Triterpenoid Saponins from *Spergularia ramosa*

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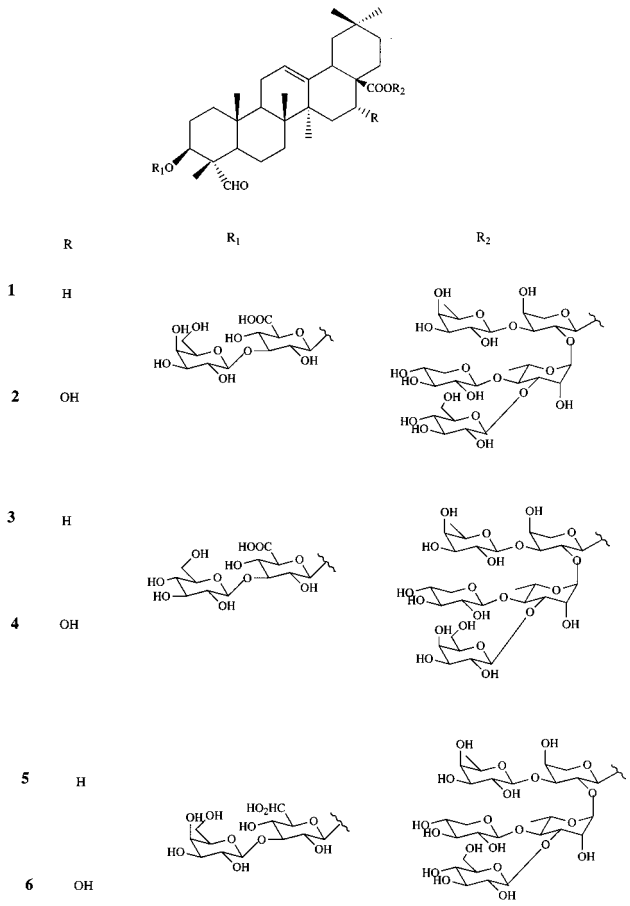
Six new oleanene glycosides were isolated from the MeOH extract of the aerial parts of *Spergularia ramosa*. They possess gypsogenin or quillaic acid as the aglycons. The disaccharide moiety linked to C-3 of the aglycons is made up of galactose (or glucose) and glucuronic acid (or glucose); the pentasaccharide moiety linked to C-28 is made up of glucose (or galactose), xylose, rhamnose, fucose, and arabinose. Their structures were elucidated by 1D and 2D NMR experiments including $^1H-^1H$ (DQF−COSY, 1D TOCSY, 2D HOHAHA, ROESY) and $^1H-^{13}C$ (HSQC, HMBC) spectroscopy.

Continuing our studies on South American medicinal plants, we have investigated the MeOH extract of the aerial parts of *Spergularia ramosa* Cambessedes (Caryophyllaceae). It is a herbaceous plant native to Paucartambo Province, Department of Cusco, Perù, where it is known as "choquetacarpo", a Quecha word for goldbar. The leaves of this species are used to feed sheep and, in the form of a decoction, as a remedy for respiratory ailments, tuberculosis, and rickets.[1]

Despite the frequent utilization of this plant as an antitubercular agent in the indigenous system of medicine, no previous phytochemical investigation has been reported on *S. ramosa*. In this paper we describe the structures and full NMR assignments of six new oleanene saponins **1**−**6** using highfield (600 MHz) spectrometry and a combination of 1D and 2D NMR techniques. Saponins **1**, **3**, and **5** possess gypsogenin[2] as the aglycon, while saponins **2**, **4**, and **6** have quillaic acid as the aglycon, which differs from gypsogenin in that it has an α-OH group at C-16.[2] All of the isolated glycosides are bidesmosides with sugar chains made up of two monosaccharide units linked to C-3 as well as sugar chains consisting of five monosaccharide units linked to C-28 (Chart 1).

