Junceosides A−C, New Triterpene Saponins from *Arenaria juncea*

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Three novel triterpenoid saponins, junceosides A (**1**), B (**2**), and C (**3**), together with two known saponins have been isolated from the roots of *Arenaria juncea*. 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 3-*O*-α-L-arabinopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-glucopyranosyl(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (**1**), 3-*O*-α-L-arabinopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (**2**), and 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (**3**).

In a continuation of our study on saponin constituents of medicinal plants of the Caryophyllaceae family,[1−3] we have examined the saponin fraction of *Arenaria juncea*. This plant is well-known in the Traditional Chinese Medicine in the Shanxi, Gansu Hubei province as a substitute for the Chinese drug Yin-Chai-Hu (root of *Stellaria dichotoma* var. *lanceolata* Bge)[4−7] and is utilized to treat fever due to Yin-deficiency and fever in infant malnutrition. No previous phytochemical investigation has been reported on *A. juncea*. In this paper, we describe the isolation and structure elucidation of three new triterpenoid saponins designated as junceosides A, B, and C (**1**−**3**), having gypsogenin as aglycon, along with two known saponins, previously isolated as G4 and G2 from *Gypsophila paniculata* and *Gypsophila arrostii*.[8]

Results and Discussion

The concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *Arenaria juncea* M. Bieb. (Caryophyllaceae) was purified by precipitation with diethyl ether. The 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 junceosides A (**1**), B (**2**), and C (**3**) (Figure 1) and the saponins G4 and G2.[8]

Compound **1** was obtained as an amorphous powder. The FABMS (glycerol matrix, negative-ion mode) of **1** showed a quasimolecular ion peak at *m*/*z* 1525 $[M - H]^-$, indicating a molecular weight of 1526, compatible with the molecular formula $C_{70}H_{110}O_{36}$. Another significant ion peak visible at *m*/*z* 1363 $[(M - H) - 162]^-$ corresponded to the loss of one hexosyl unit. Acid hydrolysis of **1** with 2 N TFA afforded an aglycon identified as gypsogenin by TLC comparison with an authentic sample. This identification was confirmed on the basis of the ^{1}H NMR, ^{13}C NMR, DEPT, HSQC, and HMBC spectra of **1**.[1,3] The sugars obtained from the saponin hydrolysates were identified as galactose, arabinose, glucose, xylose, rhamnose, fucose, and glucuronic acid (co-TLC), respectively. Alkaline hydrolysis of **1** performed with 5% KOH yielded a prosapogenin which, by further acid hydrolysis, furnished glucuronic acid, arabinose, galactose, and gypsogenin. These data indicated that **1** must be a triterpene-bidesmosidic saponin in which glucuronic acid, arabinose, and galactose were bound to the aglycon by a glycosidic linkage at C-3, while the remaining sugars must be bound to the genin by a glycosidic ester linkage at C-28. This was confirmed by the signals observed in the ^{13}C NMR spectrum of **1** at δ_C 85.0 (downfield shift of C-3 of the aglycon) and δ_C 176.4 (upfield shift of C-28 of the aglycon).

Compound **1** was shown to contain seven sugar residues from the HSQC spectrum. The anomeric ^{1}H NMR signals at δ 5.80, 5.74, 5.29, 5.25, 5.20, 5.12, and 4.64 gave correlations with ^{13}C NMR signals at δ 94.5, 101.6, 103.3, 104.4, 104.6, 103.9, and 103.1, 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. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one β-fucopyranosyl (Fuc), one α-rhamnopyranosyl (Rha), one α-arabinopyranosyl (Ara), one β-xylopyranosyl (Xyl), one β-glucopyranosyl (Glc), one β-galactopyranosyl (Gal), and one β-glucuronopyranosyl (GlcA) units, respectively. The common D-configuration for Fuc, Gal, Glc, Xyl, and GlcA and the L-configuration for Rha and Ara were assumed, being the most frequently encountered among the plant glycosides.

