Glandulosides A−D, Triterpene Saponins from *Acanthophyllum glandulosum*

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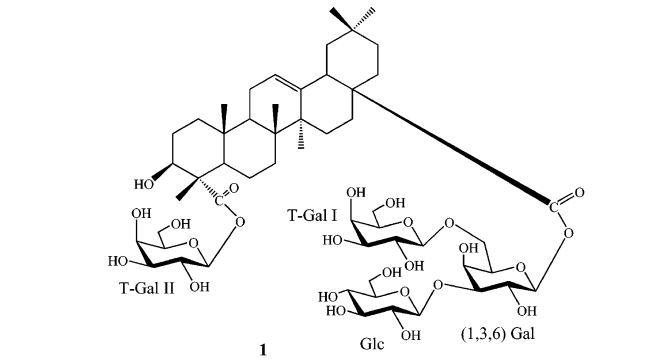
Four novel triterpenoid saponins, glandulosides A (**1**), B (**2**), C (**3**), and D (**4**), together with two known saponins (**5** and **6**) have been isolated from the roots of *Acanthophyllum glandulosum*. Their structures were elucidated using a combination of homo- and heteronuclear 2D NMR techniques (COSY, TOCSY, NOESY, HSQC, and HMBC) and by FABMS. The new compounds were characterized as 23-*O*-*β*-D-galactopyranosylgypsogenic acid-28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-galactopyranosyl-(1→6)]-*β*-D-galactopyranoside (**1**), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosylgypsogenin-28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-*O*-acetyl-*β*-D-fucopyranoside (**2**), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosylgypsogenin-28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-*β*-D-fucopyranoside (**3**), and 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosylgypsogenin-28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[3-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**4**).

In a continuation of our study on saponin constituents of medicinal plants of the Caryophyllaceae family,[1−8] we have examined the saponin fraction of *Acanthophyllum glandulosum* Bge. [syn.: *A. fontanesii* Boiss.] (Caryophyllaceae), which is one of the six species of the section *Pleiosperma* endemic to Iran.[9,10] The roots of this plant are gathered in considerable quantity in Turkmenia. They are known as "soap root" and are used as a soap substitute. It was used also as an expectorant and emetic and vulnerary for horses. No previous phytochemical investigation has been reported on *A. glandulosum*. In this paper, we describe the isolation and structure elucidation of four new triterpenoid saponins designated as glandulosides A, B, C, and D (**1**−**4**), along with two known saponins (**5** and **6**)[11,12] previously isolated from *Gypsophila capillaris* and *G. arrostii*.



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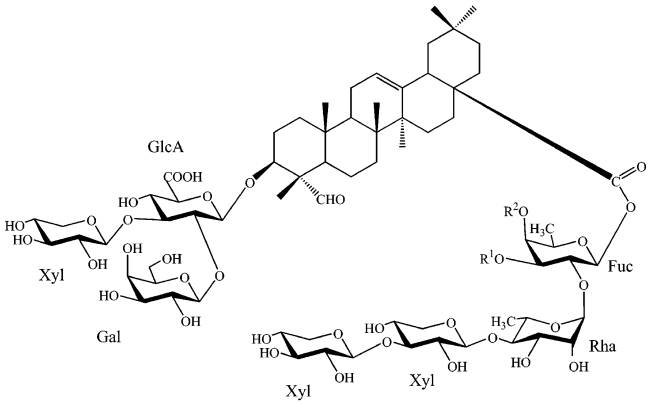

	R^1	R^2
2	Ac	H
3	Ac	Ac
4	H	

Results and Discussion

The concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *A. glandulosum* Bge. ex Boiss. was purified by precipitation with diethyl ether. The resulting crude saponin mixture was further dialyzed and subjected to multiple chromatographic steps over Sephadex LH-20 and medium-pressure liquid chromatography (MPLC) over normal silica gel and reversed-phase Si RP-18, yielding six saponins, glandulosides A (**1**), B (**2**), C (**3**), and D (**4**) and the saponins **5**[11] and **6**.[12]

