New Oleanane Saponins in *Chenopodium quinoa*

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Six triterpenoid saponins were isolated from the seeds of *Chenopodium quinoa* (Chenopodiaceae). Their structures were as follows: phytolaccagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside (**1**); spergulagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-28-*O*-*β*-D-glucopyranoside (**2**); hederagenin 3-*O*-[*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**3**); phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside (**4**); hederagenin 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside (**5**); and spergulagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside (**6**). Saponins **5** and **6** are new. The structures were characterized on the basis of hydrolysis and spectral evidence, including IR, UV, optical rotations, 1D- and 2D-NMR (HMQC and HMBC), ESIMS, and FABMS analyses.

Keywords: *Chenopodium quinoa, Chenopodiaceae, South American crop; food source; triterpene saponins; ^{1}H and ^{13}C NMR (HMBC, HMQC); FABMS*

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.; Chenopodiaceae) is an annual herb from the Andes Mountains of South America, where it has been cultivated for 3000 years and was a staple of the Incan Empire. The ancient Incas called quinoa the “mother grain” and considered it a “gift from the gods”, because it helped to sustain and nourish their peoples through the generations (*1*). Its small nutritious seeds resemble millet and are very versatile to a variety of cooking styles. The United Nations Food and Agriculture Organization (FAO) observes that quinoa is closer to the ideal protein balance than any other common grain, being at least equal to milk in protein quality content (*2*). Quinoa is high in lysine, an amino acid often lacking in cereal products, such as wheat, the cereal grain that comes closest to matching its protein content. Quinoa also has a good amount of methionine and cystine, making it a perfect complement for legumes, which are low in these essential amino acids. Quinoa’s nutritional profile has higher levels of energy, calcium, phosphorus, iron, fiber, and B-vitamins than barley, oats, rice, corn, or wheat (*3*).

The seedcoat of quinoa contains bitter-tasting saponins, which protect it from birds and insects. Therefore, quinoa is generally grown organically or naturally, without pesticides.

Saponins are found in a wide range of foods such as beans, peas, potato, tea, asparagus, sugar beet, and blackberry. Generally they have a bitter and astringent taste or are tasteless, although one exception to this is the saponin in liquorice, which is sweet tasting. The kind of taste depends on the structures of saponins present in foods. Also, biological properties are related to the chemical structure of saponins. Although extremely toxic in cold-blood animals, the oral toxicity of saponins in mammals is low. Saponins present in common foods, including quinoa, seem to be free from significant oral toxicity in humans (*4*–*8*). It has been suggested that saponins present in foodstuffs may even be beneficial in the human diet (*9*). Therefore, knowledge of the content and structural arrangement of saponins is important for understanding the taste and health effects in foods. In general, saponin levels in final products for foods are reduced to decrease possible negative biological effects and bitterness. This is accomplished by selecting “sweet” quinoa varieties (those in which saponin levels are ~$^{1}/_{10}$ those of normal varieties), processing the grains, or both. The traditional removal of the bitter taste by washing with water does not remove all saponins, some of which are better retained in the hulls of the seed due to this structural features. The final content of saponins, the number of constituents, and their structures depend on the variety of quinoa and treatment of the grains during normal processing. The saponin fraction of quinoa is a complex mixture of triterpenoid glycosides with different structures (*10*, *11*). They vary in the aglycon moiety and in the saccharide moieties (*10*).

In this work saponins were found that could not be detected by the usual analytical method, which consists of partitioning between butanol and water of the aqueous methanolic extract. Generally all saponin content is considered to be in the butanolic phase and the sugars are thought to be in the water phase, which is discarded. However, in a sample of quinoa of which the butanolic phase had already been studied, six saponins were found in the water phase, after several butanol washings. Five of these were different from those previously found in the butanol phase (*11*). This shows that the usual analytical procedure can produce a loss of components if the water-soluble fraction is disregarded.

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Nevertheless, the step of partitioning the methanolic extract between butanol and water is useful, so that saponins with similar retention times can be separated by sampling them in two different groups. The isolation and characterization of additional saponins, two of which are new, from a specimen of debittered quinoa, previously studied (*11*), are now reported.

