, 8.0)
4			69.1	4.27 (m)	71.7	3.98 (m)
5			67.1	3.73 (dd, 11.5, 5.3)	78.2	3.90 (m)
6				4.30 (d, 5.3)	64.4	4.65 (br d, 11.0)
						4.90 (br d, 11.0)
*C*OCH$_3$					170.9	
CO*CH*$_3$					20.9	2.12 (s)

C-3 of the aglycone (δ_C 84.6). Similarly, the sugar chain at C-28 was established from the following HMBC correlations: H-1 of rhamnose (δ_H 6.44) with C-2 of fucose (δ_C 73.8), H-1 of xylose (δ_H 4.95) with C-4 of rhamnose (δ_C 85.6), H-1 of arabinose (δ_H 5.19) with C-3 of xylose (δ_C 86.4), and H-1 of fucose (δ_H 6.01) with C-28 of the aglycone (δ_C 176.5). From the above evidences, the structure of **6** was elucidated as 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl gypsogenin 28-*O*-α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside.

Compound **7** displayed quasi-molecular ion peak $[M-H]^-$ at *m*/*z* 1567.6743 in the negative HRESIMS, in accordance with empirical molecular formula of $C_{72}H_{112}O_{37}$. Its negative-ion ESIMS showed a quasi-molecular ion peak at *m*/*z* 1567 $[M-H]^-$, 42 mass units

higher than that of known compound **10** (*m*/*z* 1643 $[M-H]^-$).[4a] The ^1H and ^{13}C NMR data (Table 3) of **7** assigned from TOCSY, HMQC, and HMBC experiments were similar to those of known compound **10**, except for the appearance of an additional acetyl group. The downfield signals of Glc-6 at δ_H 4.90, 4.65 gave two cross-peaks with the downfield signal of C-6 at δ_C 64.4 in the HMQC spectrum, respectively, and revealed the location of the acetyl group at this position, which was confirmed by correlations between two proton signals [δ_H 4.90 (br d, J = 11.0 Hz), 4.65 (br d, J = 11. 0 Hz)] of Glc-6 and carbonyl carbon (δ_H 170.9) of acetyl group in the HMBC spectrum, respectively. On the basis of the above results, compound **7** was determined as 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl gypsogenin 28-*O*-(6-*O*-acetyl)-β-D-glucopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside.

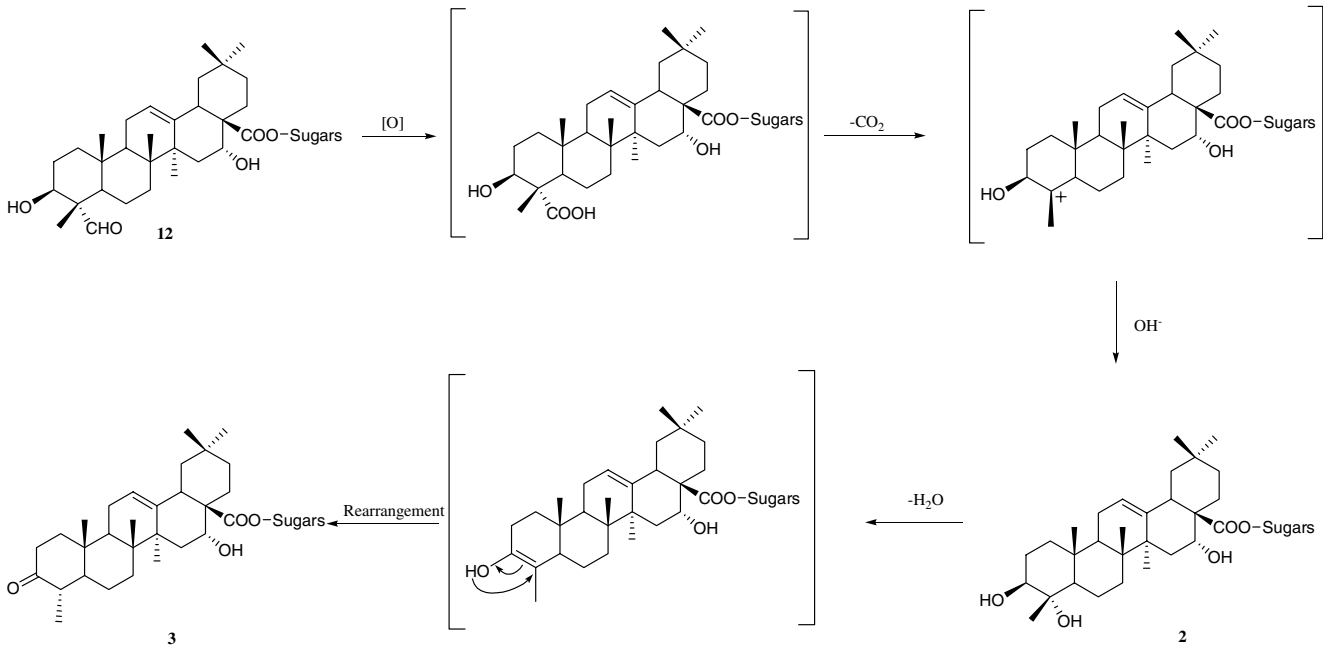
Compounds **2** and **3** are newly described triterpenoid saponins bearing identical sugar chain. As described above, acid hydrolysis of **2** furnished a rearranged aglycone and identified as 3-keto, 16α-hydroxy, 24-noroleanolic acid (**2a**), which is the aglycone of **3**. It suggested that **3** originates from **2** by a sequence of dehydrated and rearranged reactions in the plant. The fact that **2**, **3**, and **12** with the identical sugar sequences coexist in the same plant leads us to suggest a reasonably plausible biosynthetic pathway for **2** and **3** originated from **12** (Scheme 2).