Compound **1** was obtained as an amorphous powder. The high-resolution ESI mass spectrometry (HRESIMS, positive ion mode) of **1** exhibited a quasi-molecular ion peak at *m*/*z* 1157.5399 $[M + Na]^+$ (calcd 1157.5356), consistent with a molecular formula of $C_{54}H_{86}O_{25}Na$. Its negative-ion FABMS showed a quasimolecular ion peak at *m*/*z* 1133 $[M - H]^-$, indicating a molecular weight of 1134. Other significant ions visible at *m*/*z* 971 $[M - H - 162]^-$, 909 $[M - H - 162 - 162]^-$, 643 $[M - H - 162 - 162 - 162]^-$, and 485 $[(M - H) - 162 - 162 - 162 - 162]^-$ corresponded to the successive loss of four hexosyl moieties. The fragment ions at *m*/*z* 485 corresponded to the pseudomolecular ion

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Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data of Compound **1** (pyridine-d_5)[a]

gypsogenic acid	δ_C	δ_H	28-*O*-	δ_C	δ_H
1	38.5	0.98, 148	(1,3,6) Gal1	*94.6*	*6.09 (d, J = 8.4)*
2	26.8	1.84, nd	2	72.4	4.10
3	74.6	4.52	3	*87.3*	*4.22*
4	54.8		4	68.4	4.23
5	51.7	1.78	5	77.0	3.98
6	23.0	1.94, nd	6	*68.5*	*4.18, 4.50*
7	32.0	1.66, 1.78	T-Glc1	*104.9*	*5.24 (d, J = 7.7)*
8	39.8		2	75.0	3.95
9	48.0	1.66	3	77.4	4.10
10	36.5		4	71.2	4.00
11	23.4	nd, nd	5	77.9	3.90
12	122.3	5.36 (*s*)	6	62.1	4.20, 4.45
13	143.9		T-Gal I 1	*104.3*	*4.88 (d, J = 8.0)*
14	41.3		2	74.6	3.90
15	27.8	nd, nd	3	77.0	4.12
16	21.0	1.50, nd	4	71.0	4.08
17	46.7		5	77.9	3.78
18	41.8	3.06	6	62.1	4.15, 4.38
19	45.9	1.16, 1.62	23-*O*-		
20	30.4		T-Gal II 1	*96.0*	*6.29 (d, J = 8.0)*
21	33.6	1.00, 1.24	2	73.7	4.13
22	32.4	nd, nd	3	78.2	4.18
23	177.5		4	70.7	4.18
24	11.6	1.48 (s)	5	78.9	3.95
25	15.7	0.84 (s)	6	61.8	4.20, 4.30
26	17.0	0.94 (s)			
27	25.7	1.04 (s)			
28	176.3				
29	32.7	0.77 (s)			
30	23.3	0.78 (s)			

[a] ^{13}C NMR chemical shifts of substituted residues are italicized. The assignments were based on COSY, TOCSY, NOESY, HSQC, HMBC, and DEPT experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR).

of the aglycone. Mineral acid hydrolysis of **1** with 2 N TFA afforded an artifactual aglycone. The aglycone was identified as gypsogenic acid from 2D NMR spectra of **1** (Table 1).[13]

The sugars obtained from the saponin hydrolysates were identified as galactose and glucose (co-TLC), respectively. Alkaline hydrolysis of **1** performed with 5% KOH yielded an aglycone. These data indicated that no sugars were connected at C-3. This was confirmed by the signals observed in the ^{13}C NMR spectrum of **1** at δ_C 74.6 (downfield shift of C-3 of the aglycone), δ_C 177.5, and δ_C 176.4 (upfield shift of C-23 and C-28 of the aglycone, respectively) (Table 1).