Chart 1



Results and Discussion

The MeOH extract of the aerial parts of *S. ramosa* was subjected to Sephadex LH-20 column chromatography, followed by DCCC [*n*-BuOH−Me_2CO−H_2O (30:9:11), ascending mode] and then reversed-phase HPLC, to give six pure compounds (**1**−**6**). Compound **1** had a molecular formula $C_{70}H_{110}O_{36}$, as determined by ^{13}C,^{13}C DEPT NMR and negative-ion FABMS. The FABMS of **1** showed the $[M - H]^-$ ion at *m/z* 1525 and prominent fragments at *m/z* 1347 $[(M - H) - 178]^-$ and *m/z* 1363 $[(M - H) - 162]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen), at *m/z* 1201 due to the subsequent loss of one hexose unit, and at *m/z* 923 due to the sequential loss of a pentose and a deoxyhexose unit. A peak at *m/z* 469 was attributed to the aglycon moiety. The ^{13}C-NMR spectrum showed 70 signals, of which 30 were assigned to a triterpenoid moiety and 40 to the saccharide portion. The ^1H-NMR spectrum of the aglycon moiety of **1** showed signals for six tertiary methyl groups (δ 0.82, 0.94, 0.97, 1.03, 1.19 × 2) and a typical signal of H-3ax at δ 3.91 (dd, J = 11.5, 4.0 Hz) due to the presence of a β-OH group at C-3.[3] Further

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Table 1. ^{1}H- and ^{13}C-NMR Data of the Sugar Portion of Compound **1**[a,b]

sugar	position	δ_C	δ_H(J_{HH} in Hz)
ara	1	95.1	5.37 d (7.2)
	2	75.3[c]	3.77 dd (7.2, 8.5)
	3	78.0	3.68 dd (8.5, 3.0)
	4	71.7	3.58 m
	5	67.5	3.65 dd (12.0, 3.5)
			3.98 dd (12.0, 2.0)
rha	1	101.6	5.26 d (1.5)
	2	71.1	4.31 dd (1.5, 2.5)
	3	82.6	3.98 dd (2.5, 8.9)
	4	78.8	3.66 t (8.9)
	5	68.7	3.86 m
	6	18.2	1.28 d (6.5)
xyl	1	104.9	4.67 d (7.5)
	2	75.6	3.07 dd (7.5, 9.0)
	3	78.5	3.27 t (9.0)
	4	71.4	3.50 m
	5	66.7	3.17 dd, (10.0, 2.0)
			3.84 dd, (10.0, 5.0)
glu	1	105.1	4.56 d (7.6)
	2	75.1	3.32 dd (7.6, 9.5)
	3	77.9	3.24 t (9.5)
	4	70.9	3.35 t (9.5)
	5	77.7	3.36 m
	6	62.1	3.72 dd (11.0, 5.0)
			3.88 dd (11.0, 2.0)
fuc	1	103.5	4.83 d (7.4)
	2	73.4	3.50 dd (7.4, 8.5)
	3	75.1	3.46 dd (8.5, 2.5)
	4	70.5	3.85 m
	5	72.3	3.72 m
	6	16.3	1.25 d (6.0)
gluA	1	104.1	4.41 d (7.5)
	2	75.8	3.68 t (7.5, 9.0)
	3	86.4	3.72 t (9.0)
	4	72.6	3.63 t (9.0)
	5	77.9	3.68 d (9.0)
	6	176.0	
gal	1	104.5	4.60 d (7.0)
	2	73.3	3.62 dd (7.0, 8.5)
	3	74.3	3.52 dd (8.5, 4.0)
	4	69.7	3.86 dd (4.0, 2.5)
	5	76.4	3.52 m
	6	61.7	3.77 dd (12.0, 4.5)
			3.82 dd (12.0, 2.5)

[a] Assignments confirmed by 1D TOCSY, DQF−COSY, HSQC, HMBC experiments. [b] Values of the resonances of the sugar residues of **2** were almost superimposable to those of **1**. [c] Chemical shift of glycosylated carbons are underlined.

Table 2. ^{1}H- and ^{13}C-NMR Data of the Sugar Portion of Compound **3**[a,b]

