Chionaeosides A–D, Triterpene Saponins from *Paronychia chionaea*

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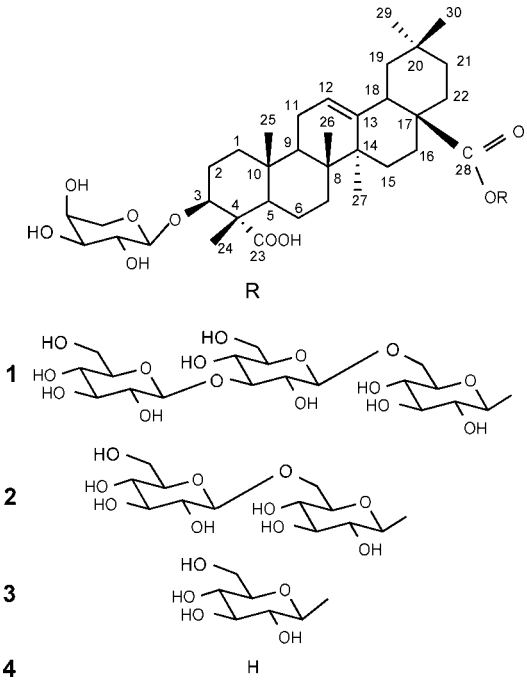
Four new triterpenoid saponins, chionaeosides A–D (**1**–**4**) were isolated from the roots of *Paronychia chionaea*. On the basis of their spectroscopic data, the structures of the new saponins were established as 3-*o*-α-L-arabinopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**), 3-*O*-α-L-arabinopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**2**), 3-*O*-α-L-arabinopyranosylgypsogenic acid 28-*O*-*β*-D-glucopyranoside (**3**), and 3-*O*-α-L-arabinopyranosylgypsogenic acid (**4**).

Paronychia, a genus of the family Illecebraceae, is represented by 28 species in the flora of Turkey. *Paronychia chionaea* Boiss. is an endemic plant, which is a prostrate caespitose herb, often woody at the base.[1] Although there are no reports of the medicinal uses of *P. chionaea*, *P. argentea* Lam. has been used in Palestinian and Jordanian traditional medicine for its hypoglycemic activity and to treat disorders of the urinary system.[2,3] Our present investigation on *P. chionaea* has demonstrated that gypsogenic acid-type saponins are the main constituents of this species. To the best of our knowledge, gypsogenic acid-type saponins are being reported for the first time in the family Illecebraceae, which has been recently separated from the family Caryophyllaceae.[4–6] This is also the first report on the isolation and the structural elucidation of triterpene glycosides (chionaeosides A–D; **1**–**4**) from a *Paronychia* species.

The MeOH extract of the dried roots of *P. chionaea* was partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, respectively. The *n*-BuOH fraction was submitted to multiple chromatographic steps involving vacuum-liquid chromatography on reversed-phase RP-18 silica gel, liquid column chromatography on normal-phase silica gel and Sephadex LH-20, and medium-pressure liquid chromatography over silica gel, to yield chionaeaosides A–D (**1**–**4**). Their structures were elucidated using chemical and spectroscopic methods, including 2D NMR techniques.[5,7,8]

