New Triterpenoid Saponins from the Roots of *Gypsophila paniculata* L.

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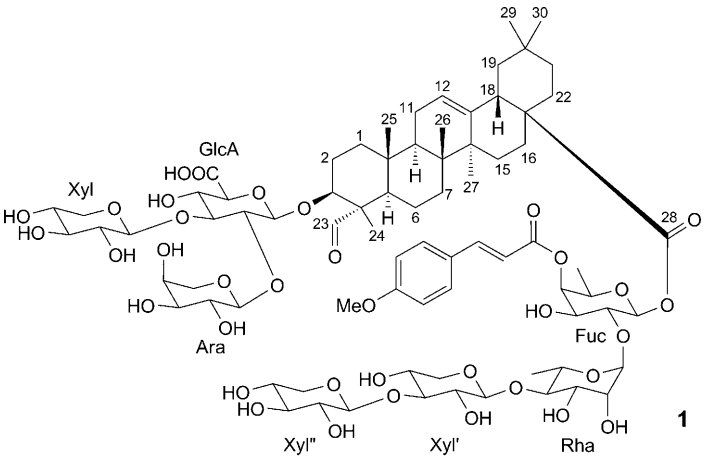
The seven new triterpenoid saponins **1**–**7** were isolated from the roots of *Gypsophila paniculata* L. Their structures were established by 1D- and 2D-NMR techniques, HR-MS, and acid hydrolysis. The isolated compounds include 3,28-*O*-bidesmosides with or without a 4-methoxycinnamoyl group (see **1** *vs.* **2** and **3**), and 3-*O*-monoglucosides **4**–**7**. All isolated saponins **1**–**7** and their aglycones were evaluated for their α-glucosidase inhibition activity. Compound **1** showed inhibitory activity against yeast α-glucosidase with an IC_{50} value of 100.9 ± 3.3 μM, whereas compounds **2**–**7** were inactive.

Introduction. – *Gypsophila paniculata* L. (Caryophyllaceae) is a small perennial herb widely distributed in the north regions of China. Its roots have been used as substitute for the traditional Chinese herb Shan-Yin-Chai-Hu (roots of *Stellaria dichotoma* var. LANCEOLATA BGE) to treat fever, consumptive disease, and infantile malnutrition syndrome [1]. Commonly known as baby's breath, it has been used as contraceptive and purgative for a very long time in Europe [2]. Many previous chemical studies reported triterpenoid saponins as the main constituents from the roots of *G. paniculata* [3–6]. We investigated the saponins from *G. oldhamiana* and their α-glucosidase inhibition activity in previous studies [7–9]. To look for more bioactive saponins from this genus, we have now focused on *G. paniculata*. The present report first deals with the isolation and structure elucidation of the seven new saponins **1**–**7**. Then, in the following bioactivity investigation, compound **1** which has an extra aromatic acyl group at the sugar chain connected to C(28) compared with the other saponins showed inhibitory activity on yeast α-glucosidase.

Results and Discussion. – The 75% EtOH extract of the roots of *G. paniculata* was partitioned with H_2O and BuOH. The BuOH-soluble fraction, chromatographed repeatedly over silica gel, *Sephadex LH-20*, and *RP-*C_{18}, followed by HPLC purification, afforded the seven new triterpenoid saponins **1**–**7**.

Compound **1** displayed a quasimolecular-ion peak at *m*/*z* 1649.7015 ($[M + Na]^+$) in the HR-MALDI-MS, which was consistent with the molecular formula $C_{78}H_{114}O_{36}$. The IR spectrum showed absorption at 3443 (OH), 1751 (ester C=O), and 1719 (C=O of COOH) cm^{-1}. Acid hydrolysis of **1** with 2M HCl afforded the aglycone and sugars. The former was identified as gypsogenin (=(3β,4α)-3-hydroxy-23-oxoolean-12-en-28-oic acid) by co-TLC comparison with an authentic sample and by comparison of the ^{1}H- and ^{13}C-NMR data (*Table 1*) with reported values [10]. The sugars were analyzed by



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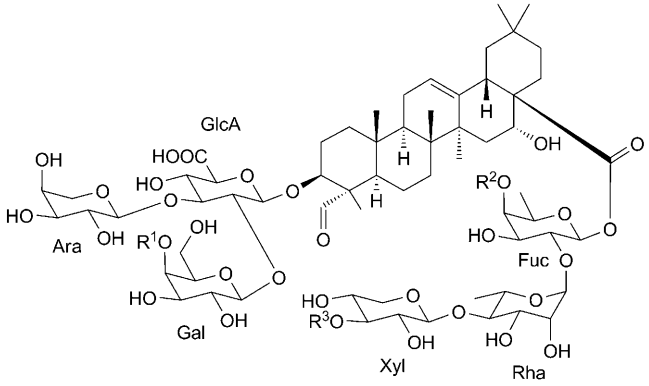
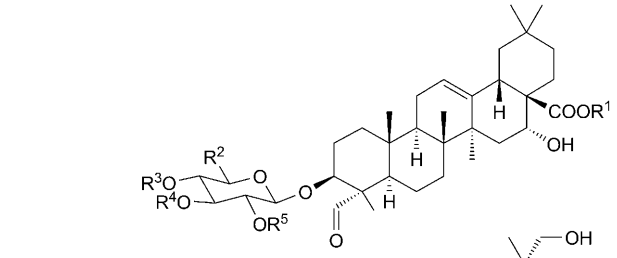


	R^1	R^2	R^3
2	Xyl	Qui	Xyl"
3	H	H	Gal'



	R^1	R^2	R^3	R^4	R^5
4	Me	$HOCH_2$	H	Xyl	Gal
6	H	COOH	H	Xyl	Glc
7	H	COOH	Gal	H	Glc

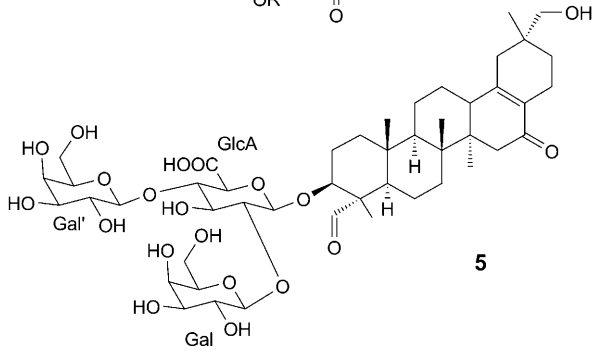


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Table 1. *^{13}C-NMR Data of the Aglycone Moieties of Compounds* **1**–**7**