sugar	position	δ_C	δ_H(J_{HH} in Hz)
ara	1	95.6	5.37 d (7.2)
	2	75.9[c]	3.79 dd (7.2, 8.5)
	3	78.3	3.69 dd (8.5, 3.0)
	4	71.0	3.57 m
	5	68.0	3.65 dd (12.0, 3.5)
			3.98 dd (12.0, 2.0)
rha	1	102.1	5.27 d (1.5)
	2	71.5	4.34 dd (1.5, 2.5)
	3	83.2	4.00 dd (2.5, 9.0)
	4	79.4	3.69 t (9.0)
	5	69.2	3.88 m
	6	18.1	1.26 d (6.5)
xyl	1	105.4	4.70 d (7.5)
	2	76.1	3.10 dd (7.5, 9.0)
	3	79.2	3.29 t (9.0)
	4	71.5	3.50 m
	5	67.1	3.20 dd (2.0, 10.0)
			3.86 dd (5.0, 10.0)
gal	1	104.9	4.63 d (7.0)
	2	73.7	3.62 dd (7.0, 8.5)
	3	74.9	3.56 dd (4.0, 8.5)
	4	70.8	3.87 t (2.5, 4.0)
	5	76.8	3.51 m
	6	62.5	3.77 dd (12.0, 4.5)
			3.80 dd (12.0, 2.5)
fuc	1	103.8	4.85 d (7.4)
	2	73.8	3.51 dd (7.4, 8.5)
	3	75.6	3.48 dd (8.5, 2.5)
	4	70.2	3.85 m
	5	72.7	3.73 m
	6	16.5	1.32 d (6.5)
gluA	1	104.7	4.41 d (7.5)
	2	76.1	3.70 dd (7.5, 9.0)
	3	86.9	3.73 t (9.0)
	4	72.4	3.59 t (9.0)
	5	77.0	3.55 d (9.0)
	6	176.1	
glu	1	105.5	4.59 d (7.6)
	2	75.4	3.33 dd (7.6, 9.1)
	3	78.4	3.46 t (9.1)
	4	71.4	3.38 t (9.1)
	5	78.1	3.33 m
	6	62.6	3.77 dd (11.0, 5.0)
			3.91 dd (11.0, 2.0)

[a] Assignments confirmed by 1D TOCSY, DQF−COSY, HSQC, HMBC experiments. [b] Values of the resonances of the sugar residues of **4** were almost superimposable to those of **3**. [c] Chemical shift of glycosylated carbons are underlined.

features were signals at δ 5.27 (1H, t, J = 3.7 Hz) and 9.5 (1H, s) ascribable, respectively, to an olefinic and an aldehidic proton. These data also indicated an oleanolic acid derivative with one of the methyl groups substituted by a −CHO function[2]

In the ^{13}C-NMR spectrum the signals at δ 123.5 and 144.8 ascribable to C-12 and C-13 confirmed the Δ^{12} oleanene skeleton.[2] A signal at δ 178.0 and the carbon resonances of rings D and E suggested the occurrence of a glycosylated −COOH group at C-28.[2] Full assignments of the proton and carbon resonances of the aglycon (see Experimental Section) were secured by ^{1}H−^{1}H DQF−COSY[4] and HSQC[5] spectra. The aldehyde function was located at C-23 on the basis of the downfield shift (+17.5 ppm) exhibited by C-4 (δ 56.3) and the highfield shifts (−7.1 ppm, −7.2 ppm, −6.0 ppm) exhibited, respectively, by C-3 (δ 85.9), C-5 (48.0), and C-24 (11.0) in comparison with the same carbon resonances in an oleanene skeleton bearing a Me-23.[2] Also, the chemical shift of Me-24 in the ^{1}H-NMR spectrum (δ_H 1.19) was diagnostic for a 23-CHO. The HMBC[6] (8 Hz) spectrum of **1** confirmed the position of the aldehyde function showing significative cross-peaks, due to $^2J_{C-H}$ and $^3J_{C-H}$ correlations between H-23 (δ 9.5) and C-3 (δ 85.9), C-4 (δ 56.3), and C-24 (δ 11.0). On the basis of the foregoing data, the aglycon of **1** was identified as 3β-hydroxy-23-oxoolean-12-en-28-oic acid, known as gypsogenin.[2] Glycosidation of the alcoholic function at C-3 and esterification of the 28-COOH group were indicated by the downfield shift (+13 ppm) and the highfield shift (−3 ppm) observed, respectively, for these carbon resonances in **1**, relative to the corresponding signals in gypsogenin.[2]

