New Triterpene Saponins from *Acanthophyllum pachystegium*

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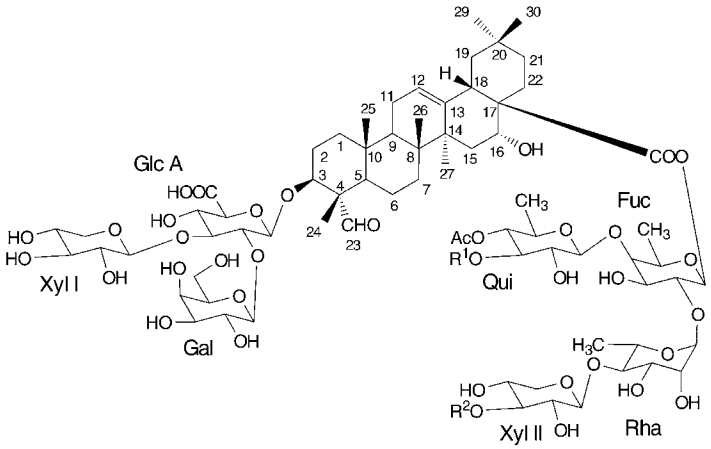
Four new triterpenoid saponins, pachystegiosides A (**1**), B (**2**), C (**3**), and D (**4**), were isolated from the roots of *Acanthophyllum pachystegium* K.H. Their structures were elucidated by means of a combination of homo- and heteronuclear 2D-NMR techniques (COSY, TOCSY, NOESY, HSQC, and HMBC) and by FAB-MS. The new compounds were characterized as 3-*O*-{*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl}quillaic acid 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[3,4-di-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranosyl}ester (**1**), 3-*O*-{*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl}quillaic acid 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[4-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranosyl} ester (**2**), 3-*O*-{*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl}quillaic acid 28-{*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[4-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranosyl} ester (**3**), and gypsogenic acid 28-[*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl] ester (**4**).

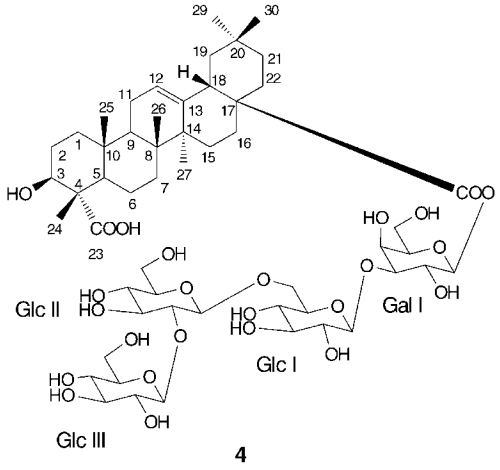
Introduction. – In a continuation of our study on saponin constituents of plants of the Caryophyllaceae family [1–9], we examined the saponin fraction of *Acanthophyllum pachystegium* K.H. All species of *Acanthophyllum* are used as soup in Khorasan province, and the aqueous extract of their roots are added to make a special type of candy. No previous phytochemical study has been reported on saponins of this plant. In this paper, we describe the isolation and structure elucidation of four new triterpenoid saponins designated as pachystegiosides A, B, C, and D (**1**–**4**).

Results and Discussion. – The methanolic extract of the roots of *A. pachystegium* was partitioned successively with $CHCl_3$ and BuOH. The concentrated BuOH-soluble fraction was purified by precipitation with Et_2O yielding the crude saponin mixture. This extract was further fractionated by column chromatography (*Sephadex LH-20*) and repeated medium-pressure liquid chromatography (MPLC; silica gel and *ODS*), yielding the four pure saponins **1**–**4** as amorphous powder.

Structural elucidation of the compounds was mainly determined by extensive spectroscopic 1D- and 2D-NMR experiments (^1H,^{13}C-COSY, TOCSY, NOESY, HSQC, and HMBC, see *Tables 1–3*) and FAB-MS. The sugars obtained from aqueous acid hydrolysis of **1**–**3** were identified by comparison with authentic samples (TLC) as glucuronic acid, galactose, xylose, rhamnose (=6-deoxymannose), and fucose (=6-



	R^1	R^2
1	Ac	Xyl III
2	H	Xyl III
3	H	H



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deoxygalactose). Quinovose (=6-deoxyglucose) was characterized by 2D-NMR data of **1**–**3**. In the case of **4**, only glucose and galactose were detected in the hydrolysate (TLC). Alkaline hydrolysis of **1**–**3** with 5% aqueous KOH solution afforded the same prosapogenin, which, by further acid hydrolysis, furnished glucuronic acid, galactose, xylose, and quillaic acid (= 3β,4α,16α)-3,16-dihydroxy-23-oxoolean-12-en-28-oic acid) (co-TLC with a reference compound). These results indicated that **1**–**3** must be bidesmosidic saponins in which glucuronic acid, galactose, and xylose were bound to

Table 1. *^{13}C-NMR* (150 MHz)[a]) *and ^{1}H-NMR* (600 MHz) *Data of the Aglycone Parts of* **1**–**4** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm.