MATERIALS AND METHODS

Materials. Plant material was supplied as a commercial product made October 3, 1997: "quinua lavada" (Mascorona Km 4 1/2 Casilla 18-01-1439 Ambato, Ecuador).

Apparatus. Optical rotations were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10-mm microcell. The NMR spectra were obtained in CD_3OD with a Bruker AMX 500 spectrometer. The DEPT experiments were performed with a pulse of 135° to obtain positive signals for CH and CH_3 and negative signals for CH_2; an average CH coupling constant of 135 Hz was assumed. ^1H detected heteronuclear multiple quantum coherence (HMQC) experiments were performed according to the procedure of Martin and Crouch (*12*), using an initial BIRD pulse to suppress ^1H resonances not coupled to ^{13}C and a GARP sequence for ^{13}C decoupling during data acquisition. The spectral width in the ^1H dimension was 2994.05 Hz; 256 experiments of 240 scans each (relaxation delay = 1.5 s, delay after BIRD pulse = 0.4 s, fixed delay t_1 = 3.3 ms) were acquired in 1K points. A sine square function was applied in the t_2 dimension, and a trapezoidal window was applied in the t_1 dimension (TM_1 = 0.03 Hz, TM_2 = 0.6 Hz) before Fourier transformation (digital resolution in F_2 dimension = 2.994 Hz/point). ^1H detected heteronuclear multiple bond correlation (HMBC) spectroscopy was performed according to the methods of Bax (*13*) and Martin and Crouch (*12*). The data processing was identical to that used for the HMQC experiment, and the final digital resolution was 2.25 Hz/point. FABMS (recorded in a glycerol matrix) was measured on a Prospec Fisons mass spectrometer. HRFABMS spectra were recorded in a glycerol matrix on a VG AUTOSPEC instrument; GC-MS was run using a Hewlett- Packard 5890 gas chromatograph equipped with an HP-5 column (25 m × 0.2 mm i.d., 0.33 μm film), fitted with an HP 5970B mass detector and an HP 59970 MS Chemstation. The FTIR spectra were obtained on a Bruker IFS-48 spectrophotometer using a KBr matrix. UV spectra were obtained on a Beckman DU70 spectrophotometer in MeOH solution. HPLC separations were performed on a Hewlett-Packard HP 1050 series apparatus with a Varian RI-4 refractive index detector, equipped with a Waters μ-Bondapack C-18 column (7.8 × 300 mm).

Extraction and Isolation. The whole flour from the seeds (790.4 g) was extracted with $MeOH/H_2O$ (90:10). The MeOH extract (44.45 g) was partitioned between $H_2O/MeOH$ (50 mL/110 mL) and $CHCl_3$ (150 mL). The methanol was evaporated from the aqueous methanolic layer, and the aqueous solution was defatted with ethyl acetate and then extracted with *n*-BuOH. The vacuum distillation residue of the H_2O-soluble fraction (6 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [Si-gel plates in *n*-BuOH/$HOAc/H_2O$ (60:15:25)]. Fractions 25−33 (1273.2 mg) containing the crude glycosidic mixture were further separated by reversed-phase HPLC with $MeOH/H_2O$ (40:60) at a flow rate of 2.5 mL/min to yield pure compounds **1** (53.2 mg; T_R, 4.4 min), **2** (33.3 mg; T_R, 5.0 min), **3** (60.2 mg; T_R, 6.8 min), **4** (1.3 mg; T_R, 14.2 min), **5** (16.6 mg; T_R, 28.8 min), and **6** (4.4 mg; T_R, 50.8 min).

Acid Hydrolysis of Compounds 1−6: Monosaccharide Composition. A solution of each compound (1 mg) in 10% H_2SO_4/EtOH (1:1, 3.5 mL) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The H_2O layer was neutralized with Amberlite MB-3 ion-exchange resin and evaporated to dryness. The resulting monosaccharides were reacted with TRISIL-Z (Pierce) and analyzed by GC-MS. Retention times were identical to those of the authentic trimethylsilylated sugars.