The sugar portion of **1** contained, in the ^{1}H-NMR spectrum (Table 2), seven anomeric proton signals (δ 4.41, d, J = 7.5 Hz; δ 4.56, d, J = 7.6 Hz; δ 4.60, d, J = 7.0 Hz; δ 4.67, d, J = 7.5 Hz; δ 4.83, d, J = 7.4 Hz; δ 5.26, d, J = 1.5 Hz; δ 5.37, d, J = 7.2 Hz) and two methyl doublets (δ 1.25, d, J = 6.0 Hz; 1.28, d, J = 6.5 Hz), suggesting the occurrence of two deoxyhexose units. The other sugar signals were overlapped in the region between δ 3.07 and 4.31. The structures of the oligosac-

charide moieties were deduced using 1D TOCSY and 2D NMR experiments. Because of the selectivity of the multistep coherence transfer, the 1D TOCSY method[7] allowed the subspectrum of a single monosaccharide unit to be extracted from the crowded overlapped region. The isolated anomeric proton signals resonating in an uncrowded region of the spectrum (between δ 4.41 and 5.37) were the starting point for the 1D TOCSY experiments. Selected 1D TOCSY obtained, irradiating each anomeric proton signal, yielded the subspectrum of each sugar residue with high digital resolution. Each subspectrum contained the scalar-coupled protons within each sugar residue. In some cases, because of the small coupling constants, the distribution of magnetization around the spin system was impeded. For this reason, for example, it was possible to identify only three protons (δ 3.77, 3.68, 3.58) coupled to the anomeric signal at δ 5.37 in the case of arabinose (Table 1). In the case of the 6-deoxyhexoses, easier identification of all of the proton signals was accomplished by also recording 1D TOCSY experiments irradiating the methyl doublets. Because in the TOCSY method both direct and relayed connectivities occur, we also recorded a DQF−COSY spectrum. The results of 1D TOCSY and DQF−COSY experiments allowed the sequential assignments of all of the proton resonances to the individual monosaccharides as reported in Table 1. Thus the shifts of the sugar resonances, summarized in Table 1, were attributable to L-arabinopyranosyl (δH−1_{ara} = 5.37) , α-L-rhamnopyranosyl (δH−1_{rha} = 5.26), β-D-fucopyranosyl (δH−1_{fuc} = 4.83), β-D-xylopyranosyl (δH−1_{xyl} = 4.67), β-D-galactopyranosyl (δH−1_{gal} = 4.60), β-D-glucopyranosyl (δH−1_{glu} = 4.56), β-D-glucuronopyranosyl (δH−1_{gluA} = 4.41) units. In the case of the arabinopyranosyl unit, the J_{H1-H2} coupling constant (7.2 Hz), midway between that observed for methyl-β-L-arabinopyranoside (4 Hz) and methyl-α-L-arabinopyranoside (8 Hz)[8] has been reported not to be diagnostic on its own, owing to the high conformational mobility of arabinopyranosides ($^4C_1 \leftrightarrow {}^1C_4$). As we reported in previous works,[3,9] evidence of α-L-arabinopyranoside was obtained from the ROESY[10] spectrum, which showed NOEs from C-1_{ara} to C-2_{ara}, C-3_{ara}, and C-5_{ara} as expected for an α-L-arabinopyranoside in rapid $^4C_1 \leftrightarrow {}^1C_4$ conformational exchange.

HSQC experiments, which correlated all the proton resonances with those of each corresponding carbon, allowed the assignments of interglycosydic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methylpyranosides. The absence of any ^{13}C-NMR glycosidation shift for the β-D-fucopyranosyl, β-D-glucopyranosyl, β-D-xylopyranosyl, and β-D-galactopyranosyl moieties suggested that these sugars were terminal units. Glycosidation shifts were observed for C-2ara (75.3), C-3ara (78.0), C-3rha (82.6), C-4rha (78.8), and C-3gluA (86.4) (Table 1). Chemical shifts of H-1ara (δ 5.37) and C-1ara (δ 95.1) indicated that this sugar unit was involved in an ester linkage with the C-28 carboxylic group.[11]