Chionaeoside A (**1**) was obtained as a white, amorphous powder. It was assigned the molecular formula $C_{53}H_{84}O_{24}$ from its positive-ion HRESIMS ($[M + Na]^+$, *m/z* 1127.5266, calcd for 1127.5250) and confirmed by ^{13}C NMR and DEPT analysis. Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 1103 $[M - H]^-$, indicating a molecular weight of 1104, corresponding to $C_{53}H_{84}O_{24}$. Further fragment ion peaks were observed at *m/z* 941 $[(M - H) - 162]^-$, *m/z* 617 $[(M - H) - 162 - 162 - 162]^-$, and *m/z* 485 $[(M - H) - 162 - 162 - 162 - 132]^-$, corresponding to the successive loss of one hexosyl, two hexosyls, and one pentosyl moiety, respectively. The fragment ion at *m/z* 485 is related to the pseudomolecular ion of the aglycon. The ^{13}C NMR and DEPT NMR spectra displayed 53 signals, of which 23 could be readily assigned to the saccharide portion and the remaining 30 to the triterpene skeleton. On acid hydrolysis with 2 N TFA, **1** gave sugar moieties that were identified as glucose and arabinose by co-TLC with authentic samples and an aglycon that was identified as gypsogenic acid from the 1D and 2D NMR spectra of **1**, when compared with literature data (Table 1).[4,9] Thus, the ^1H NMR spectrum of **1** displayed five singlets for six tertiary methyl protons at δ_H 1.44, 1.15, 0.94, 0.83, 0.78 (×2), and an olefinic proton at δ_H 5.33. The ^{13}C NMR spectrum revealed the presence of six methyl carbons giving correlations in the HMQC spectrum with the above-mentioned singlets, a pair of olefinic carbons at δ_C 122.3 (CH) and 144.0 (C), and two carboxylic groups at δ_C 176.3 and 184.0. The downfield ^{13}C NMR chemical shift at δ_C 86.4 and the upfield ^{13}C NMR chemical shift at δ_C 176.3 suggested that **1** is a bidesmosidic saponin with glycosidic linkages at C-3 through an O-heterosidic bond and at C-28 through an ester bond.



Chionaeoside A (**1**) was shown to contain four sugar residues from the HMQC spectrum. The four anomeric ^1H NMR signals at δ_H 4.91 (d, $J = 6.9$ Hz), 6.04 (d, $J = 8.3$ Hz), 4.83 (d, $J = 7.9$ Hz), and 5.19 (d, $J = 7.9$ Hz) displayed correlations with the four

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Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for the Aglycon Moieties of Compounds **1**–**4** (pyridine-d_5)[a,b,c]

	1			**2**		**3**		**4**	
position	mult.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	CH_2	38.8	1.01, 1.44	38.7	1.01, 1.44	38.5	1.01, 1.46	38.4	1.10, 1.54
2	CH_2	25.8	1.15, [c]	25.8	1.15, [c]	25.9	1.16, [c]	27.8	1.12, 2.05
3	CH	86.4	4.48	86.6	4.50	85.5	4.49	85.3	4.55
4	C	53.7		53.7		53.3		53.3	
5	CH	51.5	1.92	51.6	1.96	51.6	1.86	51.6	1.93
6	CH_2	23.5	[c]	23.5	[c]	23.5	0.78, 0.91	23.4	[c]
7	CH_2	33.5	1.00, 1.20	33.6	1.00, 1.20	33.5	0.98, 1.23	33.7	1.18, 1.40
8	C	39.9		39.9		39.8		39.6	
9	CH	47.8	1.71	47.8	1.73	47.8	1.72	47.8	1.78
10	C	36.2		36.3		36.2		36.2	
11	CH_2	23.3	1.81, 1.92	23.3	1.84, 1.94	23.0	1.84, 1.96	23.2	1.90, 2.05
12	CH	122.3	5.33	122.0	5.30	122.2	5.34	121.9	5.44
13	C	144.0		144.0		143.9		144.5	
14	C	41.8		41.8		41.7		41.8	
15	CH_2	27.7	1.00, 2.02	27.8	1.06, 2.10	27.7	1.07, 2.13	27.8	[c]
16	CH_2	21.1	1.40, 1.48	21.0	1.46, 1.52	20.9	1.43, [c]	20.8	1.53, [c]
17	C	46.7		46.7		46.7		46.3	
18	CH	41.3	3.04	41.3	3.07	41.3	3.06	41.5	3.22
19	CH_2	45.9	1.11, 1.63	46.0	1.12, 1.62	45.8	1.16, 1.64	46.1	1.24, 1.74
20	C	30.3		30.3		30.3		30.5	
21	CH_2	32.4	1.16, 1.58	32.8	1.18, 1.64	32.4	1.16, 1.54	32.9	1.74, 1.95
22	CH_2	32.4	1.59, 1.73	32.5	1.64, 1.74	32.0	1.54, 1.60	32.5	1.16, 1.60
23	C	184.0		184.0		183.0		182.0	
24	CH_3	13.1	1.44 s	13.1	1.44 s	12.6	1.41s	12.5	1.48 s
25	CH_3	15.7	0.83 s	15.7	0.83 s	15.6	0.81 s	15.5	0.86 s
26	CH_3	17.1	0.94 s	17.1	0.95 s	17.0	0.95 s	16.9	0.92 s
27	CH_3	25.8	1.15 s	25.8	1.15 s	25.8	1.16 s	25.8	1.25 s
28	C	176.3		176.8		176.2		180.2	
29	CH_3	32.8	0.78 s	32.8	0.78 s	32.7	0.80 s	32.9	0.93 s
30	CH_3	23.3	0.78 s	23.3	0.78 s	23.3	0.78 s	23.4	0.97 s