Alkaline Hydrolysis. Glycosides **1**−**6** were separately heated in 0.5 N aqueous KOH (1 mL) at 110 °C in a stoppered reaction vial for 2 h. The reaction mixture was adjusted to pH 7 with HCl and then extracted with *n*-BuOH. The organic phase was evaporated to dryness, dissolved in CD_3OD, and analyzed by ^1H NMR.

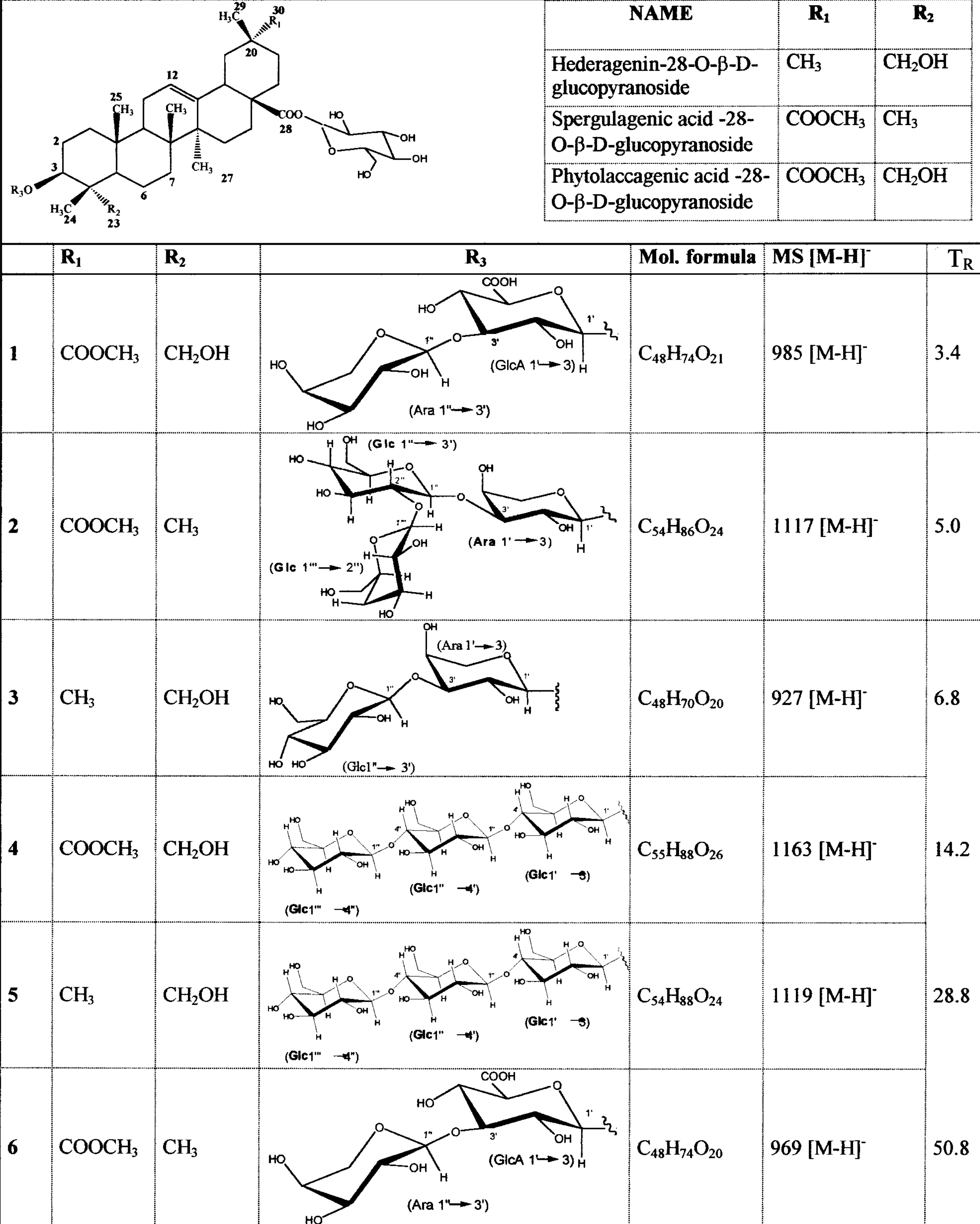
RESULTS AND DISCUSSION

C. quinoa grains were extracted with CH_3OH/H_2O (90:10). The residue of the methanolic extracts was partitioned into a mixture of *n*-BuOH and H_2O to afford the H_2O-soluble portion, which was subjected to Sephadex LH-20 chromatography. The fractions were checked by TLC, and fractions 25−37 containing the crude glycosidic mixture were submitted to reversed-phase HPLC to afford almost pure compounds (**1**−**6**) (Figure 1). The structures were determined by IR, UV, optical rotation, negative ion ESIMS, and FABMS mass spectra and by ^1H and ^{13}C DEPT NMR, 1D-HMQC, and 2D-HMBC−NMR.

Saponins **1**−**4** showed spectroscopic and hydrolytic data identical with those of phytolaccagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside (**1**), isolated from the seeds of *C. quinoa* in our previous work (*11*); spergulagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**2**) and hederagenin 3-*O*-[*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**3**), isolated be fore from the seeds of *C. quinoa* (*10*); and phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside (**4**), isolated previously from the seeds of *C. pallidicaule* (*14*). These structures were also confirmed by ESIMS, FABMS, and ^1H, ^{13}C, and ^{13}C DEPT NMR (Tables 1−3).