The positions of the sugar residues were unambiguously defined by the HMBC experiment. A cross peak due to long-range correlations between C-3 (δ 85.9) of the alycon and H-1gluA (δ 4.41) indicated that glucuronic acid was the hexose residue linked to C-3 of the aglycon and a cross peak between C-3_{gluA} (δ 86.4) and H-1 of the terminal galactose (δ 4.60) indicated that galactose was the second unit of the disaccharide chain at C-3 of the aglycon. Similarly, the sequence of the pentasaccharide chain at C-28 was indicated by the cross peaks between C-2ara (δ 75.3) and H-1rha (δ 5.26), C-3ara (δ 78.0) and H-1 of the terminal fucose (δ 4.83), C-3rha (δ 82.6) and H-1 of the terminal glucose (δ 4.56), C-4rha (δ 78.8) and H-1 of the terminal xylose (δ 4.67). A cross peak between H-1 of arabinose (δ 5.37) and the ^{13}C-NMR resonance of the aglycon carbonyl group (δ 178.0) provided definitive evidence for an ester linkage between the pentasaccharide chain and the aglycon. Thus, the terminal glucose and xylose were linked, respectively, to C-3 and C-4 of rhamnose, which was attached at C-2 of arabinose, which, in turn, linked the terminal fucose at C-3. On the basis of these evidences, compound **1** was identified as 3β-*O*-(β-D-galactopyranosyl)-(1→3)-β-D-glucuronopyranosyl-23-oxoolean-12-en-28-oic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester}.

Compound **2** showed, in the negative ion FABMS spectrum, a quasi molecular anion $[M - H]^-$ at *m/z* 1541, 16 mass units higher than that of **1** and gave ^{13}C and DEPT ^{13}C NMR consistent with a $C_{70}H_{110}O_{37}$ molecular formula. The FABMS spectrum showed a fragmentation pattern similar to that of **1**. Comparison of ^{1}H- and ^{13}C-NMR data of both compounds indicated identical saccharide chains at C-3 and C-28 and structural similarity in the aglycon moieties. The main differences were the downfield shifts of C-16 (δ 74.6) and C-15 (δ 36.0) in the ^{13}C-NMR spectrum and the downfield shift of the axial methyl group at C-14 (Me-27, δ 1.42) in the ^{1}H-NMR spectrum, implying an additional hydroxyl group at C-16 in **2**. This hypothesis was unambiguously confirmed by the HMBC spectrum, which showed cross peaks between the proton at δ 4.51 (H-16) and C-14, C-15, C-17, C-18, and C-22 (see Experimental Section). The α configuration of the C-16 hydroxyl group was evident from the chemical shift and the small *J* values of H-16 (δ 4.51, br m), characteristic of an equatorial proton.[3] Thus, the aglycon of **2** was identified as 3β,16α-dihydroxy-23-oxoolean-12-en-28-oic acid, known as quillaic acid,[2] and compound **2** was defined as 3β-*O*-(β-D-galactopyranosyl)-(1→3)-β-D-glucuronopyranosyl-16α-hydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester}.

The FABMS of **3** ($C_{70}H_{110}O_{36}$) and **4** ($C_{70}H_{110}O_{37}$) showed quasi molecular anions at *m/z* 1525 and 1541 and fragmentation patterns superimposable, respectively, to those of **1** and **2**. ^{1}H- and ^{13}C-NMR spectra of **3** and **4** suggested sugar moieties made up of a disaccharide linked to C-3 and a pentasaccharide linked to C-28 and showed signals due to the aglycon moieties very close to those observed, respectively, for gypsogenin and quillaic acid. 2D NMR analysis (2D HOHAHA,[12] DQF−COSY, HSQC) (Table 2) of the sugar portions of **3** and **4** revealed the same monosaccharide units as in **1** and **2** (Table 1); in particular, the same glycosidation sites were deduced from the HSQC experiment. These observations led us to hypothesize that the structural

difference between the sugar chains of compounds **1** and **2** with respect to those of compounds **3** and **4** was confined to the relative positions of two terminal monosaccharide units. The HMBC spectrum of **3**, which displayed correlations between the signals at δ 4.63 (H-1gal) and 83.2 (C-3rha) and between the resonances at δ 4.59 (H-1glu) and 86.9 (C-3gluA) (Table 2), indicated that the terminal β-D-galactopyranosyl unit was linked to C-3 of the α-L-rhamnopyranosyl unit on the pentasaccharide chain at C-28, and the terminal β-D-glucopyranosyl unit was linked to C-3 of the β-D-glucuronopyranosyl at C-3 of the aglycon. The other long-range correlations were almost the same as those previously reported for compound **1**. Thus, **3** was defined as 3β-*O*-(β-D-glucopyranosyl)-(1→3)-β-D-glucuronopyranosyl-23-oxoolean-12-en-28-oic acid 28-*O*-{β-D-galactopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester} and **4** as 3β-*O*-(β-D-glucopyranosyl)-(1→3)-β-D-glucuronopyranosyl-16α-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-{β-D-galactopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester}.