	1		**2**		**3**			**4**	
δ(C)	δ(H)[b])	δ(C)	δ(H)[b])	δ(C)	δ(H)[b])	δ(C)	δ(H)[b])		
CH_2(1)	37.0	0.78, 1.34	37.2	0.82, 1.34	37.0	0.82, 1.34	CH_2(1)	38.6	1.04, 1.34
CH_2(2)	23.9	1.84, 2.20	24.0	1.82, 2.20	24.0	1.82, 2.20	CH_2(2)	23.0	[c]), [c])
CH(3)	84.1	3.94	84.1	3.92	84.0	3.94	CH(3)	75.6	4.00
C(4)	54.8	–	54.1	–	54.3	–	C(4)	53.9	–
CH(5)	47.8	1.26	47.9	1.22	47.7	1.28	CH(5)	51.2	1.26
CH_2(6)	19.0	[c]),[c])	19.0	[c]),[c])	19.0	[c]),[c])	CH_2(6)	23.3	1.88, 1.92
CH_2(7)	32.0	1.42, 1.50	32.0	1.42, 1.49	31.9	1.42, 1.50	CH_2(7)	31.9	1.60, 1.74
C(8)	40.5	–	41.2	–	40.5	–	C(8)	39.9	–
CH(9)	47.5	1.18	45.6	1.18	46.0	1.17	CH(9)	47.8	1.70
C(10)	35.1	–	35.5	–	35.2	–	C(10)	36.5	–
CH_2(11)	22.8	1.82, [c])	22.9	[c]),[c])	22.6	1.78, 1.79	CH_2(11)	23.3	[c]),[c])
CH(12)	121.6	5.45	121.8	5.46	121.3	5.45	CH(12)	122.3	5.35
C(13)	143.7	–	143.7	–	143.8	–	C(13)	143.9	–
C(14)	39.2	–	39.2	–	39.2	–	C(14)	41.3	–
CH_2(15)	35.0	[c]),[c])	35.0	2.02, [c])	35.0	2.02, 2.20	CH_2(15)	26.8	1.84, [c])
CH(16)	73.8	5.13	73.0	5.00	73.0	5.00	CH(16)	21.3	1.45, 1.55
C(17)	48.0	–	48.2	–	48.2	–	C(17)	46.8	–
CH(18)	41.2	3.20 (*m*)	41.2	3.20	41.2	3.18	CH(18)	41.8	3.07 (*m*)
CH_2(19)	46.0	1.64, [c])	46.2	1.67, [c])	46.3	1.67, [c])	CH_2(19)	46.0	1.12, 1.66
C(20)	29.8	–	29.8	–	30.0	–	C(20)	30.2	–
CH_2(21)	35.0	1.12, [c])	35.0	1.14, 1.88	35.0	1.14, 1.88	CH_2(21)	33.5	1.00, 1.20
CH_2(22)	31.2	[c]), [c])	32.0	1.42, [c])	32.0	1.38, [c])	CH_2(22)	32.3	1.20, [c])
CH(23)	210.1	9.75	210.1	9.78	210.2	9.75	C(23)	184.0	–
Me(24)	9.9	1.32 (*s*)	10.5	1.35	10.1	1.32	Me(24)	12.4	1.47 (*s*)
Me(25)	14.9	0.71 (*s*)	15.1	0.71	15.0	0.71	Me(25)	15.7	0.85 (*s*)
Me(26)	16.5	0.88 (*s*)	16.4	0.91	16.4	0.90	Me(26)	17.1	0.97 (*s*)
Me(27)	26.1	1.62 (*s*)	26.6	1.62	26.1	1.62	Me(27)	26.0	1.12 (*s*)
C(28)	175.8	–	175.8	–	175.8	–	C(28)	176.4	–
Me(29)	32.0	0.82 (*s*)	32.0	0.82	31.9	0.82	Me(29)	32.6	0.76 (*s*)
Me(30)	23.9	0.94 (*s*)	23.6	0.92	23.8	0.96	Me(30)	23.1	0.78 (*s*)

[a]) Multiplicities were assigned from DEPT spectra. [b]) Overlapped ^{1}H-NMR signals are reported without designated multiplicity. [c]) Not determined.

the aglycone by a glycosidic linkage at C(3), while the remaining sugars must be bound to the aglycone by a glycosidic ester linkage at C(28). Moreover, alkaline hydrolysis of **4** under the same conditions as previously described did not afford a prosapogenin but only an aglycone which was characterized as gypsogenic acid (=(3β,4α)-3-hydroxy-olean-12-ene-23,28-dioic acid) from the 2D-NMR spectra of **4**. These results indicated that **4** must be a monodesmosidic saponin in which all sugars must be bound to the aglycone by a glycosidic ester linkage at C(28).