Compound **5** was a white powder: $[\alpha]_D$ −0.9° (*c* 0.003 in MeOH), IR ν_{max}^{KBr} cm^{-1} 3350 (OH); 1700 (COOR); FABMS (negative ion) *m*/*z* 1119 $[M - H]^-$; HRFABMS found *m*/*z* 1119.5597, calcd for $C_{54}H_{88}O_{24}$ *m*/*z* 1120.5665.

On acidic hydrolysis **5** afforded hederagenin, identified by ^1H, ^{13}C, and ^{13}C DEPT NMR spectra and by comparison with literature data (*15*), and glucose. The ^{13}C and ^{13}C DEPT NMR spectra showed 54 signals, of which 24 were assigned to the saccharide portion and 30 to a triterpenic moiety (Tables 2 and 3). In particular, hydrogens and carbons due to the C, D, and E rings of the aglycon portion of **5** resonated near the same frequencies as the corresponding signals in oleanolic acid, whereas ^1H and ^{13}C signals in the A and B rings were shifted somewhat. The NMR spectra of **5** contained one fewer methyl than oleanolic acid and two coupled doublets [^1H NMR δ 3.30 (1H, d, J = 11.8 Hz), 3.70 (1H, d, J = 11.8 Hz); ^{13}C NMR δ 64.9 (CH_2)], suggesting that one of the methyl groups of oleanolic acid was replaced by a hydroxymethyl group. In addition, one of the methyl signals (Me-24) was shifted upfield to δ 0.74 due to the presence of the CH_2OH group. The ^{13}C NMR spectrum of **5** suggested placement of the CH_2OH group at C-23, which was confirmed by the downfield shift exhibited by C-4 and the upfield shifts experienced by C-3, C-5, and Me-24. The presence of a *β*-glucose unit linked to the carboxyl group (C-28) of the aglycon was consistent with the chemical shift of the anomeric signal at δ 5.41 (1H, d, J = 8.4 Hz) in the ^1H NMR spectrum



NAME	R_1	R_2
Hederagenin-28-O-β-D-glucopyranoside	CH_3	CH_2OH
Spergulagenic acid -28-O-β-D-glucopyranoside	$COOCH_3$	CH_3
Phytolaccagenic acid -28-O-β-D-glucopyranoside	$COOCH_3$	CH_2OH

