Studies on the Constituents of *Chenopodium quinoa* Seeds: Isolation and Characterization of New Triterpene Saponins

Irene Dini,[†] Oreste Schettino,[‡] Tiziana Simioli,[†] and Antonio Dini*[,†]

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", via D. Montesano, 49, 80131 Naples, Italy, and Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli "Federico II", via D. Montesano 49, 80131 Naples, Italy

Six triterpenoid saponins were isolated from the edible grain quinoa, which is seeds of *Chenopodium quinoa* (Chenopodiaceae). Following are their structures: phytolaccagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside (**1**); phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**2**); phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1‴→3″)-*β*-D-xylopyranosyl-(1″→2′)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside (**3**); phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1‴→2″)-*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**4**); oleanolic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside (**5**); and oleanolic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**6**). The oleanane-type saponins (**5**, **6**) were isolated for the first time in this plant, two of the phytolaccagenane (**1**, **3**) were new compounds and two (**2**, **4**) were previously found in quinoa. The structures were characterized on the basis of hydrolysis and spectral evidence, including 1D- and 2-D NMR (HMQC and HMBC) and ESI-MS analyses.

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

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a plant of the Chenopodiaceae family, used as a food plant principally in the same way as wheat and rice. It is known as a pseudo-cereal, although it is not in the family of Gramineae. It has been a major crop in the Andes mountains since 3000 B.C. For the Incas it was the mother grain, but presently quinoa is cultivated in only a few regions of South America and even less in other parts of the world. Nevertheless, there is renewed interest in this plant, considered to be recently "discovered" by agricultural researchers from industrialized societies (*1* and *2*). The Food and Agriculture Organization (FAO) observes that Quinoa is closer to the ideal protein balance than any other common grain (*3*), being at least equal to milk in protein quality. The nutritional profile has higher levels of energy, calcium, phosphorus, iron, fiber, and B-vitamins than barley, oats, rice, corn, or wheat. It has a nutritionally attractive amino acid balance, being exceptionally high in lysine. Lysine is considered to be deficient in most cereal grains, making their protein profile incomplete. It is therefore necessary to combine cereals with other crops such as legumes, rich in lysine, but lacking in methionine and cystine, to obtain a suitable amino acid pattern that meets the needs for humans. Quinoa is being considered as a potential crop for the National Aeronautics and Space Administration's (NASA's) Controlled Ecological Life Support System (CELSS) (*4* and *5*). In fact, quinoa plants can remove carbon dioxide from the atmosphere and generate oxygen and water, and provide foods as seeds, leaves, and sprouts, with a substantial amount of nutritive value for long-term human space missions. The seeds are used boiled like rice, processed to provide pasta, puffed cereals, and extruded foods (in blends with corn and with oats), or in desserts and side dishes. The flour blends well with wheat flour or corn meal for bread and biscuits. Nevertheless, quinoa has a naturally bitter seed coating which encases the seed and protects it from birds and insects. Saponins impart the bitter taste and form a soapy solution in water.

Until recently, saponins have been considered to be highly toxic. Because they are toxic to various organisms, saponins are being studied for their insecticidal, antibiotic, fungicidal, and pharmacological properties. Nevertheless, although saponins are extremely toxic to cold-blooded animals their oral toxicity to mammals is low. Saponins present in common foods, including quinoa, seem to be free from significant oral toxicity in humans (*6*−*11*). On the contrary, there is a pharmacological interest in the ability of saponins to induce changes in intestinal permeability (*12*) which may aid patients in absorbing drugs (*9*). The digestibility of quinoa protein was found to be comparable to that of other high-quality food proteins, and it was also found that the saponins do not exert any negative effect on the nutritive quality of the protein (*2*). Saponins are plant glycosides, a structurally diverse group of naturally occurring compounds found in plants, mainly in the family Leguminosae (*13*). Quinoa saponins are triterpenoid glycosides. They differ in aglicon moiety, in the saccharide moieties, and also in the substitution pattern of sugars of the sapogenins (*14*−*16*). The taste

* To whom correspondence should be addressed. Tel: 039-81-678535. Fax: 039-81-678552. E-mail: andini@cds.unina.it.
[†] Dipartimento di Chimica delle Sostanze Naturali.
[‡] Dipartimento di Chimica Farmaceutica e Tossicologica.