[a] Assignments are based on HMQC, HMBC, DEPT, TOCSY, and NOESY experiments; δ in ppm; proton coupling constants (J) in Hz are given in parentheses. [b] Overlapping ^{1}H NMR signals are reported without designated multiplicity. [c] Not determined.

anomeric ^{13}C NMR signals at δ_C 105.4, 94.6, 104.5, and 104.7, respectively. This confirmed that **1** contains four sugar units. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the COSY, TOCSY, NOESY, HMQC, and HMBC NMR spectra obtained for this compound.

Following the assignments of the protons, the ^{13}C NMR resonances of each sugar unit in **1** were identified from the HMQC and further confirmed using the HMBC spectrum. Evaluation of spin–spin couplings and chemical shifts allowed the identification of three β-glucopyranosyl (Glc) and one α-arabinopyranosyl moiety (Ara). The coupling constants indicated that the glycosidic linkage of arabinose is α and those of the glucose units are β.[10] The absolute configurations of the sugar residues were determined to be D for Glc and L for Ara by optical measurement of the sugars in the acid hydrolysate of the crude saponin mixture after preparative TLC separations (see Experimental Section). The sequence of the sugar residues was subsequently determined by HMBC and NOESY experiments. A cross-peak correlation in the HMBC spectrum between signals at δ_H 4.91 (d, J = 6.9 Hz, Ara-1) and δ_C 86.4 (Agly C-3) and a reverse correlation between δ_H 4.48 (Agly H-3) and δ_C 105.4 (Ara C-1) confirmed the presence of a 3-*o*-α-L-arabinopyranosyl unit in this aglycon. This interpretation was confirmed by a NOESY cross-peak between H_3-24 and Ara H-1. After subtraction of the anomeric signal of arabinose linked at the C-3 position (δ_C 105.4) from the total HMQC spectrum, it was revealed that the remaining signals corresponded to three sugar moieties linked to the aglycon by an ester linkage.

Assignments of the ^{1}H NMR and ^{13}C NMR signals from the TOCSY, HMQC, and HMBC spectra showed that the three remaining sugars in **1** were a terminal glucopyranose (T-Glc III), a 1,3-disubstituted glucopyranose (Glc-II), and a 1,6-disubstituted glucopyranose (Glc-I) (Table 2). In the HMBC spectrum, the cross-peaks between $\delta_{H\text{-}1}$ 5.19 (d, J = 7.9 Hz, T-Glc III-1) and $\delta_{C\text{-}3}$ 87.2 (Glc-II-3), between $\delta_{H\text{-}1}$ 4.83 (d, J = 7.9 Hz, Glc-II-1) and $\delta_{C\text{-}6}$ 68.3 (Glc-I-6), and between $\delta_{H\text{-}1}$ 6.04 (d, J = 8.3 Hz, Glc-I-1) and δ_C 176.3 (Agly C-28) showed the sequence of the sugar chain at C-28 to be 28-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl].