	R_1	R_2	R_3	Mol. formula	MS [M-H]$^-$	T_R
1	$COOCH_3$	CH_2OH	(GlcA 1'→3) (Ara 1''→3')	$C_{48}H_{74}O_{21}$	985 [M-H]$^-$	3.4
2	$COOCH_3$	CH_3	(Glc 1''→3') (Ara 1'→3) (Glc 1'''→2'')	$C_{54}H_{86}O_{24}$	1117 [M-H]$^-$	5.0
3	CH_3	CH_2OH	(Ara 1'→3) (Glc1''→3')	$C_{48}H_{70}O_{20}$	927 [M-H]$^-$	6.8
4	$COOCH_3$	CH_2OH	(Glc1'→3) (Glc1''→4') (Glc1'''→4'')	$C_{55}H_{88}O_{26}$	1163 [M-H]$^-$	14.2
5	CH_3	CH_2OH	(Glc1'→3) (Glc1''→4') (Glc1'''→4'')	$C_{54}H_{88}O_{24}$	1119 [M-H]$^-$	28.8
6	$COOCH_3$	CH_3	(GlcA 1'→3) (Ara 1''→3')	$C_{48}H_{74}O_{20}$	969 [M-H]$^-$	50.8

Figure 1. Structures of compounds **1**−**6**. (T_R was calculated as described under Materials and Methods.)

and at 95.7 ppm in the ^{13}C NMR spectrum. The HMBC spectrum also showed a cross-peak between H-1 (δ 5.41) of glucose and C-28 (δ 178.2) of the aglycon. These data were in full agreement with the absence of β-D-glucose ester signals in the ^1H and ^{13}C spectra of the derivative of **5** obtained after alkaline hydrolysis. The ^1H and ^{13}C NMR spectra (Tables 1 and 3) confirmed the presence of four glucose units. The second glycosidation site was shown to be at C-3 on the basis of the downfield shift exhibited in the ^{13}C NMR spectrum by C-3 (83.0 ppm) and the upfield shift experienced by C-2 (26.4 ppm) and C-4 (42.9 ppm) when compared with corresponding signals in hederagenin (*15*) and was subsequently confirmed by 2D NMR experiments (Figure 2). Inspection of the ^1H and ^{13}C NMR spectra indicated that saponin **5** was the 3,28-*O*-bisdesmoside of hederagenin

Table 1. ^{1}H NMR Data of Compounds 1−6 Recorded in CD_3OD (500 MHz)

proton	1	2	3	4	5	6
12	5.34 m	5.34 m	5.42 m	5.34 m	5.41 m	5.34 m
18	2.72 dd J = 13.5, 2.5 Hz	2.73 dd J = 14.3, 3.7 Hz	2.88 dd J = 13.6, 3 Hz	2.73 dd J = 14.3, 3.0 Hz	2.88 dd J = 13.0, 2.9 Hz	2.73 dd J = 14.3, 3.6 Hz
23		0.97 s			3.30 d; 3.70 d J = 11.8 Hz; J = 11.8 Hz	0.98 s
24	0.72 s	0.86 s	0.74 s	0.75 s	0.74 s	0.86 s
25	1.01 s	1.06 s	1.01 s	1.00 s	1.00 s	1.08 s
26	0.81 s	0.82 s	0.83s	0.81 s	0.82 s	0.81 s
27	1.21 s	1.18 s	1.19 s	1.21 s	1.20s	1.19 s
29	1.17 s	1.17 s	0.96 s	1.17 s	0.96 s	1.17 s
30			0.94 s		0.94 s	
OCH_3	3.73 s	3.72 s		3.73 s		3.73 s
H_{anom}-Glc linked at C-28	5.37 d J = 8.0 Hz	5.34 d J = 8.0 Hz	5.42 d J = 8.5 Hz	5.41 d J = 8.4 Hz	5.41 d J = 8.4 Hz	5.37 d J = 8.3 Hz
H_{anom}-GlcA	4.52 d J = 7.24 Hz					4.41 d J = 8.0 Hz
H_{ano}- Glc_{inner}		4.40 d J = 6.6 Hz		4.59 d J = 8.1 Hz	4.59 d J = 8.1 Hz	
H_{anom}- Glc_{outer}		4.50 d J = 6.6 Hz	4.38 d J = 7.7 Hz	4.78 d J = 8.1 Hz	4.78 d J = 8.1 Hz	
H_{anom}-Ara	4.58 d J = 7.7 Hz	4.58 d J = 7.3 Hz	4.57 d J = 7.7 Hz			4.58 d J = 7.2 Hz
H_{anom}-Glc linked at C-3				4.45 d J = 7.5 Hz	4.45 d J = 7.5 Hz	