The cross-peaks in the HMBC experiment between δ_H 5.12 (d, J = 6.9 Hz) (Gal-1) and δ_C 85.2 (GlcA-3) and correlations in the NOESY experiment between signals at δ_H 4.64 (d, J = 7.3 Hz) (GlcA-1) and δ_H 3.94 (Agly-3), at δ_H 5.29 (br s) (Ara-1) and δ_H 4.18 (GlcA-2), as well as between signals at δ_H 5.12 (d, J = 6.9 Hz) (Gal-1) and δ_H 4.16 (GlcA-3), showed that the trisaccharide moiety 3-*O*-α-L-arabinopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucuronopyranosyl was linked to the gypsogenin at C-3.

Chemical shifts at δ_H 5.80 (d, J = 8.1 Hz) (Fuc-1) and δ_C 94.5 (Fuc-1) indicated that this sugar unit was involved in an ester linkage with the C-28 carboxylic group. This was confirmed by the correlation in the HMBC spectrum between the ^{1}H NMR signal at δ_H 5.80 (d, J = 8.1 Hz) (Fuc-

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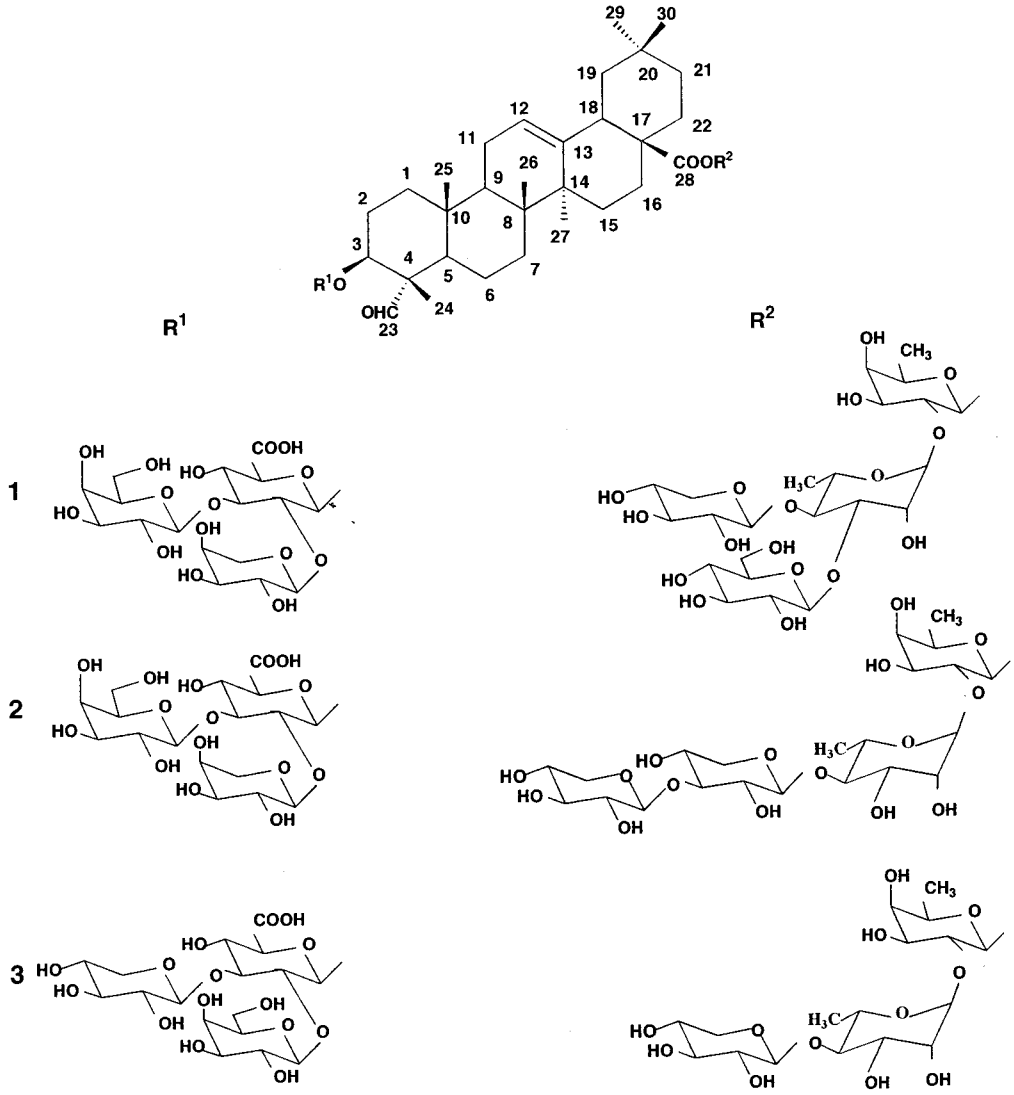

Figure 1.