Compound **1** was shown to contain four sugar residues from the HSQC spectrum: the anomeric ^{1}H NMR signals at δ 6.29 (d, J = 8.0 Hz), 6.09 (d, J = 8.4 Hz), 5.24 (d, J = 7.7 Hz), and 4.88 (d, J = 8.0 Hz) gave correlations with ^{13}C NMR signals at δ 96.0, 94.4, 104.9, and 104.3, respectively. Complete assignments of each sugar proton system were achieved by considering TOCSY and $^{1}H-^{1}H$ COSY spectra, while the carbons were assigned from HSQC and HMBC spectra. Among the four sugar units in the molecule, three were identified as galactose, because only three cross-peaks were traced from the TOCSY subspectrum corresponding to the remaining anomeric protons at δ 6.09 (d, J = 8.4 Hz), 6.29 (d, J = 8.0 Hz), and 4.88 (d, J = 8.0 Hz). This was typical for galactosyl residues in which the distribution of the scalar coupling around the system was impeded by the small $^{3}J_{4,5}$.[14] The large vicinal coupling among ring protons due to trans-diaxial orientations suggested that the fourth sugar was a glucose unit. The β-anomeric configurations for glucose and galactose were supported both by the value of $^{3}J_{1,2}$ (7−8 Hz) and by the presence of intense cross-peaks from H-1 to H-3 and H-5 in their NOESY spectrum. All sugar units were in the pyranose forms determined from their ^{13}C NMR data. The common D-configuration for Glc and Gal was determined by GC analysis of chiral derivatives of sugars in the acid hydrolysate (see Experimental Section).[15]

Assignments of the ^{1}H NMR and ^{13}C NMR signals from TOCSY, HSQC, and HMBC spectra showed that the four sugar units were two terminal Gal (T-Gal I, T-Gal II), a trisubstituted Gal-1,3,6, and a terminal Glc (T-Glc) (Table 1). The signals observed in the ^{13}C NMR spectrum of **1** at $\delta_{C-1}/\delta_{H-1}$ 96.0/6.29 (T-Gal II) and $\delta_{C-1}/\delta_{H-1}$ 94.4/6.09 (Gal-1,3,6) indicated that these sugar units were linked to the two carboxylic groups of the aglycone at C-23 and C-28 by an ester linkage. This was confirmed by correlation in the HMBC spectrum between the ^{1}H NMR signal at δ_{H-1} 6.29 (d, J = 8.0 Hz) (T-Gal II) and the ^{13}C NMR signal at δ_C 177.5 (Agly C-23) and between δ_{H-1} 6.09 (d, J = 8.4 Hz) (1,3,6-Gal) and the ^{13}C NMR signal at δ_C 176.3 (Agly C-28).

The sugar chain at C-28 was established from the following HMBC correlations: δ_{H-1} 5.24 (d, J = 7.7 Hz) (T-Glc) and δ_{C-3} 87.3 (1,3,6-Gal), δ_{H-1} 4.88 (d, J = 8.0 Hz) (T-Gal I) and δ_{C-6} 68.5 (1,3,6-Gal).

This linkage was confirmed by the NOESY correlation between δ_{H-1} 5.24 (d, J = 7.7 Hz) (T-Glc) and δ_{H-3} 4.20 (1,3,6-Gal) and between δ_{H-1} 4.88 (d, J = 8.0 Hz) (T-Gal I) and $\delta_{H-6,6'}$ 4.18, 4.50 (1,3,6-Gal).

On the basis of the above results, the structure of **1** was determined as 23-*O*-β-D-galactopyranosylgypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→6)]-β-D-galactopyranoside, a new natural compound.

Compound **2** was obtained as an amorphous powder. The high-resolution ESI mass spectrum (HRESIMS, positive-ion mode) of **2** exhibited a quasi-molecular ion peak at *m/z* 1561.7005 $[M + Na]^+$ (calcd 1561.7038), consistent with a molecular formula of $C_{72}H_{114}O_{35}Na$. Its negative-ion FABMS showed a quasimolecular ion peak at *m/z* 1537 $[M - H]^-$, indicating a molecular weight of 1538. Other significant peaks visible at *m/z* 1273 $[(M - H) - 132 - 132 - 146]^-$, 939 $[(M - H) - 132 - 132 - 146 - 146 - 42]^-$, and 469 $[(M - H) - 132 - 132 - 146 - 146 - 146 - 42 - 132 - 162 - 176]^-$ corresponded to the successive losses of two pentosyl-deoxyhexosyl groups, one deoxyhexosyl-acetyl group, and one hexosyl-pentosyl-hexosyluronic acid moiety.