	1[a])	**2**[b])	**3**[b])	**4**[b])	**5**[a])	**6**[a])	**7**[b])
C(1)	37.8	37.6	37.1	37.9	37.5	37.9	37.6
C(2)	25.7	26.7	27.0	26.9	27.0	26.9	26.7
C(3)	84.2	83.7	83.8	84.2	83.5	84.3	83.5
C(4)	54.7	54.7	55.1	54.8	54.9	54.9	54.7
C(5)	48.3	48.6	49.1	48.2	48.0	48.2	48.5
C(6)	20.1	23.1	23.7	23.5	20.1	23.5	23.2
C(7)	32.1	32.3	31.9	32.1	33.1	32.3	32.2
C(8)	39.6	39.4	39.8	39.5	39.8	39.5	39.6
C(9)	47.5	47.9	46.8	48.8	48.0	48.7	48.5
C(10)	35.9	35.8	36.1	35.6	35.8	35.7	35.6
C(11)	23.0	23.3	23.6	23.5	20.9	23.5	24.0
C(12)	122.1	121.6	122.1	121.8	24.4	121.8	122.5
C(13)	143.7	144.8	143.9	144.9	42.6	144.9	144.6
C(14)	41.7	41.6	41.3	40.9	44.7	41.2	42.3
C(15)	27.8	27.3	27.0	36.9	46.1	36.9	28.1
C(16)	23.4	73.2	73.8	75.3	199.4	74.9	23.4
C(17)	46.8	46.9	46.3	46.8	129.6	46.8	46.8
C(18)	41.8	41.6	40.8	41.6	156.8	41.9	41.6
C(19)	46.0	46.1	45.7	47.8	20.2	48.7	47.8
C(20)	30.5	30.7	30.4	30.4	36.4	32.3	30.4
C(21)	33.6	32.9	33.0	33.1	38.6	33.1	33.6
C(22)	32.7	32.3	32.4	30.6	32.4	30.7	31.9
C(23)	209.8	209.3	209.6	209.6	209.7	207.8	209.6
C(24)	10.7	10.6	11.1	10.7	10.3	10.7	10.3
C(25)	15.3	15.5	15.6	15.5	15.5	15.6	15.2
C(26)	17.0	17.0	17.3	17.2	16.2	17.2	16.9
C(27)	25.5	26.7	25.4	26.9	27.3	26.9	26.7
C(28)	176.2	175.6	175.9	179.2	–	180.2	180.5
C(29)	32.7	32.7	32.8	32.5	69.0	32.5	32.2
C(30)	23.4	24.2	23.6	24.8	18.9	24.7	24.4

[a]) Measured in (D_5)pyridine/(D_6)DMSO 10 : 1. [b]) Measured in (D_5)pyridine/D_2O 10 : 1.

TLC (silica gel, $CHCl_3$/MeOH/H_2O 8 : 5 : 1) in comparison with standard sugars. Their absolute configuration was determined by GC/MS of their thiazolidine derivatives [11]. 1D-NMR Spectra indicated obviously that compound **1** had seven sugar moieties. On the basis of the results from the above analysis, they were identified as D-xylose, L-rhamnose (=6-deoxy-L-mannose), L-arabinose, D-fucose (=6-deoxy-D-galactose), and D-glucuronic acid (3 : 1 : 1 : 1 : 1). The seven sugar anomeric C-atoms were detected at δ(C) 94.2, 101.2, 103.4, 103.8, 104.5, 105.5, and 107.1 in the ^{13}C-NMR spectrum (*Table 2*), attached to the H-atoms at δ(H) 6.08 (*d*, J = 8.1 Hz), 6.30 (br. *s*), 4.87 (*d*, J = 7.3 Hz), 5.45 (*d*, J = 7.2 Hz), 5.23 (*d*, J = 7.3 Hz), 5.12 (*d*, J = 7.4 Hz), and 4.97 (*d*, J = 7.2 Hz), respectively, as established by the HSQC experiment. The assignments of the C-atom signals of the sugar components (*Table 2*) were further determined from TOCSY, HSQC, and HMBC plots. The β-anomeric configurations for the D-glucuronic acid, D-fucose, and D-xylose units, and the α-anomeric configurations for the L-arabinose unit were determined by their large ^3J(1,2) coupling constants of 7–8 Hz and

Table 2. *^{13}C- and ^{1}H-NMR Data of the Sugar Moieties of* **1**–**3**

	1[a])		**2**[b])		**3**[a])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
3-*O*-Sugars:						
GlcA			GlcA		GlcA	
H–C(1)	103.4	4.87 (*d*, *J* = 7.3)	H–C(1)	102.3	4.67 (*d*, *J* = 7.5)	H–C(1)	102.9	4.82 (*d*, *J* = 7.6)
H–C(2)	78.1	4.22 (*dd*, *J* = 9.1, 7.5)	H–C(2)	77.9	4.20 (*dd*, *J* = 9.2, 7.5)	H–C(2)	78.2	4.30 (*dd*, *J* = 9.1, 7.5)
H–C(3)	84.5	4.25 (*t*, *J* = 9.1)	H–C(3)	82.9	4.14 (*t*, *J* = 9.2)	H–C(3)	85.8	4.22 (*t*, *J* = 9.1)
H–C(4)	70.8	4.37 (*t*, *J* = 9.3)	H–C(4)	75.9	4.09 (*t*, *J* = 9.5)	H–C(4)	72.5	3.99 (*t*, *J* = 9.3)
H–C(5)	77.8	4.45 (*d*, *J* = 9.3)	H–C(5)	73.4	4.01 (*d*, *J* = 9.7)	H–C(5)	77.2	4.15 (*d*, *J* = 9.3)
C(6)	171.5		C(6)	171.9		C(6)	171.5	
Ara			Gal			Gal		
H–C(1)	103.8	5.45 (*d*, *J* = 7.2)	H–C(1	103.4	5.27 (*d*, *J* = 7.4)	H–C(1)	103.9	5.47 (*d*, *J* = 7.2)
H–C(2)	72.1	4.51 (*dd*, *J* = 9.6, 7.7)	H–C(2)	72.4	3.91 (*dd*, *J* = 9.5, 2.7)	H–C(2)	73.0	4.42 (*dd*, *J* = 9.5, 2.7)
H–C(3)	74.1	4.34 (*dd*, *J* = 9.6, 3.2)	H–C(3)	73.0	4.11 (*dd*, *J* = 8.5, 3.3)	H–C(3)	75.5	4.12 (*dd*, *J* = 8.5, 3.3)
H–C(4)	70.0	4.11 (*d*, *J* = 3.2)	H–C(4)	78.5	4.18 (*d*, *J* = 3.3)	H–C(4)	70.1	4.53 (*d*, *J* = 3.3)
CH_2(5)	66.5	3.69 (*dd*, *J* = 11.5, 5.3), 4.21 (*d*, *J* = 5.3)	H–C(5)	75.8	3.73–3.77 (*m*)	H–C(5)	76.4	4.01–4.05 (*m*)
			CH_2(6)	62.1	4.01–4.05 (*m*), 4.08–4.12 (*m*)	CH_2(6)	61.3	4.39–4.41 (*m*), 4.48–4.52 (*m*)
Xyl			Xyl			Ara		
H–C(1)	104.5	5.23 (*d*, *J* = 7.3)	H–C(1)	104.9	5.00 (*d*, *J* = 7.3)	H–C(1)	104.3	5.31 (*d*, *J* = 7.1)
H–C(2)	75.5	3.90 (*dd*, *J* = 7.7, 8.3)	H–C(2)	75.1	3.77 (*dd*, *J* = 7.7, 8.3)	H–C(2)	75.5	3.90–3.94 (*m*)
H–C(3)	78.1	4.09 (*t*, *J* = 8.3)	H–C(3)	78.1	3.82 (*t*, *J* = 8.3)	H–C(3)	78.0	4.12 (*dd*, *J* = 9.5, 3.2)
H–C(4)	70.1	3.98–4.02 (*m*)	H–C(4)	71.2	3.90–3.94 (*m*)	H–C(4)	69.9	4.05–4.09 (*m*)
CH_2(5)	67.2	3.61 (*dd*, *J* = 12.0, 10.2), 4.20 (*dd*, *J* = 12.0, 5.4)	CH_2(5)	66.6	4.09 (*dd*, *J* = 12.0, 10.2), 4.17 (*dd*, *J* = 12.0, 5.4)	CH_2(5)	67.2	3.68 (br. *d*, *J* = 5.2), 4.23 (*dd*, *J* = 11.8, 5.2)
			Ara					
			H–C(1)	104.3	5.13 (*d*, *J* = 7.2)			
			H–C(2)	72.4	3.83–3.85 (*m*)			
			H–C(3)	74.4	3.70 (*dd*, *J* = 9.4, 3.4)			
			H–C(4)	69.9	3.91–3.95 (*m*)			
			CH_2(5)	65.7	3.74 (br. *d*, *J* = 5.1), 4.07 (*dd*, *J* = 11.4, 5.1)			

Table 2 (cont.)