The negative-ion FAB-MS of **1** showed a quasi-molecular ion peak at *m*/*z* 1741 ($[M-H]^-$), indicating a molecular mass of 1742, which suggested a molecular formula $C_{79}H_{122}O_{42}$. Three other significant ion peaks appeared at *m*/*z* 1699 ($[M-H-42]^-$), 1567 ($[M-H-42-132]^-$), and 1435 ($[M-H-42-132-132]^-$), corresponding to the successive loss of one acetyl group and two pentosyl moieties, respectively. The ^{1}H- and ^{13}C-NMR signals of the aglycone assigned from 2D-NMR spectra of **1** were

Table 2. *^{1}H-NMR* (600 MHz) *Data of the Sugar Moieties of* **1**–**4** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments.* δ(H) in ppm, J in Hz.

		δ(H)[a])				
		1	**2**	**3**		**4**
3-*O*-Sugars:						
GlcA	H–C(1)	4.68 ($d, J = 7.3$)	4.65 ($d, J = 7.3$)	4.65 ($d, J = 7.3$)		
	H–C(2)	4.21	4.13	4.21		
	H–C(3)	4.16	4.08	4.13		
	H–C(4)	4.05	4.05	4.05		
	H–C(5)	4.09	4.15	4.09		
Gal	H–C(1)	5.36 ($d, J = 7.7$)	5.30 ($d, J = 7.7$)	5.34 ($d, J = 7.7$)		
	H–C(2)	4.30	4.18	4.27		
	H–C(3)	4.02	4.02	4.00		
	H–C(4)	4.36	4.35	4.34		
	H–C(5)	3.85	3.90	3.98		
	CH_2(6)	4.16, 4.26	4.15, 4.26	4.16, 4.29		
Xyl I	H–C(1)	5.22 ($d, J = 7.7$)	5.08 ($d, J = 7.7$)	5.17 ($d, J = 7.7$)		
	H–C(2)	3.88	3.78	3.86		
	H–C(3)	4.07	4.06	4.06		
	H–C(4)	4.07	4.07	4.06		
	CH_2(5)	3.58, 4.25	3.58, 4.24	3.56, 4.18		
28-*O*-Sugars:						
Fuc	H–C(1)	5.86 ($d, J = 8.0$)	5.86 ($d, J = 8.0$)	5.80 ($d, J = 8.0$)	Gal I	H–C(1) 6.08 ($d, J = 7.8$)
	H–C(2)	4.45	4.48	4.46		H–C(2) 4.21
	H–C(3)	4.15	4.18	4.14		H–C(3) 4.24
	H–C(4)	4.04	3.90	4.04		H–C(4) 4.02
	H–C(5)	3.96	3.95	3.91		H–C(5) 3.86
	Me(6)	1.44 ($d, J = 6.1$)	1.39 ($d, J = 6.1$)	1.39 ($d, J = 6.1$)		CH_2(6) 4.12, 4.26
Rha	H–C(1)	6.12 (br. *s*)	6.14 (br. *s*)	6.12 (br. *s*)	Glc I	H–C(1) 5.26 ($d, J = 7.8$)
	H–C(2)	4.62	4.62	4.62		H–C(2) 4.01
	H–C(3)	4.52	4.44	4.44		H–C(3) 4.06
	H–C(4)	4.25	4.19	4.20		H–C(4) 4.08
	H–C(5)	4.33	4.30	4.33		H–C(5) 3.86
	Me(6)	1.54 ($d, J = 6.0$)	1.58 ($d, J = 6.0$)	1.54 ($d, J = 6.0$)		CH_2(6) 4.18, 4.42
Xyl II	H–C(1)	5.05 ($d, J = 7.7$)	4.97 ($d, J = 7.7$)	5.00 ($d, J = 7.7$)	Glc II	H–C(1) 4.86 ($d, J = 7.8$)
	H–C(2)	3.93	3.87	3.90		H–C(2) 3.98
	H–C(3)	3.90	3.90	4.00		H–C(3) 4.18
	H–C(4)	3.98	3.98	4.00		H–C(4) 4.03
	CH_2(5)	3.38, 4.10	3.38, 4.05	3.56, 4.20		H–C(5) 3.69
						CH_2(6) 4.18, 4.30
Xyl III	H–C(1)	5.00 ($d, J = 7.7$)	4.94 ($d, J = 7.7$)		Glc III	H–C(1) 5.20 ($d, J = 7.8$)
	H–C(2)	3.92	3.88			H–C(2) 4.00
	H–C(3)	4.00	4.00			H–C(3) 4.16
	H–C(4)	4.01	4.00			H–C(4) 4.09
	CH_2(5)	3.58, 4.23	3.58, 4.20			H–C(5) 3.82
						CH_2(6) 4.38, 4.40
Qui	H–C(1)	4.94 ($d, J = 7.7$)	4.94 ($d, J = 7.7$)	4.96 ($d, J = 7.7$)		
	H–C(2)	3.90	3.86	3.86		
	H–C(3)	5.50	3.95	3.95		
	H–C(4)	4.94	4.90	4.94		
	H–C(5)	3.60	3.60	3.60		
	Me(6)	1.20 ($d, J = 6.0$)	1.15 ($d, J = 6.0$)	1.15 ($d, J = 6.0$)		