Compounds **5** ($C_{70}H_{112}O_{35}$) and **6** ($C_{70}H_{112}O_{36}$) were identified, respectively, as further gypsogenin and quillaic acid derivatives possessing two sugar moieties at C-3 and C-28. FABMS exhibited fragmentation patterns consistent with the cleavage of a terminal hexopyranosyl unit (178 mass units), followed by sequential losses of a further hexopyranosyl unit (162 mass units), a pentose (132 mass units), and a deoxyhexose (146 mass units). Also in this case, the proton coupling network within each sugar residue was traced out, using a combination of DQF−COSY, 1D TOCSY, and HSQC experiments, which indicated that a β-D-glucopyranosyl unit was present instead of the β-D-glucuronopyranosyl unit observed in the disaccharide chain at C-3 of compounds **1**−**4** (Table 3). In fact, the 1D TOCSY subspectrum obtained when irradiating the anomeric proton signal at δ 4.42 showed a set of coupled protons at δ 3.68, 3.74, 3.62, 3.69 (all CH), and 3.69 and 3.85 (CH_2) assigned from H-1 to H_2-6 of a β-D-glucopyranosyl unit. Once again, direct evidence for the sugar sequence and the linkage sites was derived from a HMBC experiment. These results established the same pentasaccharidic chain as in **1** and **2**, linked to COOH group at C-28, whereas the disaccharidic chain made up by a β-D-glucopyranosyl unit substituted at position 3 by a β-D-galactopyranosyl unit was at C-3 of the aglycon. On the basis of the reported data the structures of **5** and **6** were established to be, respectively, 3β-*O*-(β-D-galactopyranosyl)-(1→3)-β-D-glucopyranosyl-23-oxoolean-12-en-28-oic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester} and 3β-*O*-(β-D-galactopyranosyl)-(1→3)-β-D-glucopyranosyl-16α-hydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester}.

Table 3. ^{1}H- and ^{13}C-NMR Data of the Sugar Portion of Compound **5**[a,b]

sugar	position	δ_C	δ_H(J_{HH} in Hz)
ara	1	95.1	5.37 d (7.2)
	2	75.4[c]	3.78 dd, (7.2, 8.5)
	3	78.0	3.66 dd (8.5, 3.0)
	4	71.7	3.58 m
	5	67.5	3.68 dd (12.0, 3.5)
			3.98 dd (12.0, 2.0)
rha	1	101.6	5.26 d (1.5)
	2	71.1	4.33 dd (1.5, 2.5)
	3	82.6	3.99 dd (2.5, 8.6)
	4	78.9	3.66 t (8.6)
	5	68.8	3.87 m
	6	18.2	1.28 d (6.5)
xyl	1	105.0	4.68 d (7.5)
	2	75.6	3.10 dd (7.5, 9.0)
	3	78.7	3.28 t (9.0)
	4	71.1	3.50 m
	5	66.7	3.19 dd (10.0, 2.0)
			3.85 dd (10.0, 5.0)
glu	1	105.1	4.57 d (7.8)
	2	75.1	3.32 dd (7.8, 9.5)
	3	77.9	3.34 t (9.5)
	4	71.1	3.36 t (9.5)
	5	77.7	3.32 m
	6	62.1	3.73 dd (12.0, 5.0)
			3.90 dd (12.0, 2.0)
fuc	1	103.4	4.84 d (7.4)
	2	73.4	3.48 dd (7.4, 8.5)
	3	75.1	3.47 dd (8.5, 2.5)
	4	70.5	3.86 m
	5	72.4	3.71 m
	6	16.3	1.25 d (6.0)
glu II	1	103.9	4.42 d (7.5)
	2	75.6	3.68 t (7.5, 9.0)
	3	86.5	3.74 t (9.0)
	4	72.6	3.62 t (9.0)
	5	78.0	3.69 m
	6	62.2	3.85 dd (12.5, 2.0)
			3.69 dd (12.5, 5.0)
gal	1	104.6	4.60 d (7.0)
	2	73.3	3.61 dd (7.0, 9.0)
	3	74.4	3.34 dd (9.0, 4.0)
	4	69.9	3.85 dd (4.0, 2.5)
	5	76.3	3.53 m
	6	61.7	3.78 dd (12.0, 4.5)
			3.80 dd (12.0, 2.5)

[a] Assignments confirmed by 1D TOCSY, DQF−COSY, HSQC, HMBC experiments. [b] Values of the resonances of the sugar residues of **6** were almost superimposable to those of **5**. [c] Chemical shift of glycosylated carbons are underlined.