10.1021/jf000971y CCC: $20.00
Published on Web 01/26/2001

and pharmacological properties are linked to the whole molecules (*12*). Anyhow, saponin levels in final products for foods are reduced to decrease possible biological negative effects and bitterness.This is accomplished by selecting "sweet" quinoa varieties (some varieties exist in which saponin levels are approximately one-tenth that of normal varieties) or by processing the grains by removing the hulls or washing with water. The aim of this work is the isolation and characterization of the intact saponins still present in a commercial quinoa sample after being "debittered" by extraction with water.

Six saponins were found: two oleanane-type saponins (**5**, **6**) were isolated for the first time in this plant, two of the phytolaccagenane (**1**, **3**) were new compounds, and two compounds (**2**, **4**) were previously found in quinoa (*14* and *15*).

MATERIALS AND METHODS

Material. The plant material was supplied as a commercial product made October 3, 1997: "quinua lavada", *Mascorona* Km 4 1/2 casilla: 18-01-1439 Ambato-equador.

Apparatus. Optical rotations were determined on a Perkin-Elmer 192 Polarimeter equipped with a sodium lamp (589 nm) and 10-cm 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 (*17*), using an initial BIRD pulse to suppress ^1H resonances not coupled to ^{13}C and GARP sequence for ^{13}C decoupling during data acquisition. The spectral width in ^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 1 k 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 (*18*) and Martin and Crouch (*17*). The data processing was identical to that used for the HMQC experiment, and the final digital resolution was 2.25 Hz/pt. FABMS (recorded in a glycerol matrix) was measured on a Prospec Fisons mass spectrometer. GCMS was run using a Hewlett-Packard 5890 gas chromatograph fitted with an HP 5970B mass detector and an HP 59970 MS Chemstation, equipped with an HP-5 column (25 m × 0.2 mm i.d., 0.33 μm film). The FTIR spectra were performed on a Bruker IFS-48 spectrophotometer using a KBr matrix. UV spectra were performed 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 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 *n*-BuOH soluble fraction (3.25 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 [Silica gel plates in *n*-BuOH/HOAc/H_2O (60:15:25)]. Fractions 22−33 (511.4 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** (4.4 mg; T_R, 4.5 min), **2** (60.2 mg; T_R, 3.2 min), **3** (9.7 mg; T_R, 3.4 min), **4** (13.9 mg; T_R, 10.0 min), **5** (10.1 mg; T_R, 3.7 min), and **6** (1.6 mg; T_R, 4.2 min).

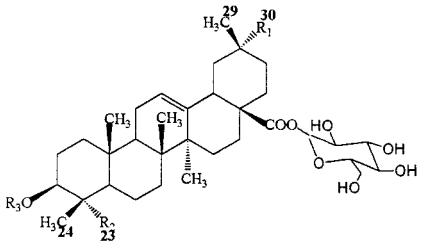
Acid Hydrolysis of Compounds 1−6: Monosaccharide Composition. A solution of each compound (1 mg) in 10% H_2SO_4/EtOH (1:1, 3.5 mL) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. 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 GCMS. Retention times were identical to those of the authentic trimethylsilylated sugars.

Alkaline Hydrolysis. Glycosides **1**, **2**, **3**, and **4** were separately heated in 0.5 M 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 were partitioned into a mixture of *n*-BuOH and H_2O to afford the *n*-BuOH soluble portion, which was subjected to Sephadex LH-20 chromatography. The fractions were checked by TLC, and fractions 22−33 (511.4 mg) containing the crude glycosidic mixture were submitted to reversed-phase HPLC to afford almost pure compounds (**1**−**6**) (Figure 1). The molecular formulas were determined by negative ion ESI−MS spectra and ^{13}C−DEPT NMR one-dimensional- and HMBC, HMQC two-dimensional- spectra.