Mineral acid hydrolysis of **2** afforded gypsogenin and sugars identified as galactose, xylose, rhamnose, fucose, and glucuronic acid (co-TLC), respectively. Alkaline hydrolysis of **2** performed with 5% KOH yielded a prosapogenin which was identified as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin from 2D NMR spectra of **2**.[3] The above data suggested that **2** should be a 3,28-*O*-bidesmoside. This was confirmed by the observation of glycosylation-induced shifts in the ^{13}C NMR spectrum at δ_C 83.7 (downfield shift of C-3) and δ_C 176.4 (upfield shift of C-28).

Compound **2** was shown to contain seven sugar residues from the HSQC spectrum and one acetoxy methyl group (Table 2). The anomeric proton signals at δ_H 6.26 (s), 5.91 (d, J = 8.4 Hz), 5.34 (d, J = 7.7 Hz), 5.22 (d, J = 7.7 Hz), 5.01 (d, J = 7.7 Hz), 4.96 (d, J = 6.9 Hz), and 4.65 (d, J = 7.1 Hz) gave correlations with anomeric carbon signals at δ_C 94.1, 101.0, 103.4, 104.0, 105.0, 106.1, and 102.7, respectively. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one α-rhamnopyranosyl (Rha), one β-fucopyranosyl (Fuc), one β-galactopyranosyl (Gal), three β-xylopyranosyl (Xyl), and one β-glucuronopyranosyl (GlcA) unit, respectively. The common D-configuration for Fuc, Gal, Xyl, and GlcA and the

Table 2. ^{13}C and ^{1}H NMR Spectroscopic Data of the Sugar Moieties of Compounds **2**−**4** (pyridine-d_5)[a]

	2		**3**		**4**	
3-*O*-sugars	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
GlcA1	*102.7*	*4.65 (d, J = 7.1)*	*103.7*	*4.65 (d, J = 7.1)*	*102.7*	*4.65 (d, J = 7.3)*
2	*77.7*	*4.20*	*77.9*	*4.20*	*77.7*	*4.20*
3	*85.1*	*4.16*	*84.1*	*4.16*	*85.1*	*4.16*
4	71.5	4.15		4.15	71.5	4.15
5	77.3	4.06	77.8	4.06	77.3	4.06
6	nd		nd		nd	
Xyl1	*104.0*	*5.22 (d, J = 7.7)*	*104.4*	*5.22 (d, J = 7.7)*	*104.0*	*5.24 (d, J = 7.7)*
2	74.6	3.86	74.7	3.90	74.6	3.86
3	76.2	4.12	77.8	4.00	76.2	4.12
4	70.3	4.06	70.3	4.08	70.3	4.06
5	66.6	3.58, 4.22	66.9	3.60, 4.26	66.6	3.58, 4.22
Gal 1	103.4	*5.34 (d, J = 7.7)*	*103.4*	*5.34 (d, J = 7.4)*	*103.4*	*5.34 (d, J = 7.4)*
2	73.3	4.32	73.3	4.32	73.3	4.32
3	74.6	4.00	74.6	4.00	74.6	4.00
4	69.8	4.30	69.8	4.30	69.8	4.30
5	76.2	3.84	76.2	3.84	76.2	3.84
6	61.4	4.20, 4.33	61.4	4.20, 4.33	61.4	4.20, 4.33
28-*O*-sugars	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Fuc 1	*94.1*	*5.91 (d, J = 8.4)*	*93.9*	*6.02 (d, J = 8.0)*	*94.2*	*5.85 (d, J = 8.0)*
2	*73.3*	*4.50*	*71.9*	*4.50*	*73.0*	*4.48*
3	*74.4*	*5.50*	*74.3*	*5.50*	75.4	4.28
4	72.7	4.28	*70.8*	*5.52*	*83.6*	*3.98*
5	70.0	4.02	69.8	4.10	71.4	3.95
6	15.5	1.18 (*d, J = 5.8*)		1.12 (*d, J = 6.0*)	16.5	1.48 (*d, J = 6.0*)
COO*C*H$_3$	20.2	1.97 (s)	20.2	2.01 (s)		
*C*OOCH$_3$	170.0		170.8			
COO*C*H$_3$			20.4	2.05 (s)		
*C*OOCH$_3$			170.0			
Rha 1	*101.0*	*6.26 (s)*	*101.5*	*5.63 (s)*	*101.2*	*6.20 (s)*
2	71.0	4.69	70.4	4.48	70.6	4.75
3	71.8	4.50	71.7	4.35	71.3	4.48
4	*84.4*	*4.22*	*84.0*	*4.23*	*83.7*	*4.18*
5	67.9	4.32	68.7	4.22	67.4	4.28
6	17.4	1.65 (*d, J = 6.0*)	17.5	1.63 (*d, J = 6.0*)	17.6	1.57 (*d, J = 6.0*)
Xyl 1	*106.1*	*4.96 (d, J = 6.9)*	*106.2*	*5.01(d, J = 7.7)*	*106.1*	*4.96 (d, J = 6.9)*
2	74.6	3.90	75.7	3.95	74.3	3.93
3	*86.7*	*3.88*	*86.7*	*3.98*	*86.1*	*3.90*
4	68.3	3.96	68.5	4.15	68.1	3.94
5	66.3	3.42, 4.12	66.5	3.86	65.6	3.42, 4.12
T-Xyl 1	*105.0*	*5.01 (d, J = 7.7)*	*105.3*	*5.06 (d, J = 7.7)*	*104.9*	*5.01 (d, J = 7.7)*
2	74.6	3.95	74.5	3.96	74.0	3.92
3	77.3	4.03	77.5	4.08	76.5	4.02
4	71.5	4.05	70.3	4.12	69.7	4.04
5	66.6	3.55, 4.18	66.9	3.58, 4.18	66.0	3.57, 4.18
Qui 1					*106.0*	*4.88 (d, J = 7.7)*
2					74.8	3.87
3					*78.0*	*5.52*
4					73.8	3.90
5					72.2	3.57
6					17.4	1.46 (d, *J* = 6.1)
COO*C*H$_3$					20.2	1.97 (s)
*C*OOCH$_3$					170.0	