Experimental Section

General Experimental Procedures. A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C and the UX-NMR software package was used for NMR measurements in CD_3OD solutions. Two-dimensional experiments—^{1}H−^{1}H DQF−COSY,[4] 2D HOHAHA,[12] inverse-detected ^{1}H−^{13}C HSQC,[5] HMBC,[6] and ROESY[10]—were obtained using UX-NMR software. The selective excitation spectra, 1D TOCSY,[7] were acquired using waveform generator-based, GAUSS-shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. Optical rotations were measured on a Perkin−Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solutions in MeOH. FABMS were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe, 2−6 kV). DCCC was performed on an apparatus manufactured by Buchi, equipped with 300 tubes. HPLC separations were performed with a Waters model 6000A pump equipped with a U6K injector and a model 401 refractive index detector.

Plant Material. The aerial parts of *Spergularia ramosa* were collected at Paucartambo Province, De-

partment of Cusco, Perù, in April 1996. A voucher sample of the plant is deposited at the herbarium of Dipartimento di Scienze Farmaceutiche, University of Salerno.

Extraction and Isolation. The air-dried leaves (300 g) were defatted with petroleum ether and $CHCl_3$ and then extracted with MeOH to give 8 g of residue. Part of the MeOH extract (4 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as eluent. Fractions (8 mL) were collected and checked by TLC [Si gel plates, *n*-BuOH−AcOH−H_2O (12:3:5)]. Fractions 13−20 (1.2 g) containing the crude glycosidic mixture were further purified by DCCC with *n*-BuOH−Me_2CO−H_2O (30:9:11) in which the stationary phase consisted of the lower phase (ascending mode, flow 12 mL/h). DCCC fractions 100−200 (350 mg) were chromatographed on a μ-Bondapak C-18 column (30 cm × 7.8 mm i. d.) eluting with MeOH−H_2O (45:55), flow 3 mL/min to yield pure **1** (30 mg, t_R = 20 min), **2** (19 mg, t_R = 15 min), **5** (21 mg, t_R = 11 min), and **6** (15 mg, t_R = 8 min). DCCC fractions 210−237 (100 mg) afforded **3** (10.5 mg, t_R = 8.5 min) and **4** (13 mg, t_R = 6.5 min) using MeOH−H_2O (1:1) as the eluent, flow 3 mL/min.

Compound 1: $[\alpha]^{25}_D$ +16.5°, (*c* 1, MeOH); ^{1}H-NMR data of the aglycon (CD_3OD, 600 MHz) δ 9.5 (1H, s, H-23), 5.27 (1H, t, *J* = 3.7 Hz, H-71), 3.91 (1H, dd, *J* = 4.0, 11.5 Hz, H-3), 2.87 (1H, dd, *J* = 4.3, 13.7 Hz, H-18), 1.19 (6H, s, Me-24 and Me-27) 1.03 (3H, s, Me-25), 0.97 (3H, s, Me-30), 0.94 (3H, s, Me-29), 0.82 (3H, s, Me-26); ^{13}C-NMR data of the aglycon (CD_3OD, 600 MHz) δ 211.6 (C-23), 178.0 (C-28), 144.8 (C-13), 123.5 (C-12), 85.9 (C-3), 56.3 (C-4), 48.1 (C-9), 48.0 (C-5), 47.0 (C-17, C-19), 42.6 (C-18), 42.0 (C-14), 40.0 (C-8), 39.0 (C-1), 36.7 (C-10), 34.6 (C-21), 33.4 (C-29), 32.8 (C-22), 30.9 (C-20), 30.5 (C-7), 28.8 (C-15), 26.9 (C-27), 25.4 (C-16), 24.9 (C-11), 23.9 (C-30), 23.5 (C-2), 21.3 (C-6), 17.6 (C-26), 16.4 (C-25), 11.0 (C-24); ^{1}H and ^{13}C NMR of the sugar moieties, see Table 1; FABMS *m/z* 1525 $[M - H]^-$, 1347 $[(M - H) - 178]^-$, 1363 $[(M - H) - 162]^-$, 1201 $[(M - H) - (162 \times 2)]^-$, 923 $[(M - H) - (162 + 162 + 132 + 146)]^-$.

