# Identification and Biological Activities of Triterpenoid Saponins from *Chenopodium quinoa*

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At least 16 saponins were detected in the seeds of *Chenopodium quinoa*. The 5 previously isolated major saponins, 3-*O*-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester, 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester, 3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester, 3-*O*-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester, 3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester, and the new saponin 3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid were isolated and characterized using mainly NMR spectroscopy, mass spectrometry, and chemical methods. The antifungal activity against *Candida albicans* and hemolytic activity on erythrocytes of these compounds and derived monodesmosides were evaluated. Both bidesmosides and derived monodesmosides showed little or no antifungal activity, whereas a comparatively higher degree of hemolytic activity could be determined for monodesmosides.

**Keywords:** *Chenopodium quinoa; Chenopodiaceae; triterpene saponins; antifungal activity; hemolytic activity*

## INTRODUCTION

*Chenopodium quinoa* Willd. (Chenopodiaceae) is a pseudo grain that is still widely cultivated and a staple crop in some areas of the Andean highlands of South America (*1*). The seeds have an almost ideal nutritional composition containing 10−16% proteins with favorable amino acid profile, 5−9% lipids, 10−15% free sugars, and 0.5−1% minerals (*2*). The plant, in addition, shows tolerance to cold, drought, and soil salinity. As a result, it has become one of the newer food crops that have been hitherto neglected but are now gaining wider recognition. Its use is, however, complicated due to the bitter taste of the seeds, which has been attributed to their content of saponin and flavonol glycosides (*3*). A total of 13 saponins were characterized in references *4* and *5* from the bran of quinoa seeds. Traditionally, the seeds are either mechanically abraded to remove the bran, where the saponins are predominantly located, or leached with water to debitter them prior to use. Gee et al. (*6*) report that quinoa saponins were found to be membranolytic against cells of the small intestine and caused increase in mucosal permeability in vitro. However, increasing attention in recent years in this group of natural products has shown varied biological properties, some of which are deleterious, but many are also beneficial to humans. Because little is known regarding the biological actions of saponins from this plant and as part of a general investigation of crop plants containing potentially beneficial saponins, work on saponins of *C. quinoa* was undertaken.

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## MATERIALS AND METHODS

**General.** $^1$H, $^{13}$C, DEPT, HMQC, COSY, and HMBC NMR spectra were recorded on a Bruker DRX 500 or AC 300 spectrometer in pyridine-$d_5$ with TMS used as internal standard. $^1$H, $^{13}$C, DEPT 135, COSY, HMQC, and HMBC (collection conditions: 2 s relaxation delay; $t_2$ domain, $^1$H, SW = 4883 Hz, 8 transients containing 2048 complex points; $t_1$ domain ($^{13}$C) 256 increments over SW = 186 ppm) spectra of each compound were measured. High-resolution FAB mass spectra were recorded using a JEOL JMS 700 spectrometer, whereas FAB and EI mass spectra were obtained using a Varian MAT 312 spectrometer. For GLC-MS, a fused silica capillary column lined with OV-1 liquid phase together with a Finnigan MAT 4500 mass spectrometer operating at 45 eV was used. Trimethylsilyl derivatives of monosaccharides were prepared using *N*-methyl-*N*-(trimethylsilyl)-2,2,2-trifluoroacetamide (MSTFA) (Merck). Semipreparative and analytical HPLC was carried out on a Knauer Eurochrom 2000 HPLC system with a Lichrospher 100, RP-18 (Merck), column of either 250 × 4 mm or 250 × 8 mm i.d. Peaks were detected at 206 nm with a linear gradient of 20−64% $CH_3CN$ in 0.1% trifluoroacetic acid in water in 30 min.