Since some triterpenes have been reported to exert antidiabetes activities[10] or potent α-glucosidase inhibitory activities,[11,12] the isolated saponins (**1**–**12**), with their aglycones quillaic acid (**1a**), 3-keto, 16α-hydroxy, 24-noroleanolic acid (**2a**) and gypsogenin (**4a**) as the references, have been evaluated for the inhibitory activities against α-glucosidase. As shown in Table 4, compounds **2**–**4**, **12**, **1a**, **2a**, and **4a** exhibited more potent α-glucosidase inhibitory activities than that of acarbose, a widely used clinically useful drug, used as a positive control (IC_{50} = 388 ± 9.6 μM). While the other saponins (**1**, **5**–**11**) were found to be inactive in this test (no inhibition at 400 μM concentration).[12] To our knowledge, **2**–**4** and **12** are the first examples of the triterpenoid saponins with the α-glucosidase inhibitory activities. Interestingly, compound **4** is a triterpenoid saponin with α-anomeric monosaccharide (α-galactose), and a similar α-anomeric structural unit exists in acarbose (α-glucose). It showed the stronger inhibitory activity with IC_{50} = 15.2 ± 1.8 μM, compared with the other saponins **2**, **3**, and **12**.

Table 4. α-Glucosidase inhibition of compounds **1**–**12** and their aglycones

Compound	α-Glucosidase inhibition constant[a] (IC_{50}) [μM]	Compound	α-Glucosidase inhibition (IC_{50}) [μM]
1	Not active[b]	**9**	Not active[b]
2	23.1 ± 1.8	**10**	Not active[b]
3	65.5 ± 4.5	**11**	Not active[b]
4	15.2 ± 1.8	**12**	78.5 ± 7.1
5	Not active[b]	**1a**	59.7 ± 3.2
6	Not active[b]	**2a**	98.2 ± 1.9
7	Not active[b]	**4a**	85.2 ± 1.2
8	Not active[b]	Acarbose[c]	388.0 ± 9.6

[a] IC_{50} is defined as the concentration that resulted in a 50% α-glucosidase inhibition and the results are means ± standard deviation of three independent replicates.
[b] No inhibition at 400 μM concentration.
[c] Positive control substance.



Scheme 2. Hypothetical biogenetic pathway for **2** and **3** originated from **12**.