Compound **1.** A white powder; $[\alpha]_D$ +10.8° (c = 0.003 in MeOH); IR ν_{max}^{KBr} cm^{-1} 3350 (OH); 1700 (COOR); FABMS (negative ion) m/z 985[M−H]$^-$; HRFABMS: found, m/z 985.4711, calcd for $C_{48}H_{74}O_{21}$, m/z 985.4723. On acidic hydrolysis, **1** afforded phytolaccagenic acid which was identified by ^1H and ^{13}C NMR spectra (Tables 1−3) and by comparison with literature data (*19*); and glucose, arabinose, and glucuronic acid as sugars. The ^1H NMR spectrum of **1** exhibited five methyl signals at δ = 0.72−1.21, together with one *O*-methyl singlet at δ = 3.73, an olefinic proton at δ = 5.34, and three anomeric protons signals at δ = 5.37 (^1H, d, J = 8.02 Hz), 4.52 (^1H, d, J = 7.24 Hz), and 4.57 (^1H, d, J = 7.24 Hz). All proton resonances were correlated with those of their corresponding carbons from HMQC spectrum. The interglycosidic and glycoside-aglycon linkages were deduced from the HMBC spectra. HMBC cross-peaks between the anomeric proton (H-1″) of a terminal arabinose at δ = 4.57 (^1H, d, J = 7.24 Hz) and the glucuronic acid signal at δ = 86.8 (C-3′) as well as between the glucuronic acid (H-1′) signal at δ = 4.52 (^1H, d, J = 7.24 Hz) and the signal at δ = 82.1 (aglycon C-3), whereas glycosidation shift on C-2 (∼2 ppm) and C-3 (∼8 ppm) of glucuronic acid indicated the linkage of a terminal arabinose via its anomeric carbon to GlcA-3 and linkage of the latter to the 3-hydroxy group of the aglycon (Figures 1 and 2). Furthermore, the ^1H, ^{13}C NMR, and DEPT spectra signals of the disaccharide moiety were in agreement with those described by Rastrelli et al. (*20*) in the similar compound **1**. The C-28-*O*-glycosidic linkage between Glc-1 and the carboxyl group of the aglycon was deduced from the chemical shifts of the anomeric proton (δ = 5.37), the carbon signals of the sugar (δ = 95.7), and of C-28 of the aglycon (δ = 177.6) (*20*), from HMBC cross-peak between the



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

	R_1	R_2	R_3
1	$COOCH_3$	CH_2OH	α-L-arabinopyranosyl-(1''→3')-β-D-glucuronopyranosyl
2	$COOCH_3$	CH_2OH	β-D-glucopyranosyl-(1''→3')-α-L-arabinopyranosyl
3	$COOCH_3$	CH_2OH	β-D-glucopyranosyl-(1'''→3'')-β-D-xylopyranosyl – (1''→2')-β-D-glucopyranosyl
4	$COOCH_3$	CH_2OH	β-D-glucopyranosyl-(1'''→2'')-β-D-glucopyranosyl-(1''→3')-α-L-arabinopyranosyl
5	CH_3	CH_3	α-L-arabinopyranosyl-(1''→3')-β-D-glucuronopyranosyl
6	CH_3	CH_3	β-D-glucopyranosyl-(1''→3')-α-L-arabinopyranosyl

Figure 1. Compounds **1**−**6**.