[a]) Overlapped ^{1}H-NMR signals are reported without designated multiplicity.

Table 3. *^{13}C-NMR* (150 MHz) *Data of the Sugar Moieties of* **1**–**4** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments*[a]). δ(C) in ppm.

		δ(C)					
		1	**2**	**3**			**4**
3-*O*-Sugars: Glc A	CH(1)	103.3	103.0	102.8			
	CH(2)	78.1	77.1	77.0			
	CH(3)	85.7	85.5	84.5			
	CH(4)	70.5	70.0	70.5			
	CH(5)	76.8	76.8	77.4			
	C(6)	170.5	170.7	170.7			
Gal	CH(1)	103.9	103.2	103.2			
	CH(2)	73.2	72.8	73.0			
	CH(3)	75.0	74.8	74.0			
	CH(4)	70.1	70.0	69.5			
	CH(5)	76.5	76.5	76.8			
	CH_2(6)	61.5	60.9	61.5			
Xyl I	CH(1)	104.6	104.0	103.6			
	CH(2)	75.0	74.1	74.5			
	CH(3)	77.6	77.0	77.0			
	CH(4)	70.5	70.0	70.0			
	CH_2(5)	67.0	66.2	66.0			
28-*O*-Sugars: Fuc	CH(1)	94.5	94.1	93.8	Gal I	CH(1)	94.5
	CH(2)	71.6	71.3	71.2		CH(2)	73.0
	CH(3)	76.0	75.5	75.5		CH(3)	86.7
	CH(4)	83.0	83.3	82.5		CH(4)	70.5
	CH(5)	71.2	70.3	70.5		CH(5)	77.8
	Me(6)	16.8	17.0	17.0		CH_2(6)	62.0
Rha	CH(1)	101.1	100.5	100.0	Glc I	CH(1)	104.8
	CH(2)	71.5	71.2	70.6		CH(2)	76.2
	CH(3)	72.2	72.0	71.0		CH(3)	77.5
	CH(4)	83.5	82.3	82.3		CH(4)	70.5
	CH(5)	68.2	68.0	67.8		CH(5)	77.8
	Me(6)	17.5	17.8	17.5		CH_2(6)	68.4
Xyl II	CH(1)	105.5	105.8	105.0	Glc II	CH(1)	102.8
	CH(2)	75.3	74.3	74.2		CH(2)	82.4
	CH(3)	87.0	86.3	77.0		CH(3)	77.6
	CH(4)	69.0	69.5	70.0		CH(4)	70.5
	CH_2(5)	66.8	66.1	65.9		CH(5)	77.8
						CH_2(6)	61.9
Xyl III	CH(1)	105.5	104.8		Glc III	CH(1)	104.8
	CH(2)	75.6	74.3			CH(2)	75.6
	CH(3)	78.0	77.0			CH(3)	77.5
	CH(4)	70.6	70.0			CH(4)	71.0
	CH_2(5)	67.0	65.5			CH(5)	77.8
						CH_2(6)	62.0
Qui	CH(1)	105.6	104.9	104.0			
	CH(2)	74.8	74.8	74.8			
	CH(3)	76.3	74.0	74.0			
	CH(4)	74.2	74.5	75.5			
	CH(5)	70.3	69.8	72.0			
	Me(6)	17.3	16.5	16.5			

[a]) Multiplicities were assigned from DEPT spectra.

superimposable to those of quillaic acid [21]. The full assignment of all the ^{1}H- and ^{13}C-NMR signals by 2D-NMR experiments of **1** resulted in the establishment of its structure as 3-*O*-{*O*-*β*-D-galactopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucuronopyranosyl}quillaic acid 28-{*O*-*β*-D-xylopyranosyl-(1 → 3)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[3,4-di-*O*-acetyl-*β*-D-quinovopyranosyl-(1 → 4)]-*β*-D-fucopyranosyl} ester, a new natural compound [1–21].