	1[a])			**2**[b])			**3**[a])	
	δ(C)	δ(H)		δ(C)	δ(H)		δ(C)	δ(H)
28-*O*-Sugars:								
Fuc			Fuc			Fuc		
H−C(1)	94.2	6.08 (*d*, *J* = 8.1)	H−C(1)	94.1	5.83 (*d*, *J* = 8.2)	H−C(1)	94.3	5.94 (*d*, *J* = 7.8)
H−C(2)	73.9	4.45 (*t*, *J* = 8.2)	H−C(2)	74.4	4.34 (*t*, *J* = 8.2)	H−C(2)	73.6	4.58 (*t*, *J* = 8.2)
H−C(3)	74.1	4.66 (*dd*, *J* = 8.4, 2.7)	H−C(3)	76.0	4.14 (*dd*, *J* = 8.2, 4.0)	H−C(3)	76.6	4.17 (*dd*, *J* = 8.4, 2.7)
H−C(4)	74.4	5.68 (*d*, *J* = 3.0)	H−C(4)	83.6	3.94 (*d*, *J* = 4.0)	H−C(4)	73.1	3.95 (*d*, *J* = 3.0)
H−C(5)	70.1	4.27–4.31 (*m*)	H−C(5)	71.5	3.89–3.91 (*m*)	H−C(5)	72.2	4.08–4.10 (*m*)
Me(6)	17.9	1.79 (*d*, *J* = 6.3)	Me(6)	16.6	1.45 (*d*, *J* = 6.3)	Me(6)	16.8	1.50 (*d*, *J* = 6.3)
Rha			Rha			Rha		
H−C(1)	101.2	6.30 (br. *s*)	H−C(1)	101.6	5.97 (br. *s*)	H−C(1)	100.9	6.37 (br. *s*)
H−C(2)	71.7	4.60 (br. *s*)	H−C(2)	71.7	4.53 (br. *s*)	H−C(2)	71.5	4.83 (br. *s*)
H−C(3)	70.0	4.78 (*dd*, *J* = 8.8, 2.3)	H−C(3)	72.1	4.43 (*dd*, *J* = 8.8, 2.3)	H−C(3)	72.7	4.60 (*dd*, *J* = 8.8, 2.3)
H−C(4)	84.9	4.24 (*t*, *J* = 8.8)	H−C(4)	84.4	4.09 (*t*, *J* = 8.8)	H−C(4)	85.4	4.28 (*t*, *J* = 8.8)
H−C(5)	68.8	4.40 (*dd*, *J* = 8.8, 6.1)	H−C(5)	68.3	4.24 (*dd*, *J* = 8.8, 6.1)	H−C(5)	67.9	4.40 (*dd*, *J* = 8.8, 6.1)
Me(6)	18.2	1.69 (*d*, *J* = 6.1)	Me(6)	18.7	1.54 (*d*, *J* = 6.1)	Me(6)	18.8	1.61 (*d*, *J* = 6.1)
Xyl′			Qui			Xyl		
H−C(1)	105.5	5.12 (*d*, *J* = 7.4)	H−C(1)	106.1	4.82 (*d*, *J* = 7.6)	H−C(1)	106.3	4.97 (*d*, *J* = 7.4)
H−C(2)	71.8	4.54 (*dd*, *J* = 8.6, 7.4)	H−C(2)	75.0	3.80–3.84 (*m*)	H−C(2)	71.9	3.94 (*dd*, *J* = 8.9, 7.4)
H−C(3)	72.6	4.39 (*dd*, *J* = 8.6, 7.1)	H−C(3)	76.1	3.42 (*dd*, *J* = 10.2, 8.5)	H−C(3)	72.4	3.94–3.96 (*m*)
H−C(4)	71.6	4.30–4.34 (*m*)	H−C(4)	74.2	3.78 (*dd*, *J* = 10.2, 8.5)	H−C(4)	71.1	3.98–4.01 (*m*)
CH_2(5)	66.7	3.73 (*dd*, *J* = 11.5, 10.3), 4.30 (*dd*, *J* = 11.5, 5.3)	H−C(5)	72.6	3.55 (*dd*, *J* = 11.3, 5.3),	CH_2(5)	66.9	3.45 (*dd*, *J* = 11.1, 10.1), 4.14 (*dd*, *J* = 11.1, 5.4)
			Me(6)	18.3	1.44 (*t*, *J* = 11.2)			
Xyl″			Xyl′			Gal′		
H−C(1)	107.1	4.97 (*d*, *J* = 7.2)	H−C(1)	104.3	4.97 (*d*, *J* = 7.3)	H−C(1)	105.8	5.10 (*d*, *J* = 7.3)
H−C(2)	71.8	3.92 (*dd*, *J* = 8.9, 7.2)	H−C(2)	72.5	4.07 (*dd*, *J* = 8.5, 7.3)	H−C(2)	73.3	4.44 (*dd*, *J* = 8.6, 7.1)
H−C(3)	72.6	3.95 (*t*, *J* = 8.9)	H−C(3)	85.5	4.13 (*t*, *J* = 8.5)	H−C(3)	75.8	4.00 (*dd*, *J* = 8.5, 3.0)
H−C(4)	71.6	3.98–4.03 (*m*)	H−C(4)	70.7	4.13–4.17 (*m*)	H−C(4)	70.6	4.20–4.22 (*m*)
CH_2(5)	67.2	3.45 (*t*, *J* = 11.1), 4.14 (*dd*, *J* = 11.1, 5.4)	CH_2(5)	66.7	3.74 (*dd*, *J* = 11.5, 10.0), 4.34 (*dd*, *J* = 11.5, 5.1)	H−C(5)	77.2	3.98 (*dd*, *J* = 11.5, 5.3)
						CH_2(6)	61.4	4.38–4.40 (*m*), 4.45–4.49 (*m*)

Table 2 (cont.)

	1[a])			**2**[b])		**3**[a])	
	δ(C)	δ(H)		δ(C)	δ(H)	δ(C)	δ(H)
(*E*)-MC			Xyl″				
C(1)	126.8		H–C(1)	106.7	4.77 (*d*, *J* = 7.4)		
H–C(2,6)	130.0	7.39 (*d*, *J* = 9.0)	H–C(2)	76.0	4.04 (*dd*, *J* = 8.9, 7.4)		
H–C(3,5)	114.5	6.96 (*d*, *J* = 9.0)	H–C(3)	72.1	4.05–4.07 (*m*)		
C(4)	161.2		H–C(4)	70.1	4.17–4.20 (*m*)		
H–C(α)	116.5	6.45 (*d*, *J* = 15.7)	CH_2(5)	66.5	3.70 (*t*, *J* = 11.5), 4.18 (*dd*, *J* = 11.5, 5.3)		
H–C(β)	145.1	7.84 (*d*, *J* = 15.7)					
C=O	167.2						
MeO	55.0	3.62 (*s*)					

[a]) Measured in (D_5)pyridine/(D_6)DMSO 10 : 1. [b]) Measured in (D_5)pyridine/D_2O 10 : 1.