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

proton	1	2	3	4	5	6
12	5.34 m	5.34 m	5.34 m	5.34 m	5.28 m	5.28 m
18	2.72 dd	2.73 dd	2.73 dd	2.72 dd	2.88 dd	2.88 dd
	J = 13.5; 2.5 Hz	J = 14.0; 2.9 Hz	J = 14.3; 3.7 Hz	J = 14.3; 3.7 Hz	J = 13.6; 2.9 Hz	J = 13.6; 3.0 Hz
23					0.97 s	0.98s
24	0.72 s	0.75 s	0.73 s	0.93 s	0.86 s	0.87 s
25	1.01 s	1.01 s	1.01 s	1.00 s	1.07 s	1.07 s
26	0.81 s	0.82 s	0.82 s	0.81 s	0.82 s	0.83s
27	1.21 s	1.21 s	1.21 s	1.20 s	1.18 s	1.18 s
29	1.17 s	1.17 s	1.17 s	1.17 s	0.96 s	0.96 s
30					0.94 s	0.94 s
OCH_3	3.73 s	3.73 s	3.73 s	3.72 s		
H_{anom}−Glc-28	5.37 d	5.38 d	5.37 d	5.34 d	5.41 d	5.38 d
	J = 8.0 Hz	J = 8.1 Hz	J = 8.1 Hz	J = 8.02 Hz	J = 7.7 Hz	J = 8.1 Hz
H_{anom}−GlcA	4.52 d				4.40 d	
	J = 7.24 Hz				J = 8.8 Hz	
H_{anom}−Glcinner			4.64 d	4.40 d		4.38 d
			J = 7.7 Hz	J = 11.0 Hz		J = 7.3 Hz
H_{anom}−Glcouter		4.38d	4.64 d	4.50 d		
		J = 7.3 Hz	J = 7.7 Hz	J = 6.6 Hz		
H_{anom}−Ara	4.58 d	4.58 d			4.58 d	4.58 d
	J = 7.7 Hz	J = 7.3 Hz			J = 7.7 Hz	J = 7.7 Hz
$H_{anom-Xyl}$			4.42 d			
			J = 7.7 Hz			

anomeric glucose proton and C-28, and 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 (see experimental), as well as ^{13}C NMR data (Table 1) indicated the β configuration at the anomeric positions for glucuronopyranosyl (J = 7.24 Hz) and glucopyranosyl (J = 8.02 Hz) units and the α configuration at the anomeric position for arabinopyranosyl unit (J = 7.24 Hz). These results suggested that **1** was a bisdesmoside that contains glucose, arabinose, and glucuronic acid. From these data, the structure of **1** was established as phytolaccagenic acid 3-*O*-[α-L-arabinopyranosyl-(1′′→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside.

Compound **2.** A white powder; $[\alpha]_D$ +5.8° (c = 0.005 in MeOH); IR ν_{max}^{KBr} cm^{-1} 3350 (OH), 1700 (COOR); FABMS (negative ion) m/z 971[M−H]$^-$; HRFABMS found m/z 971.4945, calcd for $C_{48}H_{76}O_{20}$ m/z 971.4930. On acidic hydrolysis, **2** afforded phytolaccagenic acid, and glucose and arabinose as sugars. The ^{1}H and ^{13}C

Table 2. ^{13}C NMR 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	39.6	39.5	39.6	39.8	39.7
2	CH_2	26.3	26.3	26.3	26.2	26.3	26.8
3	CH	82.1	84.2	85.1	85.5	90.7	90.7
4	C	43.8	43.3	43.3	43.3	40.2	40.2
5	CH	48.1	47.9	48.2	48.2	57.0	57.0
6	CH_2	18.8	18.9	18.9	18.9	19.3	19.3
7	CH_2	33.4	33.4	33.4	33.1	30.8	30.6
8	C	40.6	40.6	40.6	40.6	40.7	40.7
9	CH	47.6	47.4	49.2	48.0	48.0	48.0
10	C	37.6	37.7	37.7	37.7	37.8	37.8
11	CH_2	24.1	24.1	24.1	24.1	24.5	24.5
12	CH	124.3	124.3	124.3	124.4	123.9	123.8
13	C	144.5	144.5	144.5	144.5	144.5	144.6
14	C	42.9	42.9	42.9	42.9	42.6	42.8
15	CH_2	28.9	28.9	28.9	28.9	28.9	28.9
16	CH_2	24.5	24.6	24.6	24.6	24.1	24.3
17	C	47.4	47.4	47.4	47.4	47.2	47.1
18	CH	43.9	43.9	43.9	43.9	42.6	42.6
19	CH_2	43.3	43.3	43.3	43.3	47.2	47.2
20	C	44.9	45.0	45.0	44.9	31.5	31.5
21	CH_2	30.7	30.8	30.8	30.8	34.9	34.9
22	CH_2	34.3	34.3	34.4	34.4	33.2	33.2
23	CH_2	64.8	65.2	64.9	64.9		
23	CH_3					28.4	28.4
24	CH_3	13.4	13.4	13.4	13.3	16.0	16.0
25	CH_3	16.5	16.6	16.5	16.5	17.0	17.0
26	CH_3	17.7	17.8	17.7	17.7	17.7	17.7
27	CH_3	26.2	26.3	26.3	26.2	26.3	26.3
28	C	177.6	177.6	177.6	176.6	178.1	178.1
29	CH_3	28.6	28.7	28.6	28.6	33.5	33.4
30	C	178.8	178.8	178.8	177.6		
30	CH_3					24.0	24.0
OCH_3		52.3	52.4	52.4	52.3		