On the basis of the data obtained, the structure of **1** was established as 3-*O*-α-L-arabinopyranosylgypsogenic acid 28-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Chiaonaeoside B (**2**), a white, amorphous powder, exhibited in the HRESIMS (positive-ion mode) a pseudomolecular ion peak at *m/z* 965.4781 $[M + Na]^+$ (calcd 965.4722), consistent with a molecular formula of $C_{47}H_{74}O_{19}$. The negative-ion FABMS displayed a quasimolecular ion peak at *m/z* 941 $[M - H]^-$, 162 mass units lower than that of **1**, indicating a molecular weight of 942 and compatible with the molecular formula $C_{47}H_{74}O_{19}$. Other significant fragment ion peaks were observed at *m/z* 779 $[(M - H) - 162]^-$, 617 $[(M - H) - 162 - 162]^-$, and 485 $[(M - H) - 162 - 162 - 132]^-$, which revealed the subsequent loss of two hexosyl and one pentosyl moiety. All the ^{1}H and ^{13}C NMR signals assigned from the 1D and 2D NMR spectra (Table 2) of the aglycon moiety of **2** were almost superimposable on those of **1**, suggesting the aglycon to be gypsogenic acid, with sugar linkages at positions C-3 and C-28. The results of co-TLC analysis of the hydrolysate showed that the sugar components of **2** were glucose and arabinose. The occurence of three sugar units in **2** was determined by the observation of three anomeric ^{1}H NMR signals at δ_H 4.91 (d, J = 6.9 Hz), 6.06 (d, J = 8.3 Hz), and 4.87 (d, J = 7.9 Hz), giving correlations in the HMQC spectrum with anomeric carbon signals at δ_C 105.4, 95.2, and 104.4, respectively. Evaluation of spin–spin couplings, chemical shifts, and the results of acidic hydrolysis allowed the identification of two β-D-glucopyranosyl and one α-L-arabinopyranosyl unit in **2**.

The exact linkage positions for the sugar units of **2** were established using the HMBC and NOESY spectra, as described for **1**. Thus, the observation of long-range correlations in the HMBC

Table 2. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Data for the Sugar Moieties of Compounds **1**–**4** in Pyridine-d_5[a,b]

	1		**2**		**3**		**4**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-*O*-sugar								
Ara								
1	105.4	4.91 d (6.9)	105.4	4.91 d (6.9)	105.2	4.90 d (6.4)	105.3	4.93 d (6.9)
2	72.3	4.26	70.1	4.24	72.1	4.27	72.1	4.33 t (7.1)
3	73.5	4.16	73.4	4.17	73.4	4.08	73.4	4.14 dd (8.8, 3.1)
4	68.9	4.16	69.0	4.15	68.7	4.16	68.7	4.26 brs
5a	66.4	3.68 brd (12.3)	66.5	3.71 brd (11.4)	66.1	3.68 brd (11.2)	66.1	3.76 brd (10.9)
5b		4.12		4.11		4.14		4.24 dd (11.4, 2.6)
28-*O*-sugars								
Glc I								
1	94.6	6.04 d (8.3)	95.2	6.06 d (8.3)	95.2	6.10 d (5.5)		
2	73.5	4.16	73.2	4.05 t (9.2)	73.3	4.08		
3	77.5	4.08	77.5	4.09	77.9	4.20		
4	72.4	4.07	72.3	4.27	70.5	4.16		
5	77.5	3.94	77.3	3.96 m	78.6	3.92		
6a	68.3	4.16	68.7	4.21	61.6	4.22		
6b		4.47 brd (10.7)		4.58 brd (10.0)		4.32 brd (10.9)		
Glc II								
1	104.5	4.83 d (7.9)	104.4	4.87 d (7.9)				
2	74.5	3.86	74.4	3.88 t (8.5)				
3	87.2	4.18	77.7	4.18				
4	70.9	4.04	70.9	4.05				
5	77.7	3.74 m	77.7	3.75 m				
6a	61.9	4.16	61.9	4.17				
6b		4.31		4.33 brd (10.0)				
T-Glc								
1	104.7	5.19 d (7.9)						
2	74.9	3.93						
3	76.8	3.94						
4	71.0	3.96						
5	77.9	3.87						
6a	61.9	4.09						
6b		4.40 brd (11.7)						