^{13}C-NMR data [12]. The α-anomeric configuration of the L-rhamnose unit was deduced from the chemical shift of its C(5) at δ 68.8 [13][14]. The correlations between H–C(1) of glucuronic acid (δ(H) 4.87) and C(3) (δ(C) 84.2) of gypsogenin, as well as the correlation between H–C(1) of fucose (δ(H) 6.08) and C(28) (δ(C) 176.2) of gypsogenin in the HMBC spectrum, indicated that the two sugar chains were attached to C(3) and C(28) of the aglycone. The linkages of the other monosaccharides to glucuronic acid and fucose were established from the related HMBCs (*Fig. 1*). Besides those of the sugars and the aglycone, there were other signals in the ^{1}H-and ^{13}C-NMR spectra of **1**, which suggested the presence of an (*E*)-4-methoxycinnamoyl ((*E*)-MC = (2*E*)-3-(4-methoxyphenyl)-1-oxoprop-2-en-1-yl) group at δ(H) 7.84 and 6.45 (*d*, *J* = 15.7 Hz, 1 H each, H–C(β) and H–C(α)), and 7.39 and 6.96 (*d*, *J* = 9.0 Hz, 2 H each, H–C(2,6) and H–C(3,5)). The downfield signal of Fuc H–C(4) at δ(H) 5.68 gave a cross-peak with the signal of the C=O C-atom of the (*E*)-MC group at δ(C) 167.2 in the HMBC spectrum which revealed the location of the substituent group (*Fig. 1,b*). According to the above analysis, the structure of **1** was elucidated as 3-{{*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranuronosyl}oxy}-gypsogenin 28-{*O*-β-D-xylopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(*E*)-4-methoxycinnamoyl]-β-D-fucopyranosyl} ester.

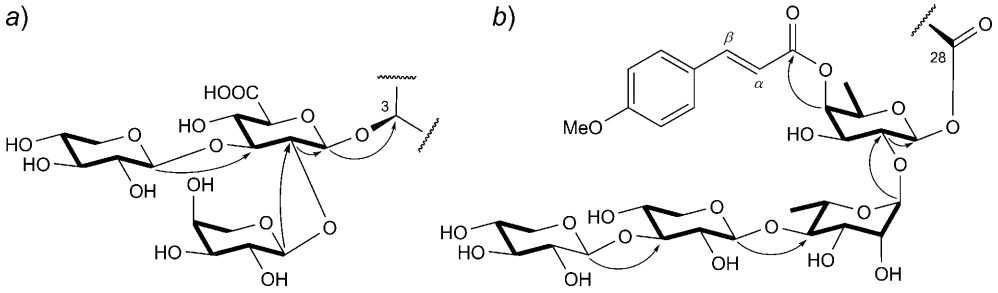

Fig. 1. *TOCSY* (—) *and HMBCs* (H → C) *of the sugar moieties of* **1** *at* a) *C(3) and* b) *C(28)*

Compound **2** was isolated as a white amorphous solid with the molecular formula $C_{80}H_{126}O_{44}$, as determined from data of the negative-ion-mode HR-ESI-MS. Acid hydrolysis of **2** with 2M HCl afforded quillaic acid (=(3β,4α,16α)-3,16-dihydroxy-23-oxoolean-12-en-28-oic acid) identified by TLC comparison with an authentic sample, together with D-fucose, D-galactose, D-xylose, D-glucuronic acid, L-rhamnose, D-quinovose, and L-arabinose (1 : 1 : 3 : 1 : 1 : 1 : 1) based on GC-MS analysis. The aglycone was further determined to be quillaic acid by comparison of its ^{1}H- and ^{13}C-NMR data (*Table 1*) with reported data [10][15]. The assignments of the signals of the sugar moieties (*Table 2*) were accomplished by a combination of TOCSY, HSQC, and HMBC experiments. The tetrasaccharide moiety attached to C(3) was established by the following HMBCs: H–C(1) of galactose (δ(H) 5.27) with C(2) of glucuronic acid (δ(C) 77.9), H–C(1) of arabinose (δ(H) 5.13) with C(3) of glucuronic acid (δ(C) 82.9), and H–C(1) of xylose (δ(H) 5.00) with C(4) of galactose (δ(C) 78.5). The NMR data of another sugar chain attached to C(28) of the aglycone were similar to those of saponarioside B [15]. The pentasaccharide moiety attached to C(28) was established by the following HMBC correlations: H–C(1) of xylose″ (δ(H) 4.77) with C(3) of xylose′

(δ(C) 85.5), H–C(1) of xylose″ (δ(H) 4.97) with C(4) of rhamnose (δ(C) 84.4), H–C(1) of rhamnose (δ(H) 5.97) with C(2) of fucose (δ(C) 74.4), H–C(1) of quinovose (δ(H) 4.82) with C(4) of fucose (δ(C) 83.6), and H–C(1) of fucose (δ(H) 5.83) with C(28) of the aglycone (δ(C) 175.6). The β-anomeric configurations of the D-fucose, D-galactose, D-xylose, D-glucuronic acid, D-quinovose (=6-deoxy-D-glucose) together with the α-anomeric configuration of the L-arabinose ($^4C_1$ configuration) unit were determined from their ^3J(1,2) coupling constants (7–8 Hz). The ^{13}C-NMR data also revealed the α-configuration of L-rhamnose. Thus, the structure of **2** was determined to be 3-{{*O*-β-D-xylopyranosyl-(1→4)-β-D-galactopyranosyl-(1→2)-*O*-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranuronosyl}oxy}quillaic acid 28-{*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranosyl} ester.

Compound **3** displayed a quasimolecular-ion peak $[M+Na]^+$ at *m*/*z* 1565.6659 in the positive-ion-mode HR-ESI-MS, in accordance with the molecular formula $C_{70}H_{110}O_{37}$. Acid hydrolysis of **3** with 2M HCl afforded quillaic acid and the monosaccharides D-fucose, L-rhamnose, D-xylose, D-galactose, L-arabinose, and D-glucuronic acid (1:1:1:2:1:1) as determined by co-TLC and GC/MS analysis. The downfield shift of C(3) (δ(C) 83.8) and upfield shift of C(28) (δ(C) 175.9) revealed that the aglycone of **3** was linked to two sugar chains at C(3) and C(28). The anomeric-H-atom signals at δ(H) 6.37 (br. *s*), 5.94 (*d*, *J* = 7.8 Hz), 5.47 (*d*, *J* = 7.2 Hz), 5.31 (*d*, *J* = 7.1 Hz), 5.10 (*d*, *J* = 7.3 Hz), 4.97 (*d*, *J* = 7.4 Hz), and 4.82 (*d*, *J* = 7.6 Hz) correlated with C-atom signals at δ(C) 100.9, 94.3, 103.9, 104.3, 105.8, 106.3, and 102.9 in the HSQC spectrum, respectively. The ^{1}H- and ^{13}C-NMR chemical-shift assignments (*Table 2*) were accomplished by a combination of TOCSY, HSQC, and HMBC experiments. The interglycosidic linkages of the sugar units at C(3) of the aglycone were established from the following HMBCs: H–C(1) of galactose (δ(H) 5.47) with C(2) of glucuronic acid (δ(C) 78.2) and H–C(1) of arabinose (δ(H) 5.31) with C(3) of glucuronic acid (δ(C) 85.8). Similarly, the sugar chain at C(28) was established from the following HMBCs: H–C(1) of rhamnose (δ(H) 6.37) with C(2) of fucose (δ(C) 73.6), H–C(1) of xylose (δ(H) 4.97) with C(4) of rhamnose (δ(C) 85.4), and H–C(1) of galactose (δ(H) 5.10) with C(3) of xylose (δ(C) 72.4). From the above analysis, the structure of **3** was elucidated as 3-{{*O*-α-L-arabinopyranosyl-(1→3)-*O*-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranuronosyl}oxy}quillaic acid 28-{*O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl} ester.