tached to the aglycone, could be classified into three series: 3-*O*-monoglucosides (**1**, **8**, **9**), 28-*O*-monoglucosides (**2**–**4**, **12**), and 3, 28-*O*-bidesmosides (**5**–**7**, **10**, **11**). On the basis of our results, the preliminary structure–activity relationships based on the position of sugar linkage attached to the aglycone may be suggested. In particular, compared with the aglycones (**1a**, **2a**, and **4a**), only the 28-*O*-monoglucosidic glycosides (**2**–**4**, **12**) still showed significant α-glucosidase inhibitory activities. The gylcosylation of aglycone at 3-OH (**1**, **5**–**11**) led to loss of α-glucosidase inhibitory activity, which was probably due to the presence of a free polar group (OH or C=O) at C-3 of aglycone and it may interact with the enzyme binding sites through intermolecular interactions. Of course, more saponins need to be tested to confirm this result.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO P-1020 polarimeter. IR (KBr-disks) spectra were recorded by Nicolet Impact-410 spectrometer. 1D and 2D NMR spectra were recorded at 300 K on Bruker, ACF-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz), with TMS as internal standard. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESIMS) and a Micro Q-TOF MS (HRESIMS), respectively. TLC was performed on precoated silica gel 60 F_{254} (Qingdao Haiyang Chemical Co. Ltd) and detection was achieved by 10% H_2SO_4-EtOH for saponins, aniline–phthalate reagents for sugars. Silica gel H (Qingdao Haiyang Chemical Co. Ltd), Sephadex LH-20 (Pharmacia), and RP-C_{18} (40–63 μm, FuJi) were used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-C_{18} column (200 × 20 mm i.d.) and 1100 Series Multiple Wavelength detector.

3.2. Plant material

The roots of *G. oldhmiana* were collected in suburbs of Jinan, Shandong Province, China, in October 2004 and identified by Prof. Yun-Yao Li of the Department of Pharmacognosy, Shandong University. A voucher specimen (No. 041022) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3. Extraction and isolation

The air-dried roots (30 kg) of *G. oldhmiana* were extracted with aq. 70% EtOH (40 l × 2 h × 3) under reflux. After removal of the solvent in vacuum, the residue was suspended in H_2O and partitioned between *n*-BuOH and water. The *n*-BuOH-soluble portion (724 g) was fractionated by a silica gel column (100–200 mesh), which was eluted with $CHCl_3$/MeOH/H_2O (100:10:0 → 100:100:1) to give five fractions (fractions 1–5), each fraction was further subjected to repeated RP-C_{18} column with MeOH/H_2O 4:6 → 8:2 as eluents. Fraction 3 was further separated by HPLC (CH_3CN–H_2O, 47:53, UV detection at 210 nm), and yielded pure **2** (10 mg, t_R = 7.7 min), **12** (30 mg, t_R = 12.2 min), and **3** (7 mg, t_R = 28.3 min), respectively. Fraction 4 was subjected to HPLC (CH_3CN-0.05% TFA in H_2O, 45:55) to afford **4** (10 mg, t_R = 3.5 min), **1** (24 mg, t_R = 6.5 min), **8** (18 mg, t_R = 7.8 min), and **9** (15 mg, t_R = 8.4 min). Fraction 5 was passed through a Sephadex LH-20 column (MeOH as eluent) to obtain **6** (20 mg) and a mixture. This mixture was further separated by HPLC (CH_3CN-0.05% TFA in H_2O, 48:52) to afford **5** (15 mg, t_R = 11.6 min), **7** (25 mg, t_R = 16.2 min), **10** (30 mg, t_R = 8.9 min), and **11** (25 mg, t_R = 6.1 min), respectively.

3.3.1. Compound 1. White powder; $[\alpha]_D^{25}$ + 17.8° (*c* 0.22; MeOH); IR ν_{max}^{KBr}: 3427, 2945, 1722, 1680, 1077 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.78, 0.94, 1.04, 1.16, 1.43, 1.79 (3H each, s, Me-25, 26, 29, 30, 24, 27), 2.79 (1H, t, *J* = 13.5 Hz, 19-H), 3.57 (1H, dd, *J* = 3.0, 13.5 Hz, 18-H), 4.04 (1H, dd, *J* = 3.0, 13.5 Hz, 3-H), 5.20 (1H, br s, 16-H), 5.57 (1H, t-like, 12-H), 9.90 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^{1}H and ^{13}C NMR data of glycosidic part, see Table 2. ESIMS *m/z*: 1009 $[M+Na]^+$; HRESIMS *m/z*: 985.4643 $[M-H]^-$ (calcd for $C_{48}H_{73}O_{21}$, 985.4649).

3.3.2. Compound 2. White powder; $[\alpha]_D^{25}$ − 6.0° (*c* 0.03; MeOH); IR ν_{max}^{KBr}: 3428, 2931, 1731, 1632, 1062 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.92, 0.96, 1.00, 1.17, 1.38, 1.70 (3H each, s, Me-25, 29, 30, 26, 24, 27), 2.79 (1H, t, *J* = 13.2 Hz, 19-H), 3.44 (1H, dd, *J* = 3.8, 14.3 Hz, 18-H), 3.84 (1H, *m*, 3-H), 5.17 (1H, br s, 16-H), 5.63 (1H, t, *J* = 3.3 Hz, 12-H); ^{13}C NMR data of the aglycone, see Table 1. ^{1}H and ^{13}C NMR data of glycosidic part, see Table 2. ESIMS *m/z*: 1161 $[M-H]^-$, HRESIMS: *m/z* 1161.5688 $[M-H]^-$ (calcd for $C_{56}H_{89}O_{25}$, 1161.5688).