[a] Assignments are based on DEPT, HMQC, HMBC, TOCSY, and NOESY experiments; δ in ppm; proton coupling constants (J) in Hz are given in parentheses. [b] Overlapping ^{1}H NMR signals are reported without designated multiplicity.

spectrum between δ_H 4.91 (Ara H-1) and δ_C 86.6 (Agly C-3) proved the arabinose unit to be attached at C-3 of gypsogenic acid. This attachment was confirmed by observation of a reverse correlation between δ_H 4.50 (Agly H-3) and δ_C 105.4 (Ara C-1). The sugar chain at C-28 was established from the following HMBC correlations: H-1 of the terminal glucose at $\delta_{H\text{-}1}$ 4.87 (d, J =7.9 Hz, T-Glc-1) and C-6 of the inner glucose at $\delta_{C\text{-}6}$ 68.7 (Glc-I), H-1 of the inner glucose at $\delta_{H\text{-}1}$ 6.06 (d, J = 8.3 Hz, Glc-I-1) and δ_C 176.8 (Agly-28). Correlations were also observed between H-6 of inner glucose at $\delta_{H\text{-}6a}$ 4.21 (Glc-I) and $\delta_{C\text{-}1}$ 104.4 (T-Glc). This linkage was also confirmed by the NOE correlation observed between $\delta_{H\text{-}1}$ 4.87 (d, J = 7.9 Hz) (T-Glc) and $\delta_{H\text{-}6a}$ 4.21 (Glc-I) in the NOESY spectrum. Therefore, the structure 3-*O*-α-L-arabinopyranosylgypsogenic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside was assigned to chionaeoside B (**2**).

Chionaeoside C (**3**) was isolated as a white, amorphous powder, and the molecular formula was determined as $C_{41}H_{64}O_{14}$ from the HRESIMS ([M + Na]$^+$ *m/z* 803.4239, calcd 803.4194) and ^{13}C NMR spectroscopic data. The negative-ion FABMS of **3** displayed a quasimolecular ion peak at *m/z* 779 [M − H]$^-$, indicating a molecular weight of 780. Further fragment ion peaks were observed at *m/z* 647 [(M − H) – 132]$^-$ and *m/z* 485 [(M − H) – 132 – 162]$^-$ due to the successive loss of one pentosyl and one hexosyl unit, respectively. Analysis of the 1D and 2D NMR data of compound **3** and comparison with those of **2** showed **3** differs from **2** only by the absence of the terminal glucopyranosyl unit (Table 2). Therefore, compound **3** was determined to be 3-*O*-α-L-arabinopyranosylgypsogenic acid 28-*O*-β-D-glucopyranoside.

Chionaeoside D (**4**), a white, amorphous powder, exhibited in the HRESIMS (positive-ion mode) a pseudomolecular ion peak at *m/z* 641.3689 [M + Na]$^+$ (calcd 641.3666), consistent with a molecular formula of $C_{35}H_{54}O_9$. Its FABMS showed a quasimolecular ion peak at *m/z* 617 [M − H]$^-$, indicating a molecular weight of 618. A fragment ion peak was observed at *m/z* 485 [(M − H) – 132]$^-$, corresponding to the elimination of one pentosyl unit. Comparison of ^{1}H and ^{13}C NMR data of the aglycon of **4** with those of **1**–**3** indicated that all compounds have the same aglycon moiety. This hypothesis was confirmed using 2D NMR experiments. Thus, the aglycon of **4** was also identified as gypsogenic acid. The ^{1}H NMR spectrum of **4** showed only one anomeric proton signal at δ_H 4.93 (d, J = 6.9 Hz), giving a correlation in the HMQC spectrum with the anomeric ^{13}C NMR signal at δ_C 105.3. Evaluation of spin couplings, chemical shifts, and the results of the acidic hydrolysis allowed the identification of one α-L-arabinopyranosyl group. The absence of any glycosylation shift for the 28-carboxyl group (δ_C 180.2) suggested that **4** had a free 28-carboxyl group. A comparison of the ^{13}C NMR spectrum of **4** with those of **1**–**3** showed that the arabinosyl unit is also attached at the C-3 position. An obvious chemical shift in the ^{13}C NMR spectrum at δ_C 85.3 assigned to C-3 of the aglycon supported this suggestion. Furthermore, the site of glycosylation was also established by HMBC experiments, which have indicated long-range correlations between δ_H 4.55 (Agly H-3) and δ_C 105.3 (Ara C-1). Therefore, the structure of **4** was determined as 3-*O*-α-L-arabinopyranosylgypsogenic acid.