Compounds **4** and **6** had the same molecular formula $C_{47}H_{72}O_{20}$, as determined from their quasimolecular-ion peaks at *m*/*z* 955.4512 ($[M-H]^-$) and 979.4509 ($[M+Na]^+$), respectively, in the HR-ESI-MS. By acid hydrolysis with 2M HCl, both of them afforded an identical aglycone identified as quillaic acid by TLC comparison with an authentic sample, which was confirmed by the ^{1}H- and ^{13}C-NMR data (*Tables 1* and *3*), HMQC, and HMBC spectra. The sugars obtained from **4** were identified as D-xylose, D-galactose, and D-glucose (1:1:1) based on GC/MS analysis of their thiazolidine derivatives, while **6** afforded D-xylose, D-glucuronic acid, and D-glucose (1:1:1) determined by the same method. The downfield chemical shifts of C(3) of the aglycone and HMBCs revealed that both of them were monodesmosidic glycosides. The trisaccharide moiety of **4** attached to C(3) was established by the following HMBCs: H–C(1) of galactose (δ(H) 5.35) with C(2) of glucose (δ(C) 78.0), H–C(1) of xylose

Table 3. *^{13}C- and ^{1}H-NMR Data of the Sugar Moieties of* **4**–**7**

	4[b])		**5**[a])		**6**[a])		**7**[b])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
Glc			GlcA		GlcA		GlcA	
H–C(1)	103.0	4.65 (*d*, *J* = 7.5)	H–C(1)	102.7	4.67 (*d*, *J* = 7.1)	103.2	4.68 (*d*, *J* = 7.1)	102.6	4.77 (*d*, *J* = 7.5)
H–C(2)	78.0	4.20 (*dd*, *J* = 8.8, 7.3)	H–C(2)	79.9	4.13 (*dd*, *J* = 9.1, 7.5)	78.0	4.41 (*dd*, *J* = 9.2, 7.5)	79.0	4.23 (*dd*, *J* = 9.1, 7.5)
H–C(3)	85.9	4.03–4.05 (*m*)	H–C(3)	76.4	4.07 (*t*, *J* = 9.1)	85.9	4.05 (*t*, *J* = 9.2)	74.9	4.20 (*t*, *J* = 9.1)
H–C(4)	72.6	4.14 (*t*, *J* = 9.5)	H–C(4)	82.5	4.26 (*t*, *J* = 9.3)	72.6	4.14 (*t*, *J* = 9.5)	81.0	4.39 (*t*, *J* = 9.3)
H–C(5)	77.5	3.91–3.97 (*m*)	H–C(5)	77.3	4.17 (*d*, *J* = 9.3)	77.5	3.97 (*d*, *J* = 9.7)	77.0	4.35 (*d*, *J* = 9.3)
CH_2(6)	62.1	4.30–4.36 (*m*), 4.36–4.39 (*m*)	C(6)	171.5		C(6)	171.1	C(6)	171.8	
Gal			Gal			Glc		Gal	
H–C(1)	104.4	5.35 (*d*, *J* = 7.4)	H–C(1)	104.8	5.19 (*d*, *J* = 7.5)	H–C(1)	104.4	5.20 (*d*, *J* = 7.5)	H–C(1)	103.8	5.11 (*d*, *J* = 7.6)
H–C(2)	73.7	4.38 (*dd*, *J* = 9.5, 7.4)	H–C(2)	73.3	4.32 (*dd*, *J* = 9.3, 7.5)	H–C(2)	73.7	4.38 (*dd*, *J* = 8.5, 7.5)	H–C(2)	73.7	4.27 (*dd*, *J* = 9.4, 7.2)
H–C(3)	75.6	4.00 (*dd*, *J* = 9.5, 3.4)	H–C(3)	75.2	3.98 (*dd*, *J* = 9.3, 3.3)	H–C(3)	75.6	4.11 (*dd*, *J* = 8.5, 8.0)	H–C(3)	75.6	4.03 (*dd*, *J* = 8.5, 3.5)
H–C(4)	68.9	4.24 (*d*, *J* = 3.4)	H–C(4)	69.4	4.42 (br. *d*, *J* = 3.3)	H–C(4)	68.9	4.11–4.15 (*m*)	H–C(4)	69.0	4.48 (br. *d*, *J* = 3.4)
H–C(5)	76.8	3.97–3.99 (*m*)	H–C(5)	76.6	4.02–4.06 (*m*)	H–C(5)	76.8	3.94–3.97 (*m*)	H–C(5)	76.6	4.00–4.04 (*m*)
CH_2(6)	61.9	4.17–4.23 (*m*), 4.39–4.40 (*m*)	CH_2(6)	61.9	4.29–4.31 (*m*), 4.41–4.46 (*m*)	CH_2(6)	61.9	4.14–4.18 (*m*), 4.36–4.40 (*m*)	CH_2(6)	61.3	4.40–4.43 (*m*), 4.48–4.52 (*m*)
Xyl			Gal′			Xyl		Glc		
H–C(1)	103.7	5.15 (*d*, *J* = 7.7)	H–C(1)	104.0	5.02 (*d*, *J* = 7.6)	H–C(1)	103.7	5.31 (*d*, *J* = 7.6)	H–C(1)	104.5	5.23 (*d*, *J* = 7.6)
H–C(2)	75.0	3.83 (*dd*, *J* = 7.7, 8.3)	H–C(2)	73.0	4.18 (*dd*, *J* = 8.5, 7.6)	H–C(2)	75.0	3.79 (*dd*, *J* = 7.6, 8.3)	H–C(2)	72.7	4.48 (*dd*, *J* = 8.8, 7.6)
H–C(3)	78.5	4.05 (*t*, *J* = 8.3)	H–C(3)	77.8	3.78 (*dd*, *J* = 8.5, 3.3)	H–C(3)	78.5	4.00–4.02 (*m*)	H–C(3)	74.5	4.06–4.09 (*m*)
H–C(4)	70.2	3.93–3.97 (*m*)	H–C(4)	69.1	4.15 (br. *d*, *J* = 3.3)	H–C(4)	70.2	3.89–3.93 (*m*)	H–C(4)	70.2	4.36–4.39 (*m*)
CH_2(5)	67.2	3.55 (*dd*, *J* = 12.0, 10.2), 4.34 (*dd*, *J* = 12.0, 5.4)	H–C(5)	74.2	3.86–3.89 (*m*)	CH_2(5)	67.2	3.58 (*dd*, *J* = 11.2, 10.1), 4.28 (*dd*, *J* = 11.2, 5.4)	H–C(5)	77.2	3.97–4.01 (*m*)
			CH_2(6)	62.0	4.27–4.29 (*m*), 4.35–4.39 (*m*)				CH_2(6)	62.0	4.10 (*dd*, *J* = 11.5, 4.2), 4.42 (br. *d*, *J* = 11.5)
MeO	51.2	3.51 (*s*)									

[a]) Measured in (D_5)pyridine/(D_6)DMSO 10 : 1. [b]) Measured in (D_5)pyridine/D_2O 10 : 1.