3.3.3. Compound 3. White powder; $[\alpha]_D^{25}$ − 4.8° (*c* 0.05; MeOH); IR ν_{max}^{KBr}: 3410–3499, 2931, 1724, 1060 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) δ 0.86, 0.88, 0.95, 1.18, 1.38 (3H each, s, Me-26, 29, 30, 25, 27), 0.99 (3H, d, *J* = 6.5 Hz, 23-Me), 2.30 (1H, t, *J* = 13.3 Hz, 19-H), 2.96 (1H, dd, *J* = 4.0, 14.3 Hz, 18-H), 4.51 (1H, br s, 16-H), 5.34 (1H, t, *J* = 3.3 Hz, 12-H); ^{13}C NMR data of the aglycone, see Table 1. ^{1}H and ^{13}C NMR data of glycosidic part, see Table 2. ESIMS *m/z*: 1143 $[M-H]^-$, 1167 $[M+Na]^+$; HRESIMS *m/z*: 1143.5560 $[M-H]^-$ (calcd for $C_{56}H_{87}O_{24}$, 1161.5592).

3.3.4. Compound 4. White powder; $[\alpha]_D^{25}$ + 50.0° (*c* 0.03; MeOH); IR ν_{max}^{KBr}: 3439, 2932, 1730, 1634, 1066 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.85, 0.86, 0.93, 1.04, 1.20, 1.34 (3H each, s, Me-30, 29, 25, 26, 27, 24), 3.14 (1H, dd, *J* = 4.1, 14.0 Hz, 18-H), 4.04 (1H, m, 3-H), 5.40 (1H, t-like, 12-H), 9.60 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^{1}H and ^{13}C NMR data of glycosidic part, see Table 2. ESIMS *m/z*: 1141 $[M + Na]^+$, HRESIMS *m/z*: 1117.5406 $[M-H]^-$ (calcd for $C_{54}H_{85}O_{24}$, 1117.5436).

3.3.5. Compound 5. White powder; $[\alpha]_D^{25}$ + 5.1° (*c* 0.04; MeOH); IR ν_{max}^{KBr}: 3421, 2941, 1727, 1649, 1048 cm^{-1}.

^{1}H NMR (500 MHz, pyridine-d_5) δ 0.79, 0.86, 0.87, 1.03, 1.24, 1.40 (3H each, s, Me-25, 29, 30, 26, 27, 24), 3.10 (1H, br d, J = 14.0 Hz, 18-H), 4.02 (1H, m, 3-H), 5.38 (1H, t-like, 12-H), 9.90 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^{1}H and ^{13}C NMR data of glycosidic part, see Table 3. ESIMS *m/z*: 1231 $[M-H]^-$; HRESIMS *m/z*: 1231.5715 $[M-H]^-$ (calcd for $C_{59}H_{91}O_{27}$, 1231.5753).

3.3.6. Compound 6. White powder; $[\alpha]_D^{25}$ + 4.8° (*c* 0.05; MeOH); IR ν_{max}^{KBr}: 3429, 2929, 1731, 1633, 1074 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.73, 0.82, 0.84, 1.01, 1.22, 1.40 (3H each, s, Me-25, 30, 29, 26, 27, 24), 3.05 (1H, br d, J = 14.0 Hz, 18-H), 4.03 (1H, m, 3-H), 5.36 (1H, t-like, 12-H), 9.91 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^{1}H and ^{13}C NMR data of glycosidic part, see Table 3. ESIMS *m/z*: 1495 $[M-H]^-$, HRESIMS *m/z*: 1495.6571 $[M-H]^-$ (calcd for $C_{69}H_{107}O_{35}$, 1495.6598).