[a] ^{13}C NMR chemical shifts of substituted residues are italicized. The assignments were based on COSY, TOCSY, NOESY, HSQC, HMBC, and DEPT experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR).

L-configuration for Rha were determined by GC analysis of chiral derivatives of sugars in the acid hydrolysate.

After subtraction of the signals of the prosapogenin from the total ^{1}H and ^{13}C NMR signals of **2**, signals of the four sugar units connected at C-28 remained. The downfield signal of Fuc-3 at δ 5.50 gave a cross-peak with the downfield signal of Fuc C-3 at δ 72.4 in the HSQC spectrum and revealed the location of the acetyl group at this position. The HMBC experiment showed long-range coupling between protons at δ_H (Fuc-6) 1.18 (d, J = 5.8 Hz) and carbons at δ_C (Fuc-5) 70.0 (^{2}J) and δ_C (Fuc-4) 72.7 (^{3}J), confirming that Fuc C-4 was not substituted.

The sugar chain attached at C-28 of the aglycone was established from the following HMBC correlations: δ_{H-1} 5.91 (d, J = 8.4 Hz) (Fuc) and δ_{C28} 176.4 (Agly), δ_{H-1} 5.01-(d, J = 7.7 Hz) (T-Xyl) and δ_{C-3} 86.7 (1,3-Xyl), δ_{H-4} 4.22 (1,4-Rha) and δ_{C-1} 106.1 (1,3-Xyl).

This linkage was confirmed by the NOESY correlation between δ_{H-1} 6.26 (s) (1,4-Rha) and δ_{H-2} 4.50 (1,2-Fuc), between δ_{H-1} 4.96 (d, J = 7.7 Hz) (1,3-Xyl) and δ_{H-4} 4.22 (1,4-Rha-4), and between δ_{H-1} 5.01 (d, J = 7.7 Hz) (T-Xyl) and δ_{H-3} 3.88 (1,3-Xyl).

On the basis of the above results, the structure of compound **2** was deduced as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl gypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-*O*-acetyl-β-D-fucopyranoside, a new natural compound.

Compound **3** was obtained as an amorphous powder. The high-resolution ESI mass spectrometry (HRESIMS, posi-

tive ion mode) of **3** exhibited a quasi-molecular ion peak at *m/z* 1603.6701 $[M + Na]^+$ (calcd 1603.6780), consistent with a molecular formula of $C_{73}H_{112}O_{37}Na$. Its negative-ion FABMS showed a quasimolecular ion peak at *m/z* 1579 $[M - H]^-$, 42 mass units higher than that **2** and indicating a molecular weight of 1580. Other significant ion peaks visible at *m/z* 1447 $[(M - H) - 132]^-$ and 1315 $[(M - H) - 132 - 132]^-$ corresponded to the successive loss of two pentosyl moieties.