1) and the ^{13}C NMR signal at δ_C 176.4 (Agly C-28). The chemical shifts of the ester glycosidic chain at C-28 assigned from the 2D NMR spectra (Tables 1 and 2) were fully superimposable with those of the ester part of G4 in *Gypsophila* species.[8]

On the basis of the above results, **1** was determined to be 3-*O*-α-L-arabinopyranosyl-(1→2)-[*β*-D-galactopyranosyl-(1→3)]-*β*-D-glucuronopyranosylgypsogenin-28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside. According to several reports on the distribution of gypsogenin glycosides in Caryophyllaceae, and after a search of its literature, **1** appears to be a new natural compound.[8−22]

Compound **2** was obtained as an amorphous powder. The FABMS (glycerol matrix, negative-ion mode) of **2** showed a quasimolecular ion peak at *m*/*z* 1495 $[M - H]^-$, indicating a molecular weight of 1496, compatible with the molecular formula $C_{69}H_{108}O_{35}$. Other significant ion peaks visible at *m*/*z* 1363 $[(M - H) - 132]^-$, 1231 $[(M - H) - 132-132]^-$, corresponded to the successive losses of two pentosyl moieties. Another fragment ion peak at *m*/*z* 939 corresponded to a hexosyl-pentosyl-hexosyluronic acid-aglycon. Mineral acid hydrolysis of **2** afforded an aglycon identified as gypsogenin by TLC comparison with an authentic sample. This identification was confirmed from the ^1H NMR, ^{13}C NMR, DEPT, HSQC, and HMBC spectra of **2**.[1−3] The sugars obtained from the saponin hydrolysates were identified as galactose, arabinose, xylose, rhamnose, fucose, and glucuronic acid (co-TLC), respectively.

Compound **2** was shown to contain seven sugar residues from the HSQC spectrum. The anomeric ^1H NMR signals at δ 6.10, 5.72, 5.24, 5.09, 4.94, 4.90, and 4.67 gave correlations with ^{13}C NMR signals at δ 100.3, 94.0, 102.6, 103.4, 104.4, 105.6, 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 α-arabinopyranosyl (Ara), one *β*-galactopyranosyl (Gal), two *β*-xylopyranosyl (Xyl), and one *β*-glucuronopyranosyl (GlcA) unit, respectively. The common D-configuration for Fuc, Gal, Xyl, and GlcA and the L-configuration for Rha and Ara were assumed.

The ^1H and ^{13}C NMR data of **2** obtained from the 2D NMR experiments (Tables 1 and 2) revealed that the carbohydrate moiety at C-3 was identical with that of **1**, indicating that the four remaining sugars were connected to C-28. The sugar chain at C-28 was established from the following HMBC correlations: H-1 of terminal xylose at δ_{H-1} 4.94 (d, $J = 7.7$ Hz) (T-Xyl) and C-3 of inner xylose at δ_{C-3} 86.1 (Xyl-1,3), H-1 of inner xylose at δ_{H-1} 4.90 (d, $J =$ 7.7 Hz) (Xyl-1,3) and C-4 of Rha at δ_{C-4} 83.7, and H-1 of Rha at δ_{H-1} 6.10 (brs) and C-2 of Fuc at δ_{C-2} 73.0. Correlations were also observed between δ_{H-3} 3.88 (Xyl-

Table 1. ^{13}C NMR Data (δ) of the Sugar Moieties of Compounds **1**−**3** (Pyridine-d_5)[a,b]