**Plant Material.** The whole seeds of *C. quinoa*, obtained commercially in Chile, were used.

**Extraction and Isolation.** Dried and powdered seeds (900 g) were defatted using petroleum ether and extracted with MeOH in a Soxhlet for 72 h. Removal of MeOH from the methanol extract yielded a brown gum, which was then suspended in $H_2O$. This suspension was partitioned with *n*-BuOH and the *n*-BuOH fraction was collected. Removal of *n*-BuOH from this fraction then gave a residue containing a saponin mixture (45 g). Silica gel column chromatography using $CHCl_3$/MeOH/$H_2O$ (8:5:1) of this mixture gave fractions I−III. Further purification of fraction I with EtOAc/EtOH/$H_2O$ (7:2:2) and of fractions II and III with EtOAc/EtOH/$H_2O$ (7:4:1) on TLC followed by HPLC afforded **1** (30 mg), **2** (11 mg), **3** (30 mg), **4** (50.5 mg), **5** (21 mg), and **6** (40 mg). Respective retention times were 29.2, 26.4, 20.9, 17.4, 25.7, and 7.3 min.

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**Basic Hydrolysis**. Saponin (2−3 mg) was placed in a 50 mL round-bottom flask, 5 mL of 5% potassium hydroxide solution in methanol was added. After a condenser had been attached, the mixture was refluxed for 2 h. After the mixture had cooled, methanol was removed under a stream of nitrogen. The residue was then taken up in water, and the suspension was adjusted to pH 6 by the addition of acetic acid. The aqueous suspension was then centrifuged and extracted with *n*-BuOH saturated with water (3 × 5 mL). Water was removed from the aqueous fraction under a stream of compressed air. The residue was taken up in pyridine and filtered. Pyridine was then removed under reduced pressure. The residue was taken up in methanol and tested using TLC with system 4. A part of the residue was derivatized with MSTFA and analyzed using GLC-MS. Removal of *n*-BuOH from the *n*-BuOH fraction, on the other hand, gave the derivatives **1a**, **2a**, **3a**, and **4a**. These were purified using RP-18 SPE columns, and their purity was confirmed using HPLC and $^{13}C$ NMR.

**Acid Hydrolysis.** Saponin (2−5 mg) and 1 mL of 1 M HCl (dioxane/$H_2O$, 1:1) were placed in an ampule. The ampule was sealed and placed in an oven at 90 °C for 2 h. The ampule was cooled, its seal was broken, and its contents were dried under a stream of nitrogen. The contents of the ampule were suspended in $H_2O$ (3 mL), and the aqueous suspension was washed with EtOAc (3 × 3 mL). After collection of the EtOAc fraction, removal of the solvent yielded the aglycon. Neutralizing the aqueous hydrolysate with $Ag_2CO_3$ and centrifugation of the precipitate gave monosaccharides.

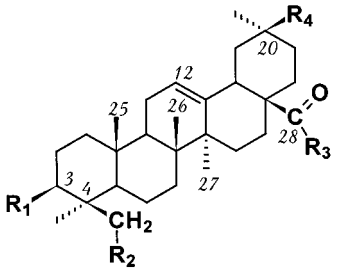
**Antifungal Assay.** Compounds **1**−**6** and the monodesmosidic derivatives **1a**, **2a**, **3a**, and **4a** were tested for antifungal activity according to a modified NCCLS (National Committee for Clinical Laboratory standards) microdilution assay procedure (*7*). All tests were carried out using sterile 96-well plates and Saburaud dextrose broth (Sigma). The log phase inoculum was obtained by incubating *Candida albicans* (ATCC 10231) for 48 h at 30 °C on Saburaud dextrose agar plates. The compounds were dissolved in either $H_2O$ or MeOH/$H_2O$ combination. Solutions of each test compound were further diluted with SDB, yielding their final concentration. Each saponin was evaluated in triplicate in a dose−response design in which the final concentrations were 1, 25, 50, 100, and 500 $\mu$g/mL and the final fungal concentration was $2 \times 10^3$ colony-forming units (cfu). To each well containing double the final saponin dilution, an equal volume of the inoculum suspension containing double the final fungal concentration was added. The minimum inhibitory concentration (MIC), the lowest concentration of the test compound resulting in the complete inhibition of growth, was determined visually. Amphotericin B (Sigma) at 1 $\mu$g/mL final concentration was used as a positive control.