The ^{1}H-NMR spectrum of **1** displayed signals for eight anomeric protons at δ(H) 6.12 (br. *s*), 5.86 (*d*, *J* = 8.0 Hz), 5.36 (*d*, *J* = 7.7 Hz), 5.22 (*d*, *J* = 7.7 Hz), 5.05 (*d*, *J* = 7.7 Hz), 5.00 (*d*, *J* = 7.7 Hz), 4.94 (*d*, *J* = 7.7 Hz), and 4.68 (*d*, *J* = 7.3 Hz), which correlated in the HSQC spectrum with δ(C) 101.1, 94.5, 103.9, 104.6, 105.5, 105.5, 105.6, and 103.3, respectively. The ring protons of the monosaccharide residues were assigned starting from the anomeric protons by means of the COSY, TOCSY, HSQC, and HMBC plots (*Table 2*), and the sequence of the oligosaccharide chains was obtained from the HMBC and NOESY experiments. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one *α*-L-rhamnopyranosyl (Rha), one *β*-D-xylopyranosyl (Xyl), one *β*-D-galactopyranosyl (Gal), one *β*-D-quinovopyranosyl (Qui), one *β*-D-fucopyranosyl (Fuc), and one *β*-D-glucuronopyranosyl (GlcA) unit; the common D-configuration for Fuc, GlcA, Gal, Xyl, and Qui, and the L-configuration for Rha were assumed, according to those most encountered among the plant glycosides in each case. The NOESY, HMBC, and HSQC experiments allowed the identification of the trisaccharide moiety linked to the 3-OH group of quillaic acid as 3-*O*-{*O*-*β*-D-galactopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucuronopyranosyl} by comparison with literature data [1][5–7]. After substraction of the signals of the 3-*O*-glycosidic part, the signals and sequence of the 28-*O*-oligosaccharidic moiety were assigned by analysis of NOESY, HSQC, and HMBC correlations. A correlation in the HSQC spectrum at δ(H) 5.86 (*d*, *J* = 8.0 Hz, Fuc H–C(1))/δ(C) 94.5 (Fuc C(1)) showed that the Fuc residue was linked to the carboxylic group of the aglycone at C(28) by an ester linkage. This conclusion was supported by the upfield shift of C(28) at δ(C) 175.8. Moreover, the HMBC correlation between δ(H) 5.00 (*d*, *J* = 7.7 Hz, Xyl III H–C(1)) and δ(C) 87.0 (Xyl II C(3)) indicated that Xyl III was linked to C(3) of Xyl II. This was also confirmed by a NOESY cross-peak between δ(H) 5.00 (*d*, *J* = 7.7 Hz, Xyl III H–C(1)) and δ(H) 3.90 (Xyl II H–C(3)). The NOESY cross-peak between δ(H) 5.05 (*d*, *J* = 7.7 Hz, Xyl II H–C(1)) and δ(H) 4.25 (Rha H–C(4)) established that Xyl II was attached to Rha by a (1 → 3) linkage. Furthermore, the HMBC correlation between δ(H) 6.12 (br. *s*, Rha H–C(1)) and δ(C) 71.6 (Fuc C(2)), together with a NOESY correlation between δ(H) 6.12 (br. *s*, Rha H–C(1)) and δ(H) 4.45 (Fuc H–C(2)) showed that the Rha was linked to C(2) of Fuc. The NOESY cross-peak between δ(H) 4.94 (*d*, *J* = 7.7 Hz, Qui H–C(1)) and δ(H) 4.04 (Fuc H–C(4)) indicated that Qui was linked to Fuc by a (1 → 4) linkage. The location of the acetyloxy groups at C(3) and C(4) of Qui (H–C(3) and H–C(4) at δ(H) 5.50 and 4.94, resp.) was determined by the TOCSY and COSY experiments, starting from the anomeric-proton signal of Qui at δ(H) 4.94 (*d*, *J* = 7.7 Hz, Qui H–C(1)). The downfield shifts observed in the HSQC spectrum at δ(H) 5.50 (Qui H–C(3))/δ(C) 76.3 (Qui C(3)) and at δ(H) 4.94 (Qui H–C(4))/δ(C) 74.2 (Qui C(4)) confirmed that the secondary OH functions at C(3) and C(4) of Qui were acetylated.