(δ(H) 5.15) with C(3) of glucose (δ(C) 85.9), and H–C(1) of glucose (δ(H) 4.65) with C(3) of the aglycone (δ(C) 84.2). The trisaccharide moiety of **6** attached to C(3) was elucidated as *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranuronosyl by a HMBC experiment too. For compound **4**, the HMBC between C(28) (δ(C) 179.2) and the H-atoms of a MeO group (δ(H) 3.51; *Table 3*) suggested that the carboxy group was esterified. To exclude the possibility of **4** being an artifact formed during the isolation procedure, the EtOH extracts of the plant roots were analyzed by HPLC-ESI-MS^n. A corresponding peak with identical MS^n pattern of the same retention time was detected in the crude extract. On the basis of the above results, compounds **4** and **6** were elucidated as 3-{{*O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl}oxy}quillaic acid methyl ester (**4**), and 3-{{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranuronosyl}oxy}quillaic acid (**6**), respectively.

Compound **5** also had the same molecular formula ($C_{47}H_{72}O_{20}$) as **4** and **6**, in agreement with the quasimolecular-ion peak at *m*/*z* 955.4483 ([*M* – H]$^-$) in the HR-ESI-MS. On acid hydrolysis, compound **5** afforded D-galactose and D-glucuronic acid (2 : 1). Besides the characteristic signals of a CHO group at δ(C) 209.7 and δ(H) 9.76, there was another C=O signal at δ(C) 199.4 (*Table 1*). Two olefinic C-atoms at δ(C) 129.6 and 156.8 showed no correlation with any H-atoms in the HSQC spectrum. This, together with the C=O at δ(C) 199.4, suggested the existence of an α,β-unsaturated C=O moiety in the aglycone skeleton. The above NMR characteristics were similar to those of villosagenin II (=(3β,4α)-3-hydroxy-16-oxo-28-norolean-17-en-23-al) described in an earlier report [16]. Moreover, the five *s* Me groups and an additional CH_2OH group at δ(C) 69.0 and δ(H) 3.53 suggested the location of an OH group at C(29), which was further confirmed by HMBC and ROESY data [17]. The α- and β-orientations of the H-atoms in the aglycone were determined by the coupling patterns and the ROESY cross-peaks (*Fig. 2*). So the uncommon triterpene aglycone of compound **5** was determined as (3β,4α,20α)-3,29-dihydroxy-16-oxo-28-norolean-17-en-23-al. Then, the sequence and interglycosidic linkage of the sugar chain, composed of three monosaccharide units at C(3), was revealed as *O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-galactopyranosyl-(1 → 4)]-β-D-glucopyranuronosyl by means of 2D-NMR data. Therefore, compound **5** was finally elucidated as (3β,4α,20α)-3,29-dihydroxy-16,23-dioxo-28-norolean-17-en-3-yl *O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-galactopyranosyl-(1 → 4)]-β-D-glucopyranuronosidic acid.

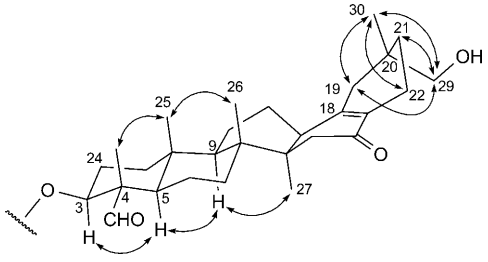

Fig. 2. *Key ROESY* (H ↔ H) *correlations of the aglycone of* **5**

Compound **7** displayed a quasimolecular-ion peak [*M* – H]$^-$ at *m*/*z* 985.4650 in the HR-ESI-MS, in accordance with the empirical formula $C_{48}H_{74}O_{21}$. GC/MS Analysis of

the acid hydrolysate demonstrated that compound **7** contained D-glucuronic acid, D-galactose, and D-glucose (1:1:1). The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *3*) of **7** were assigned from TOCSY, HSQC, and HMBC experiments The interglycosidic connectivities were determined from the observed HMBC cross-peaks from H–C(1) of galactose at δ(H) 5.11 to C(4) of glucuronic acid at δ(C) 81.0 and from H–C(1) of glucose at δ(H) 5.23 to C(2) of glucuronic acid at δ(C) 79.0. Thus, compound **7** was determined as 3-{{*O*-β-D-galactopyranosyl-(1 → 4)-*O*-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranuronosyl}oxy}quillaic acid.

α-Glucosidase catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the absorption of dietary carbohydrates and thus suppress postprandial hyperglycemia, which could be useful in the treatment of diabetes [18]. Since some triterpenoids have been reported to exert antidiabetes activities [19], all isolated saponins **1**–**7** were screened as α-glucosidase inhibitors together with the two aglycones, quillaic acid and gypsogenin. As shown in *Table 4*, compound **1** and its aglycone had more potent α-glucosidase inhibiting activity than acarbose (IC_{50} 398.1 μM), which was used as the positive control. Compounds **2**–**7** were found to be almost inactive in this test ($IC_{50} > 2000$ μM). It seems that the methoxycinnamoyl group at the sugar chain of **1** played a key role in enzyme inhibition. The above results were basically consistent with those in our earlier study [9], which suggested that 28-*O*-monodesmosides and 3,28-*O*-bidesmosides did not possess obvious α-glucosidase inhibitory activity as compared with the corresponding aglycone. More saponins with a 4-methoxycinnamoyl group need to be tested to establish further structure–activity relationships.

Table 4. *α-Glucosidase Inhibition by Compounds* **1**–**7** *and Their Aglycones*

	IC_{50} [μM][a])		IC_{50} [μM][a])
1	100.9 ± 3.3	**6**	not active
2	not active	**7**	not active
3	not active	Quillaic acid	133.7 ± 2.3
4	not active	Gypsogenin	212.8 ± 1.5
5	not active	Acarbose[b])	398.1 ± 9.6

[a]) IC_{50} is defined as the concentration that resulted in a 50% α-glucosidase inhibition, and the results are means ± the standard deviation of three independent replicates. An $IC_{50} > 400$ μM was considered to be not active. [b]) Positive control substance.