**Hemolysis Assay.** One milliliter of 1% (v/v) sheep erythrocyte (Oxoid) suspension in phosphate-buffered saline (PBS, pH 7.4) was incubated with 100 $\mu$L of saponin dilutions (1.0, 0.25, 0.05, 0.01, 0.005, and 0.001 mg/mL) in a physiological salt solution (PBS) at 37 °C for 30 min. The mixture was centrifuged (2500*g*, 30 min) and the hemoglobin content in the supernatant measured at 490 nm by a multiwell spectrophotometer (Virion autoReader A) after transfer of 200 $\mu$L to 96-well microtiter plates. Parallel measurements of saponin dilutions without erythrocytes, erythrocytes without saponin dilutions, and buffer without both erythrocytes and saponin dilutions were made. The results were then compared to a positive control sample containing erythrocytes in distilled water. The 50% hemolyzing concentration ($HC_{50}$) of each saponin was then inferred from the hemoglobin absorbance versus saponin concentration curve.

## RESULTS AND DISCUSSION

**Isolation and Identification of Saponins.** A methanolic extract of the defatted quinoa seed powder yielded the major saponins **1**−**6** (Figure 1).

Compound **1** was obtained as a white powder after HPLC purification. It gave a positive Liebermann−




| | | | | |
|---|---|---|---|---|
| **1** | $R_1$=GlcA | $R_2$=H | $R_3$=Glc | $R_4$=$CH_3$ |
| **1a** | $R_1$=GlcA | $R_2$=H | $R_3$=H | $R_4$=$CH_3$ |
| **2** | $R_1$=Ara | $R_2$=OH | $R_3$=Glc | $R_4$=$CH_3$ |
| **2a** | $R_1$=Ara | $R_2$=OH | $R_3$=H | $R_4$=$CH_3$ |
| **3** | $R_1$=Ara(3←1)Glc | $R_2$=OH | $R_3$=Glc | $R_4$=$CH_3$ |
| **3a** | $R_1$=Ara(3←1)Glc | $R_2$=OH | $R_3$=H | $R_4$=$CH_3$ |
| **4** | $R_1$=Ara | $R_2$=OH | $R_3$=Glc | $R_4$=$COOCH_3$ |
| **4a** | $R_1$=Ara | $R_2$=OH | $R_3$=H | $R_4$=COOH |
| **5** | $R_1$= Ara(3←1)Glc | $R_2$=OH | $R_3$=H | $R_4$=$COOCH_3$ |
| **6** | $R_1$= Ara(3←1)Glc | $R_2$=OH | $R_3$=Glc | $R_4$=$COOCH_3$ |

**1**: 3-*O*-β-D-glucuronopyranosyl oleanolic acid-28-*O*-β-D-glucopyranosyl ester

**2**: 3-*O*-α-L-arabinopyranosyl hederagenin-28-*O*-β-D-glucopyranosyl ester

**3**: 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin-28-*O*-β-D-glucopyranosyl ester

**4**: 3-*O*-α-L-arabinopyranosyl phytolaccagenic acid-28-*O*-β-D-glucopyranosyl ester

**5**: 3-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-arabinopyranosyl phytolaccagenic acid

**6**: 3-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-arabinopyranosyl phytolaccagenic acid-28-*O*-β-D-glucopyranosyl ester

**Figure 1.** Structures of quinoa triterpene saponins.

Burchard reaction. Positive ion high-resolution FAB MS revealed a quasimolecular ion $[M + 2Na]^+$ at *m*/*z* 839.466 and $[M + Na]^+$ at *m*/*z* 817.4371. The negative ion FAB MS gave $[M - H]^-$ at *m*/*z* 793. These established the molecular formula as $C_{42}H_{66}O_{14}$.