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

	1	2	3	4	5	6
	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	75.3	73.9	75.0	75.0	75.3
3	78.7	78.7	78.7	78.7	78.7	78.7
4	71.0	71.1	71.0	71.1	71.1	71.1
5	78.2	78.3	78.3	78.3	78.3	78.3
6	62.3	62.3	62.5	62.3	62.4	62.3
	GlcA	Glc_{outer}	Glc_{inner}	Glc_{inner}	GlcA	Glc_{outer}
1	104.6	106.1	104.5	105.5	105.5	106.1
2	73.9	73.9	83.5	85.5	73.9	73.9
3	86.8	77.9	76.1	77.2	86.4	77.9
4	71.8	71.2	70.9	71.1	71.0	71.2
5	77.6	77.7	77.7	78.3	77.6	77.7
6	177.6	62.3	62.3	62.3	177.6	62.3
	Ara	Ara	Glc_{outer}	Glc_{outer}	Ara	Ara
1	105.6	105.5	106.4	104.7	106.7	105.5
2	74.6	72.1	73.9	77.1	74.9	72.1
3	73.9	83.6	78.9	78.3	73.9	83.6
4	70.9	69.6	70.7	71.1	71.0	69.6
5	67.2	66.9	77.5	78.2	67.4	66.9
6			62.1	63.3		
			Xyl	Ara		
1			106.0	105.4		
2			71.7	72.1		
3			86.1	81.0		
4			69.1	70.7		
5			67.2	67.2		

NMR and DEPT spectra (Tables 1-3) signals were in agreement with those of phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl-]-28-*O*-*β*-D-glucopyranoside described by Mizui et al. (*15*).