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Experimental Part

General. TLC: precoated silica gel *60* F_{254} (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*) and detection by 10% H_2SO_4/EtOH (saponins) and aniline/phthalate (sugars) reagents. Column chromatography (CC): SiO_2 *H* (*Qingdao Haiyang Chemical Co., Ltd.*), *Sephadex LH-20* (*Pharmacia*), and *RP-*C_{18} (40–63 μm; *FuJi*). HPLC: *Agilent-1100* series instrument equipped with a *Shim-Pack-RP-*C_{18} column (200 × 20 mm i.d., 7 μm). Optical rotations: *Jasco-P-1020* polarimeter. UV Spectra: *Shimadzu-UV-2501-PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Impact-410* spectrometer; KBr discs; $\tilde{\nu}$ in

cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-ACF-500* (300 (^{1}H) and 125 (^{13}C) MHz) NMR instrument; at 300 K; δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS: *Agilent-1100* series LC/MSD trap mass spectrometer (HPLC-ESI-MS^n), a *Waters-Micro Q-TOF* spectrometer (HR-ESI-MS), and an *Ionspec*-4.7-Tesla-*Ultima*-FT mass spectrometer (HR-MALDI-MS), resp.; in m/z (rel. %). GC/MS: *Varian-CP-3800* gas chromatograph equipped with a *Saturn-2200* mass detector; *CP-sil-5-CB* capillary column (30 m, 0.25 mm i.d., 0.25 μm); in m/z (rel. %).

Plant Material. The roots of *G. paniculata* were collected in suburbs of Kunming, Yunnan Province, China, in October 2005, and identified by Prof. *Mian Zhang* of the Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 051022) was deposited with the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The air-dried roots (20 kg) of *G. paniculata* were extracted with 75% EtOH (3×40 l for 2 h) under reflux. After evaporation of the solvent, the residue was suspended in H_2O (5 l) and partitioned with BuOH (5×5 l). The BuOH-soluble portion (518 g) was fractionated by CC (SiO_2 (100–200 mesh), $CHCl_3$/MeOH/H_2O 100:10:0 → 100:100:1): *Fractions 1–10*. *Fr. 5* was separated by low-pressure CC (*RP-18*, MeOH/H_2O 4:6) and further purified by HPLC (MeOH/0.02% CF_3COOH in H_2O 75:25, UV detection at 210 nm): pure **1** (7 mg; t_R 25.1 min). *Fr. 8* was purified by medium-pressure CC (*RP-18*, MeOH/H_2O 3.5:6.5) and then subjected to HPLC (MeOH/0.05% CF_3COOH in H_2O 65:35): **2** (23 mg; t_R 12.4 min) and **3** (10 mg; t_R 16.8 min). *Frs. 1–3* were purified repeatedly by CC (SiO_2, gradient $CHCl_3$/MeOH/H_2O 100:10:0 → 100:30:1) and CC (*Sephadex LH-20*, MeOH/H_2O 8:2): pure **4** (12 mg), **5** (8 mg), **6** (9 mg), and **7** (11 mg).

*(3β,4α)-3-{[*O*-α-*L*-Arabinopyranosyl-(1 → 2)*-O*-[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucopyranuronosyl]oxy}-23-oxoolean-12-en-28-oic Acid 28-{*O*-β-*D*-Xylopyranosyl-(1 → 3)*-O*-β-*D*-xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O*-4-O-[(2*E*)-3-(4-methoxyphenyl-1-oxoprop-2-en-1-yl]-6-deoxy-β-*D*-galactopyranosyl} Ester* (**1**): White powder. Grey spot on TLC by spraying with *Komarowsky* reagent. $[\alpha]_D^{25} = +55.6$ ($c = 0.04$, pyridine). UV (MeOH): 311 (4.73), 291 (sh, 4.69), 230 (4.43). IR: 3443 (OH), 1751, 1719, 1675, 1636, 1075. ^{1}H-NMR (500 MHz, (D_5)pyridine/(D_6)DMSO 10:1): 0.69, 0.78, 0.87, 0.88, 0.94, 1.31 (6 *s*, Me(25), Me(26), Me(29), Me(30), Me(27), Me(24)); 4.25 (*m*, H–C(3)); 5.31 (*t*, J = 3.3, H–C(12)); 9.83 (*s*, H–C(23)); 7.39 (*d*, $J = 9.0$, 2 H, H–C(2,6) of MC); 6.96 (*d*, $J = 9.0$, 2 H, H–C(3,5) of MC); 6.45 (*d*, $J = 15.7$, H–C(α) of MC); 7.84 (*d*, $J = 15.7$, H–C(β) of MC); 3.62 (*s*, MeO of MC). ^{13}C-NMR: for the aglycone, see *Table 1*. ^{1}H- and ^{13}C-NMR: for the glycosidic part, see *Table 2*. ESI-MS (neg.): 1625 ($[M-H]^-$). HR-MALDI-MS: 1649.7015 ($[M+Na]^+$, $C_{78}H_{114}NaO_{36}^+$; calc. 1649.6987).

*(3β,4α,16α)-16-Hydroxy-23-oxo-3-{{*O*-β-*D*-xylopyranosyl-(1 → 4)-β-*D*-galactopyranosyl-(1 → 2)-O-[α-*L*-arabinopyranosyl-(1 → 3)]-β-*D*-glucopyranuronosyl}oxy}olean-12-en-28-oic Acid 28-{*O*-β-*D*-Xylopyranosyl-(1 → 3)-O-β-*D*-xylopyranosyl-(1 → 4)-O-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-O-[6-deoxy-β-*D*-glucopyranosyl-(1 → 4)]-6-deoxy-β-*D*-galactopyranosyl} Ester* (**2**): White powder. $[\alpha]_D^{25} = +4.7$ (c = 0.12, pyridine). IR: 3442 (OH), 1731, 1638, 1058. ^{1}H-NMR (500 MHz, (D_5)pyridine): 0.79, 0.82, 0.85, 0.86, 1.27, 1.45 (6 *s*, Me(29), Me(30), Me(25), Me(26), Me(27), Me(24)); 4.31 (*m*, H–C(3)); 5.15 (br. *s*, H–C(16)); 5.42 (*t*-like, H–C(12)); 9.72 (*s*, H–C(23)). ^{13}C-NMR: for the aglycone, see *Table 1*. ^{1}H- and ^{13}C-NMR: for the glycosidic part, see *Table 2*. ESI-MS (neg.): 1789 ($[M-H]^-$). HR-ESI-MS: 1789.7574 ($[M-H]^-$, $C_{80}H_{125}O_{44}^-$; calc. 1789.7544).

*(3β,4α,16α)-3-{{*O*-α-*L*-Arabinopyranosyl-(1 → 3)-O-[β-*D*-galactopyranosyl-(1 → 2)]-β-*D*-glucopyranuronosy]oxy}-16-hydroxy-23-oxoolean-12-en-28-oic Acid 28-{*O*-β-*D*-Galactopyranosyl-(1 → 3)-O-β-*D*-xylopyranosyl-(1 → 4)-O-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-6-deoxy-β-*D*-galactopyranosyl} Ester* (**3**): White powder. $[\alpha]_D^{25} = -24.6$ ($c = 0.13$, pyridine). IR: 3422 (OH), 1731, 1634, 1057. ^{1}H-NMR (500 MHz, (D_5)pyridine): 0.78, 0.88, 0.91, 0.96, 1.29, 1.47 (6 *s*, Me(25), Me(29), Me(30), Me(26), Me(27), Me(24)); 4.34 (*m*, H–C(3)); 5.53 (*t*-like, H–C(12)); 5.21 (br. *s*, H–C(16)); 9.78 (*s*, H–C(23)). ^{13}C-NMR: for the aglycone, see *Table 1*. ^{1}H- and ^{13}C-NMR: for the glycosidic part, see *Table 2*. ESI-MS (neg.): 1541 ($[M-H]^-$). HR-ESI-MS: 1565.6659 ($[M+Na]^+$, $C_{70}H_{110}NaO_{37}^+$; calc. 1665.6624).