1		**2**		**3**	
3-*O*-sugars		3-*O*-sugars		3-*O*-sugars	
GlcA1	*103.1*	GlcA1	*102.7*	GlcA1	*103.2*
2	*77.5*	2	*77.1*	2	*77.4*
3	*85.2*	3	*84.2*	3	*85.6*
4	68.4	4	68.6	4	69.5
5	77.2	5	76.6	5	76.4
6	171.9	6	171.9	6	171.9
Ara 1	*103.3*	Ara 1	*102.6*	Xyl 1	*104.1*
2	72.1	2	72.2	2	75.6
3	74.0	3	74.0	3	77.4
4	69.3	4	70.9	4	69.7
5	66.5	5	66.7	5	66.5
Gal 1	*103.9*	Gal 1	*103.4*	Gal 1	*103.4*
2	71.8	2	71.8	2	72.8
3	74.0	3	73.2	3	74.5
4	70.9	4	69.7	4	67.8
5	76.2	5	75.8	5	75.9
6	61.9	6	61.3	6	61.4
28-*O*-sugars		28-*O*-sugars		28-*O*-sugars	
Fuc 1	*94.5*	Fuc 1	*94.0*	Fuc 1	*94.3*
2	*74.8*	2	*73.0*	2	*73.6*
3	74.0	3	75.4	3	75.6
4	71.8	4	72.0	4	72.5
5	71.4	5	71.6	5	71.9
6	16.3	6	16.1	6	16.4
Rha 1	*101.6*	Rha 1	*100.3*	Rha 1	*100.8*
2	69.8	2	70.6	2	71.1
3	*81.8*	3	71.3	3	71.8
4	*78.5*	4	*83.7*	4	*84.5*
5	68.4	5	67.4	5	67.9
6	18.4	6	17.6	6	18.0
T-Glc 1	*104.6*	Xyl 1	*105.6*	T-Xyl 1	*106.8*
2	74.0	2	74.3	2	74.5
3	77.2	3	*86.1*	3	77.7
4	70.4	4	68.1	4	70.2
5	77.1	5	65.6	5	66.8
6	61.4				
T-Xyl 1	*104.4*	T-Xyl 1	*104.4*		
2	74.5	2	74.0		
3	77.5	3	76.5		
4	69.2	4	69.7		
5	66.3	5	66.0		

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

1,3) and δ_{C-3} 104.4 (T-Xyl), at δ_{H-4} 4.10 (Rha) and δ_{C-1} 105.6 (Xyl-1,3), and at δ_{H-2} 4.44 (Fuc) and δ_{C-1} 100.3 (Rha). This linkage was confirmed by the NOESY correlation between δ_H 6.10 (brs) (Rha-1) and δ_H 4.44 (Fuc-2), at δ_{H-1} 4.90 (d, J = 7.7 Hz) (Xyl-1,3) and δ_H 4.10 (Rha-4), and at δ_H 4.94 (d, J = 7.7 Hz) (T-Xyl-1) and δ_{H-3} 3.88 (Xyl-1,3). The NMR data of this saponin were very similar to those obtained for **1**, except for the absence of NMR data of the glucosyl group attached at Rha-3 in compound **1** and appearance of a set of additional signals, corresponding to a terminal β-D-xylopyranosyl group in **2** which was attached at Xyl(1−3). On the basis of the above results, compound **2** is 3-*O*-α-L-arabinopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, a new natural compound.[8−22]

Compound **3** was obtained as an amorphous powder. Its FABMS (glycerol matrix, negative-ion mode) showed a quasimolecular ion peak at m/z 1363 $[M - H]^-$, indicating a molecular weight of 1364, compatible with the molecular formula $C_{64}H_{100}O_{31}$. Mineral acid hydrolysis of **3** afforded gypsogenin and sugars identified as galactose, xylose, rhamnose, fucose, and glucuronic acid (co-TLC), respectively. Alkaline hydrolysis of **3** performed with 5% KOH yielded a prosapogenin which furnished, by further acid hydrolysis, galactose, xylose, glucuronic acid, and gypsogenin, of which 2D NMR data were in good agreement with those of the prosapogenin of squarroside A identified as 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosylgypsogenin.[3]