Table 2. ^{13}C NMR Data of Data of Aglycon Moieties of Compounds 1−6 Recorded in CD_3OD

C	DEPT	1	2	3	4	5	6
1	CH_2	39.6	39.8	39.6	39.6	39.6	39.8
2	CH_2	26.3	26.9	26.4	26.2	26.4	26.9
3	CH	82.1	91.8	84.0	85.5	83.0	90.8
4	C	43.8	40.4	42.9	43.3	42.9	40.7
5	CH	48.1	57.0	48.0	48.2	48.0	57.0
6	CH_2	18.8	19.3	18.8	18.9	18.8	19.3
7	CH_2	33.4	30.7	31.5	33.1	31.5	30.7
8	C	40.6	40.7	40.7	40.6	40.7	40.7
9	CH	47.6	48.0	48.0	48.0	48.0	48.0
10	C	37.6	37.9	37.6	37.7	37.6	37.9
11	CH_2	24.1	24.1	23.9	24.1	23.9	24.1
12	CH	124.3	124.4	123.9	124.4	123.9	124.4
13	C	144.5	144.4	145.0	144.5	145.0	144.4
14	C	42.9	42.8	43.0	42.9	43.0	42.8
15	CH_2	28.9	28.9	28.9	28.9	28.9	28.9
16	CH_2	24.5	24.5	24.6	24.6	24.6	24.5
17	C	47.4	47.4	47.2	47.4	48.0	47.4
18	CH	43.9	43.9	42.6	43.9	42.6	43.9
19	CH_2	43.3	43.3	47.2	43.3	47.2	43.3
20	C	44.9	45.0	31.5	44.9	31.5	45.0
21	CH_2	30.7	31.3	34.9	30.8	34.9	31.3
22	CH_2	34.3	34.4	33.2	34.4	33.2	34.4
23	CH_2	64.8		64.9	64.9	64.9	
	CH_3		26.2				26.2
24	CH_3	13.4	16.0	13.3	13.3	13.3	16.0
25	CH_3	16.5	17.0	16.5	16.5	16.5	17.0
26	CH_3	17.7	17.7	17.7	17.7	17.7	17.7
27	CH_3	26.2	26.2	26.4	26.2	26.4	26.2
28	C	177.6	178.9	178.2	176.6	178.2	178.9
29	CH_3	28.6	28.6	33.5	28.6	33.5	28.6
30	C	178.8	177.6		177.6		177.6
	CH_3			23.9		23.9	
OCH_3		52.3	52.4		52.3		52.4

Table 3. ^{13}C NMR Data of Sugar Moieties of Compounds 1−6 Recorded in CD_3OD

C	1	2	3	4	5	6
C-28 sugar	**Glc-28**	**Glc-28**	**Glc-28**	**Glc-28**	**Glc-28**	**Glc-28**
1	95.7	95.7	95.7	95.7	95.7	95.7
2	75.0	75.0	75.3	75.1	75.1	75.1
3	78.7	78.7	78.7	78.7	78.7	78.7
4	71.0	71.1	71.1	71.1	71.1	71.0
5	78.2	78.3	78.3	78.3	78.3	78.2
6	62.3	62.3	62.3	62.4	62.4	62.3
C-3 sugar	**GlcA**	**Ara**	**Ara**	**Glc**	**Glc**	**GlcA**
1	104.6	105.4	105.5	104.7	104.7	105.6
2	74.4	72.1	72.1	73.9	73.9	72.2
3	86.8	81.0	83.6	77.7	77.7	86.6
4	71.8	70.7	69.6	80.4	80.4	71.0
5	77.6	67.2	66.9	77.2	77.2	77.6
6	177.6			62.9	62.9	176.6
	Ara	**Glc_{inner}**	**Glc**	**Glc_{inner}**	**Glc_{inner}**	**Ara**
1	105.6	105.5	106.1	105.5	105.5	106.3
2	74.6	85.5	73.9	72.0	72.0	74.9
3	73.9	77.2	77.9	77.7	77.7	73.8
4	70.9	71.1	71.2	80.4	80.4	69.0
5	67.2	78.3	77.7	76.2	76.2	67.1
6		62.3	62.3	62.4	62.4	
		Glc_{outer}		**Glc_{outer}**	**Glc_{outer}**	
1		104.7		104.4	104.4	
2		77.1		76.2	76.2	
3		78.3		78.3	78.3	
4		71.1		71.1	71.1	
5		78.2		78.2	78.2	
6		63.3		62.4	62.4	