The negative-ion FAB-MS of **2** showed a quasi-molecular ion peak at *m*/*z* 1699 ($[M-H]^-$), indicating a molecular mass of 1700, which suggested a molecular formula $C_{77}H_{120}O_{41}$. Three other significant ion peaks appeared at *m*/*z* 1567 ($[M-H-132]^-$) and 1435 ($[M-H-132-132]^-$), corresponding to the successive loss of two pentosyl moieties, respectively. Moreover, a significant ion peak appeared at *m*/*z* 955, corresponding to the prosapogenin. The full assignment of all the ^{1}H- and ^{13}C-NMR signals by 2D-NMR experiments of **2** resulted in the establishment of the structure as 3-*O*-{*O*-*β*-D-galactopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucuronopyranosyl}quillaic acid 28-{*O*-*β*-D-xylopyranosyl-(1 → 3)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[4-*O*-acetyl-*β*-D-quinovopyranosyl-(1 → 4)]-*β*-D-fucopyranosyl} ester, a new natural compound [1–21].

The ^{1}H- and ^{13}C-NMR spectra of **2** allowed the identification of quillaic acid as aglycone (*Table 1*), and the presence of eight monosaccharide units was suggested by the eight anomeric protons at δ 6.14 (br. *s*), 5.86

($d, J = 8.0$ Hz), 5.30 ($d, J = 7.7$ Hz), 5.08 ($d, J = 7.7$ Hz), 4.97 ($d, J = 7.7$ Hz), 4.94 ($d, J = 7.7$ Hz), 4.94 ($d, J =$ 7.7 Hz), and 4.65 ($d, J = 7.3$ Hz), which correlated in the HSQC spectrum with δ(C) 100.5, 94.1, 103.2, 104.0, 105.8, 104.8, 104.9, and 103.0, respectively.

The ^{1}H- and ^{13}C-NMR signals of **2** assigned from the 2D-NMR spectra were almost superimposable on those of **1**, except for the disappearance of the signals of one acetyl group at C(3) of the Qui unit (*Tables 2* and *3*), in accordance with the difference in molecular mass (42 mass units) between **1** and **2**.

The negative-ion FAB-MS of **3** showed a quasi-molecular ion peak at *m*/*z* 1567 ($[M-H]^-$), indicating a molecular mass of 1568, which suggested a molecular formula $C_{72}H_{112}O_{37}$. On the basis of the 2D-NMR spectral data and hydrolysis, the structure of **3** was established as 3-*O*-{*O*-β-D-galactopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl}quillaic acid 28-{*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[4-*O*-acetyl-β-D-quinovopyranosyl-(1 → 4)]-β-D-fucopyranosyl} ester, a new natural compound [1–21].

The ^{1}H- and ^{13}C-NMR spectra of **3** allowed the identification of quillaic acid as aglycone (*Table 1*) and seven anomeric protons at δ(H) 6.12 (br. *s*), 5.80 ($d, J = 8.0$ Hz), 5.34 ($d, J = 7.7$ Hz), 5.17 ($d, J = 7.7$ Hz), 5.00 ($d, J = 7.7$ Hz), 4.96 ($d, J = 7.7$ Hz), and 4.65 ($d, J = 7.3$ Hz), which correlated in the HSQC spectrum with ^{13}C-NMR signals at δ 100.0, 93.8, 103.2, 103.6, 105.0, 104.0, and 102.8, respectively. Comparison of the 2D-NMR signals of **3** and **2** indicated the loss of the signals of Xyl III at C(3) of Xyl II. This was confirmed by the ^{1}H- and ^{13}C-NMR data (*Tables 2* and *3*) of **3**. The Xyl in **3** was terminal (C(2) at δ(C) 74.2, and C(3) at δ(C) 77.0) instead of being 1,3-substituted (*cf.* **2**: C(2) at δ(C) 74.3 and C(3) at δ(C) 86.3) and was in accordance with the difference in the molecular mass (132 mass units) between **2** and **3**.

The negative-ion FAB-MS of **4** showed a quasi-molecular ion peak at *m*/*z* 1133 ($[M-H]^-$), indicating a molecular mass of 1134, which suggested a molecular formula $C_{54}H_{86}O_{25}$. On the basis of the 2D-NMR spectral data and hydrolysis, the structure of **4** was established as gypsogenic acid 28-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl} ester, a new natural compound [1–21].