Compound **3** was investigated by TOCSY, COSY, NOESY, HSQC, and HMBC NMR experiments and full assignments of all ^{1}H and ^{13}C resonances were obtained (Tables 1 and 2). The anomeric ^{1}H NMR signals at δ_H 6.25, 5.87, 5.35, 5.15, 4.90, and 4.64 gave correlations with anomeric ^{13}C NMR signals at δ_C 100.8, 94.3, 103.4, 104.1, 106.8, and 103.2, respectively. The NMR spectral data of **3** at C-28 were similar to those of **2** except the absence of signals due to the xylosyl moiety at the position 3 of xylose. On the basis of the above results, compound **3** is 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, a new natural compound.[8−22]

Two additional compounds were isolated and identified by analysis of their spectral data (FABMS and 2D NMR) as 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-glucopyranosyl (1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside and 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosylquillaic acid-α-L-arabinopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, known saponins previously characterized as G4 and G2 from *Gypsophila paniculata* and *Gypsophila arrostii*.[8]

Experimental Section

General Experimental Procedures. The 1D and 2D NMR spectra ($^{1}H-^{1}H$ COSY, TOCSY, NOESY, HSQC, and HMBC) were performed using a UNITY-600 spectrometer at the operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz for ^{1}H and 150 MHz for ^{13}C spectra). Conventional pulse sequences were used for COSY, HSQC, and HMBC. TOCSY spectra were acquired using the standard MLEV17 spin-locking sequence and 90 ms mixing time. The mixing time in the NOESY experiment was set to 500 ms. The carbon type (CH_3, CH_2, CH) was determined by DEPT experiments. All chemical shifts (δ) are given in ppm, and the samples were solubilized in pyridine-d_5 (δ 148.9). Fast-atom bombardment (FABMS) (negative-ion mode, glycerol matrix) was conducted on a JEOL SX 102. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. IR spectra (KBr disk) were recorded on a Perkin-Elmer 281 spectrophotometer. TLC and HPTLC employed precoated Si gel plates 60 F_{254} (Merck). The following TLC solvent systems were used: for saponins (a) $CHCl_3$−MeOH−AcOH−H_2O (15:8:3:2); for sapogenins (b) toluene−Me_2-CO (4:1); for monosaccharides (c) $CHCl_3$−MeOH−H_2O (8:5:1). Spray reagents for the saponins were Komarowsky reagent, a mixture (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and H_2SO_4 50%; for the sugars, diphenylaminephosphoric acid reagent. Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Gilson pump M 303, head pump 25 SC, manometric module M 802, Büchi column (460 × 25 mm and 460 × 15 mm), Büchi precolumn (110 × 15 mm)].

Plant Material. The roots of *Arenaria juncea* were collected in July 1990 in Hubei Province, People's Republic of China. A voucher specimen (No. 48-11) is deposited in the Herbarium

Table 2. ^{1}H NMR Data (δ) of the Sugar Moieties of Compounds **1**−**3** (Pyridine-d_5)[a,b]