Acid hydrolysis of the intact saponin enabled the isolation and identification of the aglycon as oleanolic acid. EI MS of the aglycon gave $M^+$ at *m*/*z* 456 with the typical retro Diels−Alder fragments at *m*/*z* 248 and 203 (*8*). $^1H$ NMR showed signals for seven methyl groups at $\delta$ 0.82, 0.92, 0.95, 1.00, 1.08, 1.22, and 1.27, a trisubstituted olefinic proton at $\delta$ 5.40 (1H, *m*, H-12), and a methine proton signal at $\delta$ 3.36 (1H, *dd*, $J = 11.7$, 4.0 Hz, H-3) in the aglycon, which are characteristic of an oleanene-type triterpene (*9*). The $^{13}C$ NMR spectrum confirmed the oleanene nature through the shifts for C-12 and C-13 at $\delta$ 122.8 and 144.1, respectively (*10*). The shifts of the aglycon resonances are summarized in Table 1.

Acid hydrolysis of **1** afforded sugars that were identified as glucuronic acid and glucose through mass spectral data of the derivatized monosaccharides as well as by co-TLC with authentic material. In addition, the anomeric $^{13}C$ signals at $\delta$ 107.2 and 95.7 and their corresponding protons at $\delta$ 5.03 (1H, *d*, $J = 7.6$ Hz) and 6.31 (1H, *d*, $J = 7.9$ Hz), respectively, were strongly indicative of two hexoses. The COSY spectrum together with the HMQC and HMBC spectra allowed the identification of the spin systems of the two monosaccharide residues. The shifts of the sugar resonances are summarized in Table 2. The linkage sites of the monosac-