The ^{1}H- and ^{13}C-NMR signals of the aglycone assigned from 2D-NMR spectra of **4** were superimposable to those of gypsogenic acid [21]. The ^{1}H-NMR spectrum of **4** displayed signals for four anomeric protons at δ(H) 6.08 ($d, J = 7.8$ Hz), 5.26 ($d, J = 7.8$ Hz), 5.20 ($d, J = 7.8$ Hz), and 4.86 ($d, J = 7.8$ Hz), which correlated in the HSQC spectrum δ(C) 94.5, 104.8, 104.8, and 102.8, respectively. The ring protons of the monosaccharide residues were assigned starting from the anomeric protons by means of the COSY, TOCSY, HSQC, and HMBC plots (*Table 2*), and the sequence of the oligosaccharide chains was obtained from the HMBC and NOESY experiments. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one β-D-galactopyranosyl (Gal), and three β-D-glucopyranosyl (Glc) units, the common D-configuration for Glc and Gal being assumed, according to those most encountered among the plant glycosides in each case. The hexoses with the anomeric-proton signals at δ(H) 5.26, 5.20, and 4.86 ($d, J = 7.8$) were deduced to be glucose and not galactose since δ(H) 4.06 (Glc I H–C(3)), 4.18 (Glc II H–C(3)), and 4.16 (Glc III H–C(3)) showed up as *t* due to equal couplings with vicinal protons [22]. The HMBC and NOESY plots allowed us to establish the sequence of the sugars that were linked at C(28) (*cf.* δ(C) 75.6 (Agly C(3)), 184.0 (Agly C(23), 176.4 (Agly C(28)). A correlation in the HMQC spectrum at δ(H) 6.08 ($d, J = 7.8$ Hz, Gal I H–C(1))/δ(C) 94.5 (Gal I C(1)) showed that the Gal I residue was linked to the carboxylic group of the aglycone at C(28) by an ester linkage. This conclusion was supported by the HMBC correlation between δ(H) 6.08 ($d, J = 7.8$ Hz, Gal I H–C(1)) and δ(C) 176.4 (Agly C(28)). Moreover, the HMBC correlation between δ(H) 5.20 ($d, J = 7.8$ Hz, Glc III H–C(1)) and δ(C) 82.4 (Glc II C(2)), together with a NOESY cross-peak between δ(H) 5.20 ($d, J = 7.8$ Hz, Glc III H–C(1)) and δ(H) 3.98 (Glc II H–C(2)) showed that Glc III was linked to C(2) of Glc II. The HMBC correlation between δ(H) 4.86 ($d, J = 7.8$ Hz, Glc II H–C(1)) and δ(C) 68.4 (Glc I C(6)) as well as a NOESY cross-peak between δ(H) 4.86 ($d, J = 7.8$ Hz, Glc II H–C(1)) and 4.42 (Glc 1 H–C(6)) showed that Glc II was linked to Glc

I by a (1 → 6) linkage. Furthermore, the HMBC correlation between δ(H) 5.26 (*d*, *J* = 7.8 Hz, Glc I H–C(1)) and δ(C) 86.7 (Gal I C(3)), together with a NOESY cross-peak between δ(H) 5.26 (*d*, *J* = 7.8 Hz, Glc I H–C(1)) and δ(H) 4.24 (Gal I H–C(3)) showed that Glc I was linked to C(3) of Gal I.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*). Medium-pressure liquid chromatography (MPLC): silica gel *60* (*Merck*, 15–40 μm), *Gilson* pump *M 305*, *Büchi* column (460 × 25 mm and 460 × 15 mm), *Büchi* precolumn (110 × 15 mm). TLC and HPTLC: silica gel *60* F_{254} (*Merck*); solvent systems: for saponins, $CHCl_3$/MeOH/AcOH/H_2O 15 : 8 : 3 : 2 (*a*); for sapogenins, $CHCl_3$/MeOH 9 : 1 (*b*); for monosaccharides, $CHCl_3$/MeOH/H_2O 8 : 5 : 1 (*c*); spray reagents: for saponins, *Komarowsky* reagent, a 5 : 1 mixture of 2% 4-hydroxybenzaldehyde in MeOH and 50% H_2SO_4 soln.; for sugars; diphenylamine/phosphoric acid reagent. IR Spectra (KBr disc): *Perkin-Elmer 281* IR spectrophotometer; in cm^{-1}. 1D- and 2D-NMR spectra (^1H,^1H-COSY, TOCSY, NOESY, HSQC, and HMBC); *Unity-600* spectrometer at the operating frequency of 600 MHz on a *Varian nova-600* instrument equipped with a *SUN-4-L-X* computer system (600 MHz for ^1H and 150 MHz for ^{13}C); conventional pulse sequences for COSY, HSQC, and HMBC; standard MLEV17 spin-locking sequence and 90 ms mixing time for TOCSY; 500 ms mixing time for NOESY; C-type (Me, CH_2, CH) by DEPT experiments; chemical shifts δ in ppm, *J* in Hz; (D_5)pyridine solns. (δ(C) 150.3, 155.9, 123.9). Fast-atom-bombardment (FAB) MS: negative mode; *Jeol SX-102*.