The ^1H and ^{13}C NMR data of **3** (Table 1) assigned from TOCSY, HSQC, and HMBC experiments were similar to those of compound **2**, except for the appearance of an additional acetyl group.

The location of the two acetyl groups at Fuc-3 and Fuc-4 (δ_H 5.12 and δ_H 5.02) was determined by the TOCSY and COSY spectra, starting from the anomeric ^1H NMR signal of fucose at δ 5.54 (d, $J = 7.9$ Hz). The downfield shifts observed in the HSQC spectrum for the Fuc H-3/Fuc C-3 and Fuc H-4/Fuc C-4 resonances at δ_H 5.12/δ_C 71.4 and at δ_H 5.02/δ_C 70.0 proved the secondary alcoholic functions Fuc-3-OH and Fuc-4-OH to be acetylated.

On the basis of the above results, the structure of compound **3** was elucidated as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-β-D-fucopyranoside, a new natural compound.

Compound **4** was obtained as an amorphous powder. The high-resolution ESI mass spectrometry (HRESIMS, positive-ion mode) of **4** exhibited a quasi-molecular ion peak at *m/z* 1707.7199 $[M + Na]^+$ (calcd 1707.7254), consistent with a molecular formula of $C_{77}H_{120}O_{40}Na$. Its negative-ion FABMS showed a quasimolecular ion peak at *m/z* 1683 $[M - H]^-$, 162 mass units higher than that of **2** and indicating a molecular weight of 1684, compatible with the molecular formula $C_{77}H_{120}O_{40}$. Another significant ion peak at *m/z* 1537 $[(M - H) - 146]^-$ corresponded to the loss of one deoxyhexosyl moiety.

The ^1H and ^{13}C NMR spectra of **4** allowed the identification of gypsogenin as an aglycone, one acetoxy methyl group at δ_H1.97/δ_C 20.2, and eight sugar residues. The anomeric proton signals at δ_H 5.85 (d, $J = 8.0$ Hz), 6.20 (s), 5.34 (d, $J = 7.4$ Hz), 5.24 (d, $J = 7.7$ Hz), 5.01 (d, $J = 7.7$ Hz), 4.96 (d, $J = 6.9$ Hz), 4.88 (d, $J = 7.7$ Hz), and 4.65 (d, $J = 7.3$ Hz) correlated in the HSQC spectrum with carbon signals at δ_C 94.2, 101.2, 103.5, 104.0, 104.9, 106.1, 106.2 and 102.7, respectively. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one β-fucopyranosyl (Fuc), one α-rhamnopyranosyl (Rha), one β-galactopyranosyl (Gal), three β-xylopyranosyl (Xyl), one β-quinovopyranosyl, and one β-glucuronopyranosyl (GlcA) unit, respectively. The common D-configuration for Qui was assumed, according to that most encountered among the plant glycosides.

From the TOCSY spectrum, the chemical shifts of the quinovose protons were assigned from the anomeric proton at δ 4.88 (d, $J = 7.7$ Hz). It showed five cross-peaks at δ_H 3.87, 5.52, 3.90, 3.57, and 1.46 ppm (d, $J = 6.1$ Hz) assigned to H-2−H-6, respectively, which were confirmed by correlations in the COSY spectrum. The cross-peaks from these protons observed in the HSQC spectrum allowed the ^{13}C NMR assignments of the quinovose residue. The downfield signal of Qui-3 at δ 5.52 gave a cross-peak with the downfield signal of C-3 at δ 78.0 in the HSQC spectrum and revealed the location of the acetyl group at this position. The HMBC experiment showed long-range coupling between ^1H NMR signals at δ_H (Qui-6) 1.46 and ^{13}C NMR signals at δ_C (Qui-5) 72.2 (^2J) and δ_C (Qui-4) 73.8 (^3J), confirming that Qui C-4 was not substituted.