**Table 1. $^1$H and $^{13}$C NMR Spectral Data of the Aglycon Moieties of Saponins 1−6**

| carbon no. | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 38.6 | 0.88/1.40 | 39.7 | 1.06/1.56 | 38.8 | 1.01/1.53 | 38.7 | 1.02/1.56 | 38.8 | 1.06/1.58 | 38.8 | 1.03/1.54 |
| 2 | 26.6 | 1.93/2.23 | 26.9 | 2.05/2.28 | 26.1 | 1.94/2.19 | 26.1 | 2.07/2.22 | 26.2 | 2.05/2.22 | 26.2 | 2.19/2.00 |
| 3 | 89.0 | 3.36 | 82.7 | 4.29 | 81.7 | 4.26 | 81.8 | 4.28 | 81.8 | 4.29 | 81.8 | 4.27 |
| 4 | 39.5 | | 44.3 | | 43.5 | | 43.4 | | 43.6 | | 43.5 | |
| 5 | 55.7 | 0.79 | 48.4 | 1.68 | 47.5 | 1.68 | 47.5 | 1.68 | 47.5 | 1.72 | 47.5 | 1.66 |
| 6 | 18.4 | 1.28/1.53 | 19.0 | 1.34/1.67 | 18.6 | 1.28/1.66 | 18.1 | 1.36/1.70 | 18.1 | 1.36/1.72 | 17.8 | 1.35/1.69 |
| 7 | 32.5 | 1.33/1.72 | 33.4 | 1.32/1.72 | 32.7 | 1.29/1.62 | 32.9 | 1.32/1.60 | 32.9 | 1.30/1.64 | 32.7 | 1.31/1.61 |
| 8 | 39.9 | | 40.8 | | 39.9 | | 39.9 | | 39.7 | | 39.9 | |
| 9 | 47.9 | 1.6 | 49.0 | 1.79 | 48.1 | 1.76 | 48.1 | 1.80 | 48.2 | 1.80 | 48.1 | 1.74 |
| 10 | 36.9 | | 37.8 | | 36.8 | | 36.8 | | 36.9 | | 36.9 | |
| 11 | 23.6 | 1.92/1.92 | 24.2 | 1.95/2.04 | 23.0 | 1.92/2.04 | 23.7 | 1.92/2.04 | 23.8 | 1.94/2.02 | 23.9 | 1.87/1.96 |
| 12 | 122.8 | 5.40 | 123.8 | 5.44 | 122.9 | 5.47 | 123.5 | 5.58 | 123.2 | 5.62 | 123.5 | 5.42 |
| 13 | 144.1 | | 144.9 | | 144.1 | | 143.5 | | 144.4 | | 144.0 | |
| 14 | 42.1 | | 42.9 | | 42.1 | | 41.9 | | 42.0 | | 42.1 | |
| 15 | 28.2 | 1.13/2.31 | 29.1 | 1.11/2.33 | 28.2 | 1.09/2.30 | 28.2 | 1.19/2.39 | 28.4 | 1.15/2.14 | 28.2 | 1.16/2.32 |
| 16 | 23.6 | 1.83/1.98 | 24.7 | 1.08/1.93 | 23.9 | 0.99/1.89 | 23.4 | 2.04/2.12 | 23.8 | 1.97/2.11 | 23.9 | 1.87/ 2.00 |
| 17 | 46.9 | | 47.8 | | 46.9 | | 46.4 | | 46.1 | | 47.0 | |
| 18 | 41.7 | 3.15 | 42.6 | 3.19 | 41.7 | 3.14 | 43.1 | 3.19 | 43.4 | 3.30 | 42.3 | 3.33 |
| 19 | 46.1 | 1.23/1.80 | 47.0 | 1.25/1.79 | 46.4 | 1.24/1.72 | 42.3 | 1.77/2.24 | 42.6 | 1.80/2.26 | 41.3 | 1.78/2.22 |
| 20 | 30.7 | | 31.6 | | 30.7 | | 43.9 | | 44.2 | | 46.2 | |
| 21 | 33.9 | 1.11/1.40 | 33.7 | 1.09/1.32 | 33.9 | 1.05/1.30 | 30.2 | 1.36/2.12 | 30.8 | 1.46/2.21 | 29.7 | 1.40/2.18 |
| 22 | 33.2 | 1.76/1.85 | 33.4 | 1.79/1.93 | 32.5 | 1.75/1.82 | 33.9 | 1.79/1.92 | 34.5 | 1.99/2.14 | 35.6 | 1.78/1.92 |
| 23 | 28.1 | 1.22 | 65.3 | 3.68/4.31 | 64.1 | 3.69/4.29 | 64.4 | 3.69/4.28 | 64.2 | 3.73/4.37 | 64.7 | 3.67/4.32 |
| 24 | 16.9 | 0.95 | 14.5 | 0.93 | 13.6 | 0.92 | 13.6 | 0.82 | 13.7 | 0.94 | 13.6 | 0.93 |
| 25 | 15.5 | 0.82 | 17.0 | 0.97 | 16.1 | 0.94 | 16.1 | 0.99 | 16.1 | 0.93 | 16.1 | 0.93 |
| 26 | 17.4 | 1.08 | 18.4 | 1.14 | 17.5 | 1.11 | 17.4 | 1.09 | 17.4 | 1.02 | 17.5 | 1.11 |
| 27 | 26.1 | 1.27 | 27.0 | 1.19 | 26.0 | 1.19 | 26.0 | 1.14 | 26.1 | 1.26 | 26.1 | 1.24 |
| 28 | 176.4 | | 177.3 | | 176.4 | | 176.9 | | 179.8 | | 176.9 | |
| 29 | 33.1 | 0.92 | 33.9 | 0.89 | 33.0 | 0.88 | 28.2 | 1.16 | 28.4 | 1.22 | 28.5 | 1.16 |
| 30 | 23.7 | 1.00 | 24.5 | 0.88 | 23.6 | 0.87 | 176.0 | | 177.2 | | 176.0 | |
| $-COOC\underline{H}_3$ | | | | | | | 51.7 | 3.66 | 51.7 | 3.66 | 51.6 | 3.63 |