having four monosaccharide units. The interglycosidic linkages and the sequential arrangement of the trisaccharide moiety linked to C-3 of the aglycon were determined using 2D $^{1}H-^{13}C$ correlation (HMBC) (Figure 2). On the basis of these assignments, it was possible to determine the position of glycosidic bonds. Diagnostic correlation peaks were observed between H-1 of glucose (δ 4.45) and C-3 of the aglycon (δ 83.0), between H-1 of glucose inner (δ 4.59) and C-4 of glucose (δ 80.4), and between H-1 of glucose outer (δ 4.78) and C-4 of glucose inner (δ 80.4). These data led to the assignment of **5** as hederagenin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside (Figure 1).

Compound **6** was a white powder: $[\alpha]_D$ 2.3° (c 0.003 in MeOH), IR ν_{max}^{KBr} cm^{-1} 3350 (OH); 1700 (COOR); FABMS (negative ion) m/z 969 $[M - H]^-$; HRFABMS found m/z 969.4702, calcd for $C_{48}H_{74}O_{20}$ m/z 969.4773.

On acidic hydrolysis, **6** afforded spergulagenic acid, which was identified by ^{1}H and ^{13}C NMR spectra (Tables 1−3) and by comparison with literature data (*10*), and glucose, arabinose, and glucuronic acid as sugars. The ^{1}H NMR spectrum of **6** exhibited six methyl signals at

Figure 2. Selected HMBC (→) and HMQC (····>) correlations in compounds **1**−**6**.

δ 0.81−1.19, together with one *O*-methyl singlet at δ 3.73, an olefinic proton at δ 5.34, and three anomeric proton signals at δ 5.37 (1H, d, J = 8.3 Hz), 4.58 (1H, d, J = 7.2 Hz), and 4.41 (1H, d, J = 8.0 Hz). All proton resonances were correlated with those of their corresponding carbons from the HMQC experiment. The interglycosidic and sequential arrangements of the disaccharide moiety linked at C-3 of the aglycon linkages were deduced from the HMBC spectra. HMBC cross-peaks were detected between the anomeric proton (H-1) of a terminal arabinose at δ 4.58 (1H, d, J = 7.2 Hz) and the glucuronic acid signal at δ 86.6 (C-3) as well as between the glucuronic acid (H-1) signal at δ 4.41 (1H, d, J = 8.0 Hz) and the signal at δ 90.8 (aglycon C-3). A glycosylation shift of C-3 (8 ppm) of glucuronic acid (*11*) indicated the linkage of a terminal arabinose to GlcA and linkage of the latter to the 3-hydroxyl group of the aglycon (Figures 1 and 2). Furthermore, the ^{1}H, ^{13}C NMR, and DEPT data of the disaccharide moiety were in full agreement with those described by Rastrelli et al. (*14*), who identified the same disaccharide moiety when C-23 and C-24 of the aglycon are CH_3. The C-28-*O*-glycosidic linkage between the anomeric proton of glucose and the carboxyl group of the aglycon was deduced from the chemical shifts of the anomeric proton (δ 5.37), its carbon signal (δ 95.7), and the resonance of C-28 of the aglycon (δ 178.9) (*14*). The HMBC cross-peak between the anomeric glucose proton and C-28 (Figure 2) was in full agreement with the results of alkaline hydrolyses giving a monodesmoside. Chemical shifts, multiplicity of the signals, absolute values of the coupling constants, and their magnitude in the ^{1}H NMR spectrum as well as ^{13}C NMR data (Tables 1−3) indicated the β configuration at the anomeric positions for glucuronopyranosyl (J = 8.0 Hz) and glucopyranosyl (J = 8.3 Hz) units and the α configuration at the anomeric position for the arabinopyranosyl unit (J = 7.2 Hz). These results suggested that **6** was a bisdesmoside that contains glucose, arabinose, and glucuronic acid. From these data, the structure of **6** was established as spergulagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranoside (Figure 1).