Compound **3.** A white powder; $[\alpha]_D$ +3° (c = 0.004 in MeOH); IR ν_{max}^{KBr} cm^{-1} 3350 (OH), 1700 (COOR); FABMS (negative ion) m/z 1133[M−H]$^-$; HRFABMS found m/z 1133.5441, calcd for $C_{54}H_{86}O_{25}$ m/z 1133.5458. The ^{13}C NMR spectra showed 54 signals of which 23 were assigned to the saccharide portion and 31 to a triterpenic moiety. On acidic hydrolysis, **3** afforded phytolaccagenic acid which was identified by ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), and the sugars glucose and xylose in a 3:1 ratio. These results suggested that **3** was a bisdesmoside that contained glucose and a trisaccharide unit containing glucose and xylose in a 2:1 ratio. Chemical shifts, multiplicity of the signals, absolute values of the coupling constants and their magnitude in the ^{1}H NMR spectrum (Table 1), as well as ^{13}C NMR data (Tables 2 and 3) indicated the *β* configuration at the anomeric positions for xylopyranosyl (J = 7.7 Hz), glucopyranosyl inner and outer (J = 7.7 Hz), and glucopyranosyl 28 (J = 8.1 Hz) units. The presence of a *β*-glucose unit linked at the carboxyl group (C-28) of the aglycon was consistent with the anomeric signals at *δ* 5.37 (1H, d, J = 8.1 Hz) in the ^{1}H spectrum and at *δ* 95.7 in the ^{13}C spectrum and in full agreement with the results of alkaline hydrolysis of **3**. The ether glycosidation site was shown to be at C-3 on the basis of the downfield shift exhibited in the ^{13}C spectrum by for C-3 (48.2 ppm), and the upfield shifts experienced by C-2 (26.3 ppm) for C-4 (43.3 ppm),when compared with corresponding signals in phytolaccagenic acid, and was subsequently confirmed by 2-D NMR experiments. The interglycosidic linkage and the sequential arrangement of the trisaccharide moiety linked at C-3 of the aglycon was determined by ^{13}C NMR and HMBC spectra. The ^{13}C showed significant glycosidation shifts: for the C-2′ (+8 ppm), the C-1′ (−2 ppm), and the C-3′ (−2.7 ppm) of the Glc_{inner} moiety; and for the C-3″ (+8 ppm), the C-2″ (−2.2 ppm), and the C-4″ (−2 ppm) of the Xyl moiety. In the HMBC experiment carried out to confirm the position of glycosyl bond were observed diagnostic HMBC cross-peaks between the anomeric proton (H-1‴) of a terminal $glucose_{outer}$ at *δ* = 4.64 (^{1}H, d, J = 7.7 Hz) and the xylose (C-3″) signal at *δ* = 86.1 as well as between the xylose (H-1″) signal at *δ* = 4.42 (^{1}H, d, J = 7.7 Hz) and the signal (C-2′) at *δ* = 83.5 (Glc_{inner}-2), and between the glucose (H-1′) signal at *δ* = 4.64 (^{1}H, d, J = 7.7 Hz) and signal (C-3) at *δ* = 85.1 (aglycon C-3), indicating linkage of a terminal glucose via its anomeric carbon to Xyl-3 linked to Glc-2 and linkage of the latter to the 3-hydroxy group of the aglycon (Figures 1 and 2). Compound **3** was identified as phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1‴→3″)-*β*-D-xylopyranosyl-(1″→2′)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside.

Compound **4.** A white powder; $[\alpha]_D$ -1° (c = 0.002 in MeOH); IR ν_{max}^{KBr} cm^{-1} 3350 (OH), 1700 (COOR); FABMS (negative ion) m/z 1133[M−H]$^-$; HRFABMS found m/z 1133.5469, calcd for $C_{54}H_{86}O_{25}$ m/z 1133.5458. The ^{13}C NMR spectra showed 54 signals of which 23 were assigned to the saccharide portion and 31 to a triterpenic moiety. On acidic hydrolysis, **4** afforded phytolaccagenic acid and glucose and arabinose as sugars in a ratio 2:1. The ^{1}H and ^{13}C NMR and DEPT spectra (Tables 1−3) signals were in agreement with those for phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1‴→2″)-*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside described by Mizui et al. (*15*).

Compound **5.** A white powder; $[\alpha]_D$ +2° (c = 0.002 in MeOH); IR ν_{max}^{KBr} cm^{-1} 3350 (OH), 1700 (COOR); FABMS (negative ion) m/z 925[M−H]$^-$; HRFABMS found m/z