3.3.7. Compound 7. White powder; $[\alpha]_D^{25}$ − 1.5° (*c* 0.20; MeOH); IR ν_{max}^{KBr} 3440, 1734, 1041 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.86, 0.87, 0.89, 1.10, 1.21, 1.52 (3H each, s, Me-25, 29, 30, 26, 27, 24), 3.09 (1H, br d, J = 14.0 Hz, 18-H), 4.05 (1H, m, 3-H), 5.36 (1H, t-like, 12-H), 9.93 (1H, s, 23-H); ^{13}C NMR data of the aglycone, see Table 1. ^{1}H and ^{13}C NMR data of glycosidic part, see Table 3. ESIMS *m/z*: 1567 $[M-H]^-$, HRESIMS *m/z*: 1567.6743 $[M-H]^-$ (calcd for $C_{72}H_{111}O_{37}$, 1567.6809).

3.4. Acid hydrolysis and sterochemistry of sugars of 1–7

Each compound (5 mg) was heated in 2 M HCl (5 mL) at 90 °C for 4 h. The reaction mixture was extracted with EtOAc (3 × 5 mL). The EtOAc extract was purified by chromatography on Sephadex LH-20 (2.0 × 100 cm). Comparing TLC with authentic samples, the aglycone of **1** was determined to be quillaic acid (**1a**, R_f: 0.25, $CHCl_3$–MeOH, 20:1), while those of **4**–**7** were gypsogenin (**4a**, R_f: 0.43, $CHCl_3$–MeOH, 20:1). In addition, 3-keto, 16α-hydroxy, 24-noroleanolic acid (**2a**) was obtained from **2** and **3**. The H_2O layer was concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 mL), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 mL) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried in vacuo, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL each) and the *n*-hexane extract was analyzed by GC–MS (Varian 3800GC, Varian 2200MS, 70 eV) under the following conditions: capillary column, SE30 (30 m × 0.25 mm × 0.25 μm); the column temperature, 170–250 °C with the rate of 5 °C/min. The carrier gas: N_2 (30 mL/min). In the acid hydrolysate of **1**–**7**, L-arabinose, D-fucose, L-rhamnose, D-xylose, D-glucuronic acid, D-galactose, and D-glucose were confirmed by comparison of their retention times of their derivatives with those of authentic L-arabinose (t_R 4.416 min), D-fucose (t_R 4.573 min), L-rhamnose (t_R 5.086 min), D-xylose (t_R 5.645 min), D-glucuronic acid (t_R 6.613 min), D-galactose (t_R 7.538 min), and D-glucose (t_R 8.032 min) derivatives prepared in the same way, respectively.

3.4.1. 3-Keto, 16α-hydroxy, 24-noroleanolic acid (2a). An amorphous solid, $[\alpha]_D^{25}$ + 30.5° (*c* 0.10; $CHCl_3$); IR ν_{max}^{KBr} 3434, 1778, 1706, 1457 cm^{-1}; ESIMS *m/z*: 457 $[M+H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.84, 0.85, 0.91, 1.12, 1.14 (3H each, s, Me-30, 26, 29, 25, 27-H), 1.00 (3H, d, J = 6.5 Hz, 23-H) 2.54 (1H, t, J = 14.0 Hz, H-19), 3.23 (1H, dd, J = 3.3, 14.0 Hz, 18-H), 5.12 (1H, br s, H-16), 5.34 (1H, t-like, 12-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.2 (C-1), 37.4 (C-2), 213.6 (C-3), 44.8 (C-4), 53.7 (C-5), 24.0 (C-6), 31.7 (C-7), 39.2 (C-8), 45.4 (C-9), 36.7 (C-10), 22.1 (C-11), 122. 6 (C-12), 143.9 (C-13), 41.9 (C-14), 36.0 (C-15), 74.1 (C-16), 48.6 (C-17), 41.3 (C-18), 46.9 (C-19), 30.7 (C-20), 35.9 (C-21), 32.4 (C-22), 11.7 (C-23), 13.2 (C-25), 17.1 (C-26), 26.8 (C-27), 180.6 (C-28), 33.1 (C-29), 23.6 (C-30).

3.5. Enzyme inhibition assay

The α-glucosidase inhibition assay was performed according to the slightly modified method of Pierre et al.[13] α-Glucosidase (E.C.3.2.1.20) was purchased from Sigma company (No. G-5003, Lot. 081k7415). The inhibition was measured spectro-photometrically at pH 6.8 and at 37 °C for 10 min, using 0.01 M *p*-nitrophenyl α-D-glucopyranoside (PNPG) as a substrate and 1 U/mL of enzyme, in 0.067 M KH_2PO_4–Na_2HPO_4 buffer. Acarbose were used as positive controls. The increment in absorption at 410 nm due to the hydrolysis of PNPG by α-glucosidase was monitored continuously with an auto multi-functional microplate reader (BIO-RAD680).

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