The sugar chain attached at C-28 of the aglycone was established from the following HMBC correlations: δ_{H-1} 4.88 (d, $J = 7.7$ Hz) (T-Qui) and δ_{C-4} 83.6 (Fuc), δ_{H-1} 5.01 (d, $J = 7.7$ Hz) (T-Xyl) and C-3 (1,3-Xyl) δ_{C-3} 86.1, δ_{H-4} 4.18 (1,4-Rha) and δ_{C-1} 106.1 (1,3-Xyl), δ_{H-3} 3.90 (1,3-Xyl) and δ_{C-1} 104.9 (T-Xyl). This linkage was confirmed by the NOESY correlation between δ_{H-1} 6.26 (s) (Rha) and δ_{H-2} 4.48 (Fuc), δ_{H-1} 4.96 (d, $J = 6.9$ Hz) (1,3-Xyl) and δ_{H-4} 4.18 (Rha), δ_{H-1} 5.01 (d, $J = 7.7$ Hz) (T-Xyl) and δ_{H-3} 3.90 (1,3-Xyl), δ_{H-1} 4.88 (d, $J = 7.7$ Hz) (Qui) and δ_{H-4} 3.98 (Fuc).

NMR spectral data of **4** were similar to those of **2** except for the presence of signals due to the 3-*O*-acetyl-β-D-quinovopyranosyl moiety at Fuc-4 and loss of the acetyl group that was attached at Fuc-3 in **2**.

On the basis of the above results, compound **4** was determined as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl gypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[3-*O*-acetyl-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranoside, a new natural compound.

Two additional compounds were isolated and identified by analysis of their spectral data (FABMS and 2D NMR) as gypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→6)]-β-D-galactopyranoside (**5**)[11] and 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranoside (**6**),[12] known saponins previously characterized from *G. capillaris* and *G. arrostii*.

Experimental Section

General Experimental Procedures. All physical data of the isolated compounds were obtained on the same instruments as those used in a previous paper.[7]

Plant Material. The roots of *Acanthophyllum glandulosum* Bge. [syn.: *A. fontanesii* Boiss.] (Caryophyllaceae) were collected from the northeastern part of Iran, Khorasan Province, and identified by Mr. Joharchi. A voucher specimen under the reference no. 4200 is deposited in the Herbarium of the Laboratory of the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Extraction and Isolation. Dried, powdered roots of *A. glandulosum* (500 g) were defatted with *n*-hexane and extracted successively with $CHCl_3$ and MeOH. After removal of the solvent by evaporation, the MeOH extract (30 g) was obtained. This extract was suspended in H_2O (400 mL) and submitted to extraction with H_2O-saturated n-BuOH (3 × 200 mL) to give after evaporation of the solvent in vacuum the n-BuOH fraction (10 g). It was solubilized in MeOH (10 mL) and precipitated in Et_2O (3 × 250 mL), yielding 7 g of a crude saponin fraction. Of this mixture, 2 g was dialyzed for 3 days and then lyophilized. The residue obtained (1.1 g) was dissolved in MeOH and submitted to column chromatography on Sephadex LH-20 (MeOH), yielding 0.8 g of a white powder. This mixture was first fractionated by MPLC on silica gel 60 (15−40 μm) using as eluent $CHCl_3$−MeOH−H_2O (13:7:2, lower phase). Further separations were performed by successive MPLC using as eluent $CHCl_3$−MeOH−H_2O (8:5:1) and on reversed-phase material, Lichroprep RP-18, Merck (40−63 μm), eluted with MeOH−H_2O (50%) to give compounds **1** (20 mg), **2** (10 mg), **3** (9 mg), **4** (12 mg), **5** (18 mg),[11] and **6** (22 mg).[12]

Compound 1: white amorphous powder; TLC R_f 0.4 (system a); blue spots by spraying with Komarowsky reagent; $[\alpha]^{20}_D$ −6° (*c* 0.10, MeOH); IR ν_{max} 3398 (OH), 2928 (CH), 1735 (C=O ester), 1718 (CO carboxylic acid), 1615, 1386 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR of gypsogenic acid and sugar moieties, Table 1; HRESIMS, positive-ion mode

negative *m*/*z* 1157.5399 $[M + Na]^+$ (calcd for $C_{54}H_{86}O_{25}Na$ 1157.5356); negative FABMS (glycerol matrix) *m*/*z* 1133 $[M - H]^-$, 971 $[M - H - 162]^-$, 909 $[M - H - 162 - 162]^-$, 643 $[M - H - 162 - 162 - 162]^-$, and 485 $[(M - H) - 162 - 162 - 162 - 162]^-$.