Experimental Section

General Experimental Procedures. Optical rotations were measured using an AA-10R automatic polarimeter. IR spectra (KBr disks) were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. The 1D and 2D NMR spectra (^{1}H–^{1}H COSY, NOESY, HMQC, and HMBC) were performed using a UNITY-600 spectrometer at an operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4L-X computer system (600 MHz for ^{1}H and 150 MHz for ^{13}C spectra). Conventional pulse sequences were used for COSY, HMBC, and HMQC NMR spectra. The carbon type (CH_3,

CH_2, CH) was determined by DEPT experiments. All chemical shifts (δ) are given in ppm units with reference to tetramethylsilane (TMS) as the internal standard, and the coupling constants (J) are in Hz. Samples were measured in pyridine-d_5. HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer. FABMS (negative-ion mode) were obtained on a JEOL-SX-102 spectrometer, using glycerol as matrix. Vacuum-liquid chromatography (VLC) was carried out using RP-C_{18} silica gel (Merck 9303, 25–40 μm). Silica gel (63–200 μm Merck 64271) and Sephadex LH-20 (Sigma 9041-37-6) were used for open column chromatography. Medium-pressure liquid chromatography (MPLC) was performed with a Gilson M 305 pump, a Buchi column (46 × 1.5 cm), and silica gel 60 (Merck 7734, 15–40 μm). TLC and HPTLC were carried out on precoated Kieselgel 60 F_{254} (Merck 5628) plates. The following TLC solvent systems were used: (a) for saponins $CHCl_3$–MeOH–H_2O (61:32:7); (b) for sapogenins CH_2Cl_2–MeOH (10:1); and (c) for monosaccharides $CHCl_3$–MeOH–H_2O (64:40:8). The spray reagent used for the saponins and aglycons was the Komarowsky reagent (a 5:1 solution of 2% 4-hydroxybenzaldehyde in MeOH and ethanolic 50% H_2SO_4 solution), and diphenylaminophosphoric acid reagent was used for the detection of sugars.

Plant Material. The roots of *Paronychia chionaea* Boiss. were collected in July 1993 at Spil Mountain, Manisa, Turkey, and identified by Serdar G. Şenol (Deparment of Biology, Faculty of Sciences, Ege University, Izmir, Turkey). A voucher specimen (EGE 19558) is deposited in the Ege University Botanical Garden and Herbarium Research and Application Center, Izmir, Turkey.

Extraction and Isolation. Dried, ground roots of *P. chionaea* (1.1 kg) were extracted with MeOH (4 × 5 L) at room temperature and evaporated until dryness, yielding 30 g of a MeOH extract. This was dissolved in H_2O (500 mL) and successively partitioned with *n*-hexane (3 × 300 mL), $CHCl_3$ (3 × 300 mL), EtOAc (3 × 300 mL), and *n*-BuOH saturated with H_2O (3 × 300 mL). After evaporation of the solvent, the *n*-BuOH extract (18 g) was submitted to VLC (Lichroprep RP-18, 25–40 μm, 200 g) using a H_2O–MeOH gradient (100:0 → 0:100), yielding 10 fractions. Fraction 10 (320.4 mg) was subjected to column chromatography (Sephadex LH-20, MeOH) to afford **4** (32.8 mg). Fraction 8 (246.8 mg) was separated by flash column chromatography on reversed-phase silica gel C_{18} using a H_2O–MeOH gradient (100:0 → 0:100), to give five subfractions. Subfraction 5 (68.5 mg) was separated over normal-phase silica gel to give **1** (27.3 mg) and **2** (16.9 mg). Fraction 9 (126.8 mg) was finally purified by MPLC [silica gel (15–40 μm), $CHCl_3$–MeOH–H_2O (13:7:2, lower phase)], yielding **3** (12.1 mg).