Compound 2: $[\alpha]^{25}_D$ +23.2°, (*c* 1, MeOH); ^{1}H-NMR data of the aglycon (CD_3OD, 600 MHz) δ 9.5 (1H, s, H-23), 5.25 (1H, t, *J* = 3.9 Hz, H-12), 4.51 (1H, br m, H-16), 3.95 (1H, dd, *J* = 3.5, 12.0 Hz, H-3), 2.86 (1H, dd, *J* = 3.5, 13.5 Hz, H-18), 1.42 (3H, s, Me-27), 1.19 (3H, s, Me-24) 1.04 (3H, s, Me-25), 0.98 (3H, s, Me-30), 0.92 (3H, s, Me-29), 0.82 (3H, s, Me-26); ^{13}C-NMR data of the aglycon (CD_3OD, 600 MHz) δ 211.1 (C-23), 176.6 (C-28), 143.8 (C-13), 125.7 (C-12), 85.3 (C-3), 74.6 (C-16), 56.7 (C-4), 49.8 (C-17), 48.0 (C-9), 47.6 (C-5, C-19), 42.4 (C-14), 41.9 (C-18), 40.8 (C-8), 38.9 (C-1), 36.7 (C-10), 36.0 (C-15, C-21), 33.4 (C-29), 33.1 (C-22), 31.0 (C-20), 30.6 (C-7), 28.0 (C-27), 25.4 (C-2), 24.3 (C-30), 24.1 (C-11), 21.0 (C-6), 17.5 (C-26), 16.4 (C-25), 10.9 (C-24); ^{1}H and ^{13}C NMR of the sugar moieties, see Table 1; FABMS *m/z* 1541 $[M - H]^-$, 1363 $[(M - H) - 178]^-$, 1379 $[(M - H) - 162]^-$, 939 $[(M - H) - (162 + 162 + 132 + 146)]^-$.

Compound 3: $[\alpha]^{25}_D$ +12.4°, (*c* 1, MeOH); ^{1}H and ^{13}C NMR of the aglycon were almost superimposable on those reported for **1**; for ^{1}H and ^{13}C NMR of the sugar moieties, see Table 2; FABMS *m/z* 1525 $[M - H]^-$, 1347 $[(M - H) - 178]^-$, 1363 $[(M - H) - 162]^-$, 1201 $[(M - H) - (162 \times 2)]^-$, 923 $[(M - H) - (162 + 162 + 132 + 146)]^-$.

Compound 4: $[\alpha]^{25}_D$ +20.8, (*c* 1, MeOH); ^{1}H and ^{13}C NMR of the aglycon were almost superimposable on those reported for **2**; for ^{1}H and ^{13}C NMR of the sugar moieties, see Table 2; FABMS *m/z* 1541 $[M - H]^-$, 1363 $[(M - H) - 178]^-$, 1379 $[(M - H) - 162]^-$, 1217 $[(M - H) - (162 \times 2)]^-$, 939 $[(M - H) - (162 + 162 + 132 + 146)]^-$.

Compound 5: $[\alpha]^{25}_D$ + 8.5°, (*c* 1, MeOH); ^{1}H and ^{13}C NMR of the aglycon were almost superimposable on those reported for **1**; for ^{1}H and ^{13}C NMR of the sugar moieties, see Table 3; FABMS *m/z* 1511 $[M - H]^-$, 1333 $[(M - H) - 178]^-$, 1349 $[(M - H) - 162]^-$, 1187 $[(M - H) - (162 \times 2)]^-$, 909 $[(M - H) - (162 + 162 + 132 + 146)]^-$.

Compound 6: $[\alpha]^{25}_D$ + 17.0° (*c* 1, MeOH); ^{1}H and ^{13}C NMR of the aglycon were almost superimposable on those reported for **2**; for ^{1}H and ^{13}C NMR of the sugar moieties, see Table 3; FABMS *m/z* 1527 $[M - H]^-$, 1349 $[(M - H) - 178]^-$, 1365 $[(M - H) - 162]^-$, 1203 $[(M - H) - (162 \times 2)]^-$, 925 $[(M - H) - (162 + 162 + 132 + 146)]^-$.

References and Notes

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