Compound 2: white amorphous powder; TLC R_f 0.54 (system a); blue spots by spraying with Komarowsky reagent; $[\alpha]^{20}_D$ +5° (*c* 0.10, MeOH); IR ν_{max} 3401 (OH), 2925 (CH), 1734 (C=O ester), 1615, 1417 cm^{-1}; ^{13}C NMR of gypsogenin,[5] ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) of sugar moieties, Table 2; HRESIMS, positive-ion mode negative *m*/*z* 1561.7005 $[M + Na]^+$ (calcd for $C_{72}H_{114}O_{35}Na$ 1561.7038); negative FABMS (glycerol matrix) *m*/*z* 1537 $[M - H]^-$, 1273 $[(M - H) - 132 - 132 - 146]^-$, 939 $[(M - H) - 132 - 132 - 146 - 146 - 42]^-$, and 469 $[(M - H) - 132 - 132 - 146 - 146 - 146 - 42 - 132 - 162 - 176]^-$.

Compound 3: white amorphous powder; TLC R_f 0.56 (system a); blue spots by spraying with Komarowsky reagent; $[\alpha]^{20}_D$ +13° (*c* 0.10, MeOH); IR ν_{max} 3401 (OH), 2932 (CH), 1734 (C=O ester), 1615, 1383 cm^{-1}; ^{13}C NMR of gypsogenin,[5] ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) of sugar moieties, Table 2; HRESIMS, positive-ion mode negative *m*/*z* 1603.6701 $[M + Na]^+$ (calcd for $C_{73}H_{112}O_{37}Na$ 1603.6780); negative FABMS (glycerol matrix) *m*/*z* 1579 $[M - H]^-$, 1447 $[(M - H) - 132]^-$, 1315 $[(M - H) - 132 - 132]^-$.

Compound 4: white amorphous powder; TLC R_f 0.60 (system a); blue spots by spraying with Komarowsky reagent; $[\alpha]^{20}_D$ +13° (*c* 0.10, MeOH); IR ν_{max} 3401 (OH), 2932 (CH), 1734 (C=O ester), 1615, 1383 cm^{-1}; ^{13}C NMR of gypsogenin,[5] ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) of sugar moieties, Table 2; HRESIMS, positive-ion mode negative *m*/*z* 1707.7199 $[M + Na]^+$ (calcd for $C_{77}H_{120}O_{40}Na$ 1707.7254); negative FABMS (glycerol matrix) *m*/*z* 1683 $[M - H]^-$, 1537 $[(M - H) - 146]^-$.

Compounds 5 and 6. The spectroscopic data were in full agreement with previously published data.[11,12]

Acid Hydrolysis. A solution of individual saponin (3 mg) in 2 N aqueous CF_3COOH (5 mL) was refluxed on a water bath for 3 h. After this period, the reaction mixture was diluted with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 extracts were washed with H_2O and then evaporated to dryness in a vacuum. Evaporation of the solvent gave gypsogenin for **2**−**4** (co-TLC with an authentic sample). After evaporation to dryness of the aqueous layer with MeOH, the sugars were analyzed by silica gel TLC by comparison with standard sugars (solvent system c), and the absolute configuration of sugar residues was determined by GC analysis as described in a previous paper.[15]

Alkaline Hydrolysis. The saponin (7 mg) was refluxed with 5% aqueous KOH (10 mL) for 1 h. The reaction mixture was adjusted to pH 6 with dilute HCl and then extracted with H_2O-saturated n-BuOH (3 × 10 mL). The combined n-BuOH extracts were washed (H_2O). Evaporation of the n-BuOH gave the prosapogenin. The acidic hydrolysis of prosapogenin in 2 N aqueous CF_3COOH for 2 h at 120 °C furnished gypsogenin and glucuronic acid, galactose, and xylose for **2**−**4** (co-TLC with authentic samples).

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