Chionaeoside A (1): white, amorphous powder; $[\alpha]_D^{22}$ −15.0 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500–3390 (OH), 2950 (CH), 1740 (C═O ester), 1655 (C═C) cm^{-1}; ^1H (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; FABMS (negative) *m/z* 1103 $[M - H]^-$, 967 $[M - H) - 162]^-$, 617 $[(M - H) - 3 \times 162]^-$, 485 $[(M - H) - 3 \times 162 - 132]^-$; HRESIMS *m/z* 1127.5266 $[M + Na]^+$ (calcd for 1127.5250, $C_{53}H_{84}O_{24}Na$).

Chionaeoside B (2): white, amorphous powder; $[\alpha]_D^{22}$ −27.5 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500–3390 (OH), 2950 (CH), 1742 (C═O ester), 1655 (C═C) cm^{-1}; ^1H (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; FABMS (negative) *m/z* 941 $[M - H]^-$, 779 $[(M - H) - 162]^-$, 617 $[(M - H) - 2 \times 162]^-$, 485 $[(M - H) - 2 \times 162 - 132]^-$; HRESIMS *m/z* 965.4781 $[M + Na]^+$ (calcd for 965.4722, $C_{47}H_{74}O_{19}Na$).

Chionaeoside C (3): white, amorphous powder; $[\alpha]_D^{22}$ −33 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500–3390 (OH), 2950 (CH), 1740 (C═O ester), 1656 (C═C) cm^{-1}; ^1H (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; FABMS (negative) *m/z* 779 $[M - H]^-$, 647 $[(M - H) - 132]^-$, 485 $[(M - H) - 132 - 162]^-$; HRESIMS *m/z* 803.4239 $[M + Na]^+$ (calcd for 803.4194, $C_{41}H_{64}O_{14}Na$).

Chionaeoside D (4): white, amorphous powder; $[\alpha]_D^{22}$ −18.1 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500–3390 (OH), 2950 (CH), 1750 (CO carboxyl group), 1655 (C═C) cm^{-1}; ^1H (pyridine-d_5, 600 MHz), and ^{13}C NMR (pyridine-d_5, 150 MHz) see Tables 1 and 2; FABMS (negative) *m/z* 617 $[M - H]^-$, 485 $[(M - H) - 132]^-$; HRESIMS *m/z* 641.3689 $[M + Na]^+$ (calcd for 641.3666, $C_{35}H_{54}O_9Na$).

Acid Hydrolysis of Compounds 1–4 and the Saponin Mixture. A solution of each compound (3 mg) was refluxed in 2 N aqueous CF_3COOH (5 mL) in a water bath for 3 h. Then, 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 evaporated to dryness to afford the aglycon. After repeated evaporation of the aqueous layer by adding MeOH until neutral, the crude sugar residue was analyzed by TLC [silica gel, solvent system (c)], in comparison with standard sugars. A solution of the crude saponin mixture (1 g) was refluxed in 50 mL of 2 N HCl for 5 h. After extraction of the aglycon by CH_2Cl_2, the aqueous layer was neutralized with 0.5 M NaOH and freeze-dried. Two sugars were identified by TLC on silica gel [system (c)] by comparing with authentic samples. Purification of these sugars was achieved by preparative TLC using silica gel plates [system (c)] to afford glucose (R_f 0.29, $[\alpha]^{20}_D$ +10; *c* 0.13) and arabinose (R_f 0.40, $[\alpha]^{20}_D$ +22; *c* 0.15).

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