1		**2**		**3**	
3-*O*-sugars	(*J* Hz)	3-*O*-sugars	(*J* Hz)	3-*O*-sugars	(*J* Hz)
GlcA1	*4.64 (d, J = 7.3)*	GlcA1	*4.67 (d, J = 6.9)*	GlcA1	*4.64 (d, J = 7.3)*
2	*4.18*	2	*4.16*	2	*4.18*
3	*4.16*	3	*4.18*	3	*4.10*
4	4.12	4	4.10	4	4.34
5	4.04	5	4.08	5	4.15
6	-	6	-	6	-
Ara 1	*5.29 (br s)*	Ara 1	*5.24 (br s)*	Xyl 1	*5.15 (d, J = 7.7)*
2	4.18	2	4.14	2	3.85
3	3.95	3	3.95	3	4.04
4	4.22	4	4.08	4	4.05
5	3.68, 4.22	5	3.70, 4.18	5	3.58, 4.24
Gal 1	*5.12 (d, J = 6.9)*	Gal 1	*5.09 (d, J = 7.3)*	Gal 1	*5.35 (d, J = 7.7)*
2	4.25	2	4.20	2	4.28
3	3.78	3	3.98	3	4.02
4	4.10	4	4.00	4	4.33
5	3.78	5	3.78	5	3.88
6	4.04, 4.32	6	4.08, 4.24	6	4.25, 4.33
28-*O*-sugars		28-*O*-sugars		28-*O*-sugars	
Fuc 1	*5.80 (d, J = 8.1)*	Fuc 1	*5.72 (d, J = 8.0)*	Fuc 1	*5.87 (d, J = 8.0)*
2	*4.32*	2	*4.44*	2	*4.55*
3	4.02	3	4.03	3	4.09
4	3.95	4	3.96	4	3.95
5	3.78	5	3.82	5	3.85
6	1.35 (d, *J* = 6.4)	6	1.38 (d, *J* = 6.4)	6	1.40 (d, *J* = 6.4)
Rha 1	*5.74 (s)*	Rha 1	*6.10 (s)*	Rha 1	*6.25 (s)*
2	5.06 (d, *J* = 2.9)	2	4.60	2	4.74 (d, *J* = 2.9)
3	*4.68*	3	4.38	3	4.52
4	*4.16*	4	*4.10*	4	*4.19*
5	4.28	5	4.22	5	4.32
6	1.52 (d, *J* = 6.1)	6	1.53 (d, *J* = 6.1)	6	1.58 (d, *J* = 6.2)
T- Glc 1	*5.20 (d, J = 7.7)*	Xyl 1	*4.90 (d, J = 7.7)*	T-Xyl 1	*4.90 (d, J = 7.7)*
2	3.92	2	3.80	2	3.88
3	4.01	3	*3.88*	3	3.98
4	3.89	4	3.90	4	4.08
5	3.80	5	3.42, 4.08	5	3.44, 4.12
6	4.12, 4.28				
T- Xyl 1	*5.25 (d, J = 8.0)*	T-Xyl 1	*4.94 (d, J = 7.7)*		
2	3.74	2	3.86		
3	3.96	3	3.96		
4	4.18	4	4.18		
5	3.34, 4.01	5	3.56, 4.12		

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

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Extraction and Isolation. Dried powdered roots of *Arenaria juncea* (639 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 *n*-BuOH (3 × 200 mL). After evaporation of the solvent under reduced pressure, 23 g of the *n*-BuOH extract was obtained. The *n*-BuOH extract was solubilized in MeOH (10 mL) and precipitated in Et_2O (3 × 250 mL), yielding 7 g of a crude saponin fraction, of which 4 g was dialyzed for 3 days and submitted to column chromatography on Sephadex LH-20 eluted by MeOH, yielding 0.8 g of a white powder. This mixture was first fractionated by MPLC silica gel 60 (15−40 μm) using as eluent $CHCl_3$−MeOH−H_2O (8:5:1). Further separations were performed by successive MPLC on reversed-phase material, Lichroprep RP-18, Merck (40−63 μm) eluted with MeOH−H_2O (linear gradient 50−60%), to give compounds **1** (13 mg), **2** (11 mg), **3** (13 mg), saponin G-4 (25 mg),[8] and saponin G-2 (22 mg).[8]

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}; ^{13}C NMR of gypsogenin,[8] ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) of sugar moieties, Tables 1 and 2; negative FABMS (glycerol matrix) *m*/*z* 1525 [M − H]$^-$, 1363 [(M − H) − 162]$^-$.

Compound 2: white amorphous powder; TLC R_f 0.48 (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,[8] ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) of sugar moieties, Tables 1 and 2; negative FABMS (glycerol matrix) *m*/*z* 1495 [M − H]$^-$, 1363 [(M − H) − 132]$^-$, 1231 [(M − H) − 132 − 132]$^-$.

Compound 3: white amorphous powder; TLC R_f 0.53 (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,[8] ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) of sugar moieties, Tables 1 and 2; negative FABMS (glycerol matrix) *m*/*z* 1363 [M − H]$^-$.

Saponin G-4: the spectral data were in full agreement with previously published data.[8]

Saponin G-2: the spectral data were in full agreement with previously published data.[8]

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 vacuo. Evaporation of the solvent

gave gypsogenin (co-TLC with an authentic sample). After evaporation to dryness of the aqueous layer with MeOH until neutral, the sugars were analyzed by silica gel TLC by comparison with standard sugars (solvent system c).

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 arabinose for **1** and **2**, and glucuronic acid, galactose, and xylose for **3** (co-TLC with authentic samples).

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