Plant Material. The roots of *Acanthophyllum pachystegium* K. H. were collected from the northeastern part of Iran, Khorasan province, and identified by Mr. *Joharchi*. A voucher specimen under the reference No. 003-0116-1 is deposited in the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Extraction and Isolation. Dried powdered roots (500 g) were defatted in a *Soxhlet* apparatus with 3 l of petroleum ether for 24 h. The air-dried plant was extracted with 5 l of MeOH for 48 h yielding, after evaporation, a syrupy brown residue (30 g). The MeOH extract was dissolved in 400 ml of H_2O and partitioned with H_2O-sat. BuOH (3 × 300 ml) to give, after evaporation, the BuOH fraction (10 g). It was solubilized in MeOH (10 ml) and precipitated in Et_2O (3 × 250 ml): 7.5 g of crude saponin mixture. Of this mixture, a sample (2 g) was submitted to dialysis for 3 days and then lyophilized. The residue obtained (1.2 g) was dissolved in MeOH and submitted to CC (*Sephadex LH-20*, MeOH) and then to successive MPLC (silica gel *60* (15–40 μm); $CHCl_3$/MeOH/H_2O 8 : 5 : 1): **1** (10 mg), **2** (8.5 mg), **3** (8 mg), and **4** (9 mg).

*(3β,4α,16α)-3-{*O*-β-*D*-Galactopyranosyl-(1 → 2)*-O*-[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucuronopyranosyl-oxy}-16-hydroxy-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*-[3,4-di-O*-acetyl-6-deoxy-β-*D*-glucopyranosyl-(1 → 4)]-6-deoxy-β-*D*-galactopyranosyl} Ester* (**1**). White amorphous powder: TLC: R_f 0.31. $[\alpha]_D^{25} = -27.2$ (*c* = 0.05, MeOH). IR (KBr): 3500–3300, 2930, 1740, 1615, 1390. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1699 ($[M - H - 42]^-$), 1567 ($[M - H - 42 - 132]^-$), 1435 ($[M - H - 42 - 132 - 132]^-$).

*(3β,4α,16α)-3-{*O*-β-*D*-Galactopyranosyl-(1 → 2)*-O*-[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucuronopyranosyl-oxy}-16-hydroxy-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*-acetyl-6-deoxy-β-*D*-glucopyranosyl-(1 → 4)]-6-deoxy-β-*D*-galactopyranosyl} Ester* (**2**). White amorphous powder: TLC: R_f 0.17. $[\alpha]_D^{25} = -32.2$ (*c* = 0.05, MeOH). IR (KBr): 3500–3300, 2926, 1734, 1615, 1417. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1567 ($[M - H - 132]^-$), 1435 ($[M - H - 132 - 132]^-$).

*(3β,4α,16α)-3-{*O*-β-*D*-Galactopyranosyl-(1 → 2)*-O*-[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucuronopyranosyl-oxy}-16-hydroxy-23-oxoolean-12-en-28-oic Acid 28-{*O*-β-*D*-Xylopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O*-[4-O*-acetyl-6-deoxy-β-*D*-glucopyranosyl-(1 → 4)-6-deoxy-β-*D*-galactopyranosyl} Ester* (**3**). White amorphous powder: TLC: R_f 0.22. $[\alpha]_D^{25} = -10.4$ (*c* = 0.05, MeOH). IR (KBr): 3500–3300, 2932, 1734, 1616, 1399. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1567 ($[M - H]^-$).

*(3β,4α)-3-Hydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-β-*D*-Glucopyranosyl-(1 → 2)*-O*-[β-*D*-glucopyranosyl-(1 → 6)]-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranosyl} Ester* (**4**). White amorphous powder: TLC: R_f 0.35. $[\alpha]_D^{25} = -18.6$ (*c* = 0.05, MeOH). IR (KBr): 3500–3300, 2930, 1740, 1615, 1390. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1133 ($[M - H]^-$).

Acid Hydrolysis. A mixture of saponin (5 mg) in H_2O (2 ml) and 2N aq. CF_3COOH (5 ml) was refluxed on a water bath for 3 h. After extraction with $CHCl_3$ (3 × 5 ml), the aq. layer was repeatedly evaporated with MeOH until neutral and then analyzed by TLC (solvent *c*) by comparison with standard sugars.

Alkaline Hydrolysis. The saponin (5 mg) was refluxed in the presence of 5% aq. KOH soln. (10 ml) for 2 h. The mixture was adjusted to pH 6 with dil. HCl soln. and then extracted with H_2O-sat. BuOH (3×10 ml). The combined BuOH extracts were washed with H_2O and evaporated: prosapogenin.

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