Six saponins were found in the sample of quinoa from the discarded water layer after the BuOH/H_2O partition of the residue obtained from the MeOH extract. All six of the isolated saponins are bisdesmosides with sugar chains made up of one glucose moiety linked to C-28 and two or three monosaccharide units linked to C-3.

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LITERATURE CITED

(1) National Research Council. *Lost Crops of the Incas: Little Known Plants of the Andes with Promise for Worldwide Cultivation*; National Academy Press: Washington, DC, 1989; pp 149−162.
(2) Schlick, G.; Bubenheim, D. L. Quinoa: An emerging “new” crop with potential for CELSS. *NASA Technol. Paper* **1993**, 3422.
(3) Koziol, M. J. Chemical composition and nutritional evaluation of Quinoa. *J. Food Compos. Anal.* **1992**, *5*, 35−68.
(4) Oser, B. An evaluation of *Yucca mohavensis* as a source of food grade saponin. *Food Cosmet. Toxicol.* **1966**, *4*, 57−61.
(5) Ishaaya, I.; Birk, Y.; Bondi, A.; Tencer, Y. Soyabean saponins IX. Studies of their effect on birds, mammals and cold blooded organisms. *J. Sci. Food Agric.* **1969**, *20*, 433−436.
(6) Phillips, J. K.; Butterworth, I.; Gaunt, J.; Evans, A.; Grasso, P. Long-term toxicity study of quillaja extract in mice. *Food Cosmet. Toxicol.* **1979**, *17*, 23−27.

(7) Malinow, M. W.; McNulty, D.; Houghton, S.; Kessler, P.; Stenzel, S.; Goodnight, E.; Bardana, J.; Polatay, P.; McLaughlin, P.; Livingston, A. Lack of toxicity of alfalfa saponins in monkeys. *J. Med. Primatol.* **1982**, *11*, 106–118.

(8) Malinow, M.; McLaughlin, P.; Bardana, E.; Craig, S. Elimination of toxicity from diets containing alfalfa seeds. *Food Cosmet. Toxicol.* **1984**, *22*, 583–587.

(9) Oakenfull, D.; Sidhu, G. Could saponins be a useful treatment for hypercholesterolaemia? *Eur. J. Clin. Nutr.* **1990**, *44*, 79–88.

(10) Mizui, F.; Kasai, R.; Otani, K.; Tanaka, O. Saponins from brans of Quinoa, *Chenopodium quinoa* Wiild. II. *Chem. Pharm. Bull.* **1990**, *38*, 375–377.

(11) Dini, I.; Schettino, O.; Simioli, T.; Dini, A. Studies on the constituents of *Chenopodium quinoa* seeds: Isolation and characterization of new triterpene saponins. *J. Agric. Food Chem.* **2001**, *49*, 741–746.

(12) Martin, G. E.; Crouch, R. C. Inverse detected two-dimensional NMR methods application in natural products chemistry. *J. Nat. Prod.* **1991**, *54*, 1–70.

(13) Bax, A.; Aszavalos, A.; Dinya, Z.; Sudo, K. Structure elucidation of the antibiotic desertomycin thought the use of new two-dimensional NMR techniques. *J. Am. Chem. Soc.* **1986**, *108*, 8056.

(14) Rastrelli, L.; De Simone, F.; Schettino, O.; Dini, A. Constituents of *Chenopodium pallidicaule* (Canihua) seeds: Isolation and characterization of new triterpene saponins. *J. Agric. Food Chem.* **1996**, *44*, 3528–3533.

(15) Tori, K.; Seo, S.; Shimaoka, A.; Tomita, Y. Carbon-13 NMR spectra of olean-12-enes. Full signal assignments including quaternary carbon signals assigned by use of indirect ^{13}C, ^{1}H spin couplings. *Tetrahedron Lett.* **1974**, *48*, 4227–4230.

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