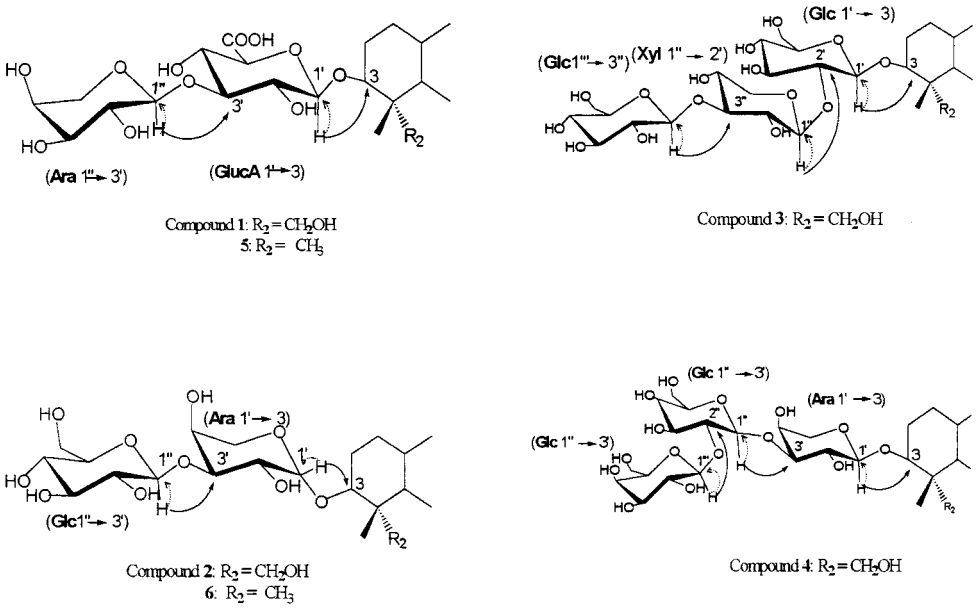


Figure 2. Selected HMBC (→) and HMQC (····>) correlations in compounds **1**−**6**.

925.4886, calcd for $C_{47}H_{74}O_{18}$ *m/z* 925.4875. The ^{13}C NMR spectra showed 47 signals, of which 17 were assigned to the saccharide portion and 30 to a triterpenic moiety. On acidic hydrolysis, **5** afforded oleanolic acid and glucose, arabinose, and glucuronic acid as sugars in a ratio 1:1:1. The ^{1}H and ^{13}C NMR and DEPT spectra (Tables 1-3) signals were in agreement with those of oleanolic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside, previously isolated from the root of *Momordica cochinchinensis* (*21*).

Compound **6.** A white powder; $[\alpha]_D$ +3° (*c* = 0.002 in MeOH); IR ν_{max}^{KBr} cm^{-1} 3350 (OH), 1700 (COOR); FABMS (negative ion) *m/z* 911[M−H]$^-$; HRFABMS found *m/z* 911.5094, calcd for $C_{47}H_{76}O_{17}$ *m/z* 911.5082. The ^{13}C NMR spectra showed 47 signals, of which 17 were assigned to the saccharide portion and 30 to a triterpenic moiety. On acidic hydrolysis, **5** afforded oleanolic acid and as sugars glucose and arabinose in a ratio of 2:1. The ^{1}H and ^{13}C NMR and DEPT spectra (Tables 1-3) signals were in agreement with those of oleanolic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside isolated before from *Fagonia indica* (*22*).

Six saponins were found in the sample of quinoa despite the fact that it was washed with water. The results show that this process for debittering was not sufficient to reduce saponins at all, particularly the saponins **1-4**, which contain phytolaccagenic acid as aglycon moiety. The occurrence of phytolaccagenane−saponin type is very uncommon in the plant kingdom (*13*). The results appear important to evaluate what kind of saponins can remain after "debittering" quinoa with water.

ACKNOWLEDGMENT

The NMR and GCMS spectra were performed at the "Centro di Ricerca Interdipartimentale di Analisi Strumentale" and FABMS spectra were performed at the "Servizio di Spettrometria di Massa" of the University "Federico II" Napoli. The assistance of the staff of these organizations is gratefully appreciated.

LITERATURE CITED

(1) Risi, J.; Galwey N. W. The *Chenopodium* grains of the Andes: Inca crops for modern agriculture. *Adv. Appl. Biol.* **1984**, *10*, 145−217.

(2) Ruales, J.; Nair B. M. Nutritional quality of the protein in quinoa (*Chenopodium quinoa* Willd.) seeds. Plant Foods. *Hum. Nutr.* **1992,** *42*, 1-12.

(3) Koziol, M. J. Chemical composition and nutritional evaluation of Quinoa. *J. Food Comp. Anal.* **1992**, *5*, 35−68.

(4) Bubenheim, D. Plants for water recycling, oxygen regeneration, and food production. *Waste Management Res.* **1991**, *9*, 435−443.