*(3β,4α,16α)-3-{{*O*-β-*D*-Galactopyranosyl-(1 → 2)-O-[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucopyranosyl}oxy}-16-hydroxy-23-oxoolean-12-en-28-oic Acid Methyl Ester* (**4**): White powder. $[\alpha]_D^{25} = -11.0$ (c = 0.12, pyridine). IR: 3421, 1717, 1622, 1077. ^{1}H-NMR (500 MHz, (D_5)pyridine/D_2O 10:1): 0.74, 0.87, 0.91, 1.10, 1.29, 1.45 (6 *s*, Me(25), Me(29), Me(30), Me(26), Me(27), Me(24)); 4.09 (*m*, H–C(3)); 5.47 (*t*-like,

H–C(12)); 5.11 (br. *s*, H–C(16)); 9.78 (*s*, H–C(23)). ^{13}C-NMR: for the aglycone, see *Table 1*. ^{1}H- and ^{13}C-NMR: for the glycosidic part, see *Table 3*. ESI-MS (neg.): 955 ($[M-H]^-$). HR-ESI-MS: 955.4512 ($[M-H]^-$, $C_{47}H_{71}O_{20}^-$; calc. 955.4539).

(3β,4α,20α)-3,29-Dihydroxy-16,23-dioxo-28-norolean-17-en-3-yl O-*β*-D-*Galactopyranosyl-(1 → 2)*-O-*[β*-D-*galactopyranosyl-(1 → 4)]-β*-D-*glucopyranuronosidic Acid* (**5**): White powder. $[\alpha]_D^{25} = -0.3$ ($c = 0.07$, pyridine). UV (MeOH): 255 (3.6). IR: 3422, 1729, 1636, 1058. ^{1}H-NMR (500 MHz, (D_5)pyridine/D_2O 10 : 1): 0.65, 0.78, 0.90, 1.09, 1.33 (5 *s*, Me(26), Me(30), Me(25), Me(27), Me(24)); 3.53 (br. *s*, 2 H–C(29)); 4.11 (*m*, H–C(3)); 9.76 (*s*, H–C(23)). ^{13}C-NMR: for the aglycone, see *Table 1*. ^{1}H- and ^{13}C-NMR: for the glycosidic part, see *Table 3*. ESI-MS (neg.): 955 ($[M-H]^-$). HR-ESI-MS: 955.4483 ($[M-H]^-$, $C_{47}H_{71}O_{20}^-$; calc. 955.4539).

*(3β,4α,16α)-3-{[*O-*β*-D-*Glucopyranosyl-(1 → 2)*-O-*[β*-D-*xylopyranosyl-(1 → 3)]-β*-D-*glucopyranuronosyl]oxy}-16-hydroxy-23-oxoolean-12-en-28-oic Acid* (**6**): White powder. $[\alpha]_D^{25} = -6.0$ ($c = 0.16$, pyridine). IR: 3444, 1731, 1637, 1058. ^{1}H-NMR (500 MHz, (D_5)pyridine): 0.70, 0.86, 0.97, 1.10, 1.21, 1.37 (6 *s*, Me(25), Me(29), Me(30), Me(26), Me(27), Me(24)); 4.11 (*m*, H–C(3)); 5.47 (*t*-like, H–C(12)); 5.05 (br. *s*, H–C(16)); 9.78 (*s*, H–C(23)). ^{13}C-NMR: for the aglycone, see *Table 1*. ^{1}H- and ^{13}C-NMR: for the glycosidic part, see *Table 3*. ESI-MS (neg.): 955 ($[M-H]^-$). HR-ESI-MS: 979.4491 ($[M+Na]^+$, $C_{47}H_{72}NaO_{20}^+$; calc. 979.4515).

*(3β,4α,16α)-3-{[*O-*β*-D-*Galactopyranosyl-(1 → 4)*-O-*[β*-D-*glucopyranosyl-(1 → 2)]-β*-D-*glucopyranuronosyl]oxy}-16-hydroxy-23-oxoolean-12-en-28-oic Acid* (**7**): White powder. $[\alpha]_D^{25} = -0.4$ ($c = 0.11$, pyridine). IR: 3423, 1715, 1630, 1075. ^{1}H-NMR (500 MHz, (D_5)pyridine/D_2O 10 : 1): 0.66, 0.79, 0.91, 1.03, 1.27, 1.45 (6 *s*, Me(25), Me(29), Me(30), Me(26), Me(27), Me(24)); 4.52 (*m*, H–C(3)); 5.21 (br. *s*, H–C(16)); 5.36 (*t*-like, H–C(12)); 9.65 (*s*, H–C(23)). ^{13}C-NMR: for the aglycone, see *Table 1*. ^{1}H- and ^{13}C-NMR: for the glycosidic part, see *Table 3*. ESI-MS (neg.): 985 ($[M-H]^-$). HR-ESI-MS: 985.4650 ($[M-H]^-$, $C_{48}H_{73}O_{21}^-$; calc. 985.4644).

Acid Hydrolysis and GC/MS Analysis. Each compound was heated in 2M HCl (5 ml) at 90° for 4 h. The mixture was then extracted with AcOEt (3 × 5 ml) and the AcOEt extract purified by CC (*Sephadex LH-20* (2.0 × 100 cm), MeOH). After co-TLC comparison with authentic samples, the agylcones of compounds **2**–**4**, **6**, and **7** were determined to be quillaic acid (R_f 0.25, $CHCl_3$/MeOH 20 : 1), while that of **1** was gypsogenin (R_f 0.43, $CHCl_3$/MeOH 20 : 1). In addition, (3*β*,4*α*)-3,29-dihydroxy-16-oxo-28-norolean-17-en-23-al was obtained from **5**. The aq. layer was neutralized by passing it through an ion-exchange resin (*Amberlite MB-3*) column and concentrated to yield the crude sugar mixture. The thiazolidine derivatives were prepared by reaction with L-cysteine hydrochloride as described previously [9]. The final sample was subjected to GC/MS analysis under the following conditions (see also *General*) detection temp. 220°; column temp. 150–260° with the rate of 8°/min; carrier gas He (0.8 ml/min); split ratio 1/10; injection temp. 250°; injection volume 0.5 µl. The absolute configurations of the sugars were confirmed to be D-quinovose, L-rhamnose, L-arabinose, D-galactose, D-fucose, D-xylose, and D-glucose by comparison of the retention times with those of standard samples: D-quinovose (12.51 min), L-rhamnose (12.67 min), L-arabinose (11.86 min), D-galactose (14.31 min), D-fucose (12.85 min), D-xylose (11.89 min), and D-glucose (14.01 min), resp.

Enzyme Inhibition Assay [9]. *α*-Glucosidase (from baker's yeast, E.C.3.2.1.20) was purchased from *Sigma* company (No. G-5003, Lot. 081k7415). The reaction was carried out at pH 6.8 and 37° for 10 min, with 0.01M 4-nitrophenyl *α*-D-glucopyranoside (PNPG) as a substrate and 1 unit/ml of enzyme, in 0.067M KH_2PO_4/Na_2HPO_4 buffer. Acarbose was used as a pos. control. The increment in absorption at 410 nm due to the hydrolysis of PNPG by *α*-glucosidase was monitored continuously with an auto multifunctional microplate reader (*Bio-Rad 680*).

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