(5) Schlick, G.; Bubenheim, D. L. Quinoa: An emerging "new" crop with potential for CELSS; NASA Technical Paper 3422; National Aeronautics and Space Administration, Ames Research Center: Moffett Field, CA, 1993.

(6) 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 Agr.* **1993**, *63*, 201−209.

(7) Malinow, M. W.; McNulty, D.; Houghton, S.; Kessler, P.; Stenzel, S.; Goodnight, E.; Bardana, J.; Polatay, P.; McLaughlin.; 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) Oser, B. An evaluation of *Yucca mohavensis* as a source of food grade saponin. *Food. Cosmet. Toxicol.* **1966**, *4*, 57−61.

(11) 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.

(12) Gee, J. M.; Price, K. R.; Ridout, C. L.; Wortley, G. M.; Hurrell, R. F.; Johson, I. T. Saponins of quinoa (*Chenopodium quinoa*): effects of processing on their abundance in quinoa products and their biological effects on intestinal mucosal tissue *J. Sci. Food Agr.* **1969**, *20*, 433−436.

(13) Price, K. R.; Johnson, I. T.; Fenwick, G. R.; The chemistry and biological significance of saponins in foods and feedingstuffs. *CRC Crit. Rev. Food Sci. Nutr.* **1987**, *26*, 27−135.
(14) Mizui, F.; Kasai, R.; Otani, K.; Tanaka, O. Saponins from brans of Quinoa, *Chenopodium quinoa* Willd. I. *Chem. Pharm. Bull.* **1988**, *36*, 1415−1418.
(15) Mizui, F.; Kasai, R.; Otani, K.; Tanaka, O. Saponins from brans of Quinoa, *Chenopodium quinoa* Willd. II. *Chem. Pharm. Bull.* **1990**, *38*, 375−377.
(16) Ma, W. W.; Heinstein, P. F.; McLaughlin, J. L. Additional toxic, bitter saponins from the seeds of *Chenopodium quinoa*. *J. Nat. Prod.* **1989**, *52*, 1132−1135.
(17) Martin, G. E.; Crouch, R. C. Inverse detected two-dimensional NMR methods application in natural products chemistry. *J. Nat. Prod.* **1991**, *54*, 1−70.
(18) Bax, A.; Aszavalos, A.; Dinya, Z.; Sudo, K. Structure elucidation of the antibiotic desertomycin through the use of new two-dimensional NMR techniques. *J. Am. Chem. Soc.* **1986**, *108*, 8056.
(19) Bandara, M. B.; Jayasinghe, L.; Karunaratne, V.; Wannigama, G. P.; Kraus, W.; Bokel, M.; Sotheeswaran, S. Dispoclisin, a bisdesmosidic triterpenoid saponin from *Dispoclisia glucences*. *Phytochemistry* **1989**, *28*, 2783−2785.
(20) 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.
(21) Iwamoto, M.; Okabe, H.; Yamauchi, T. Studies on the constituents of *Momordica cochinchinensis* Spreng. II. Isolation and characterization of the rot saponins, momordins I, II and III. *Chem. Pharm. Bull.* **1985**, *33*, 1−7.
(22) Shaker, K. H.; Berharnhardt, M.; Elgamal, M.; Hani. A.; Seifert, K. Triterpenoid saponins from *Fagonia indica*. *Phytochemistry* **1999**, *51*, 1049−1053.

Received for review August 2, 2000. Revised manuscript received November 21, 2000. Accepted December 1, 2000. This research was supported by 40% and 60% grants from the Ministero della Ricerca Scientifica e Tecnologica. Italy. The work is supported by a grant of Programma Operativo Plurifondo 1994−1999; Sottoprogramma 5 − Misura 5.4; Azione 5.4.2; Centri Pubblici di Ricerca; Bando di gara annualita' 1997; Progetto di ricerca sottoposto alla valutazione per il finanziamento da parte della Regione Campania ai sensi della l.r. 31.12.96, n.°10738 pubblicato sul B. U. R. C. n.3 del 16 gennaio 1997 modificato ed integrato il 5/3/1997 (delibera n.919 burc n.13 del 10/3/1997.

JF000971Y