**Table 2. $^1$H and $^{13}$C Spectral Data of the Monosaccharide Moieties of Saponins 1−6**

| carbon no. | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\delta_C$ | $\delta_H$ ($J$, Hz)[a] | $\delta_C$ | $\delta_H$ ($J$, Hz) | $\delta_C$ | $\delta_H$ ($J$, Hz) | $\delta_C$ | $\delta_H$ ($J$, Hz) | $\delta_C$ | $\delta_H$ ($J$, Hz) | $\delta_C$ | $\delta_H$ ($J$, Hz) |
| C-3 | | | | | | | | | | | | | |
| GlcA | 1 | 107.2 | 5.03 *d* (7.6) | | | | | | | | | | |
| | 2 | 75.5 | 4.13 | | | | | | | | | | |
| | 3 | 78.1 | 4.35 | | | | | | | | | | |
| | 4 | 73.4 | 4.61 | | | | | | | | | | |
| | 5 | 77.8 | 4.67 | | | | | | | | | | |
| | 6 | 172.8 | | | | | | | | | | | |
| Ara | 1 | | | 107.6 | 4.98 *d* (7.2) | 106.5 | 4.96 *d* (7.4) | 106.7 | 4.99 *d* (7.2) | 106.6 | 4.98 *d* (7.4) | 106.5 | 4.95 *d* (7.4) |
| | 2 | | | 74.0 | 4.44 | 71.9 | 4.57 | 73.0 | 4.45 | 72.0 | 4.59 | 71.9 | 4.56 |
| | 3 | | | 75.6 | 4.07 | 84.1 | 4.00 | 74.7 | 4.07 | 84.3 | 4.09 | 84.1 | 4.05 |
| | 4 | | | 70.5 | 4.26 | 69.2 | 4.40 | 69.6 | 4.28 | 69.3 | 4.41 | 69.2 | 4.35 |
| | 5 | | | 67.9 | 3.72, 4.23 | 67.1 | 3.62, 4.15 | 67.0 | 3.76, 4.28 | 67.1 | 3.62, 4.21 | 67.5 | 3.60, 4.14 |
| Glc | 1 | | | | | 106.2 | 5.30 *d* (7.8) | | | 106.4 | 5.33 *d* (7.8) | 106.2 | 5.30 *d* (7.8) |
| | 2 | | | | | 75.6 | 4.03 | | | 75.7 | 4.03 | 75.6 | 4.02 |
| | 3 | | | | | 78.3 | 4.23 | | | 78.4 | 4.26 | 78.3 | 4.24 |
| | 4 | | | | | 71.5 | 4.22 | | | 71.6 | 4.23 | 71.5 | 4.23 |
| | 5 | | | | | 78.6 | 4.22 | | | 78.8 | 4.01 | 78.6 | 4.23 |
| | 6 | | | | | 62.6 | 4.35, 4.52 | | | 62.7 | 4.40, 4.58 | 62.6 | 4.37, 4.29 |
| C-28 | | | | | | | | | | | | | |
| Glc | 1 | 95.7 | 6.31 *d* (7.9) | 95.7 | 6.34 *d* (7.9) | 95.7 | 6.31 *d* (7.9) | 95.7 | 6.31 *d* (7.9) | | | 95.7 | 6.28 *d* (7.9) |
| | 2 | 74.1 | 4.22 | 75.0 | 4.23 | 74.1 | 4.19 | 74.0 | 4.17 | | | 74.1 | 4.20 |
| | 3 | 79.3 | 4.04 | 80.2 | 4.04 | 79.3 | 4.03 | 79.3 | 3.97 | | | 79.2 | 3.92 |
| | 4 | 71.1 | 4.36 | 71.9 | 4.35 | 71.0 | 4.32 | 70.8 | 4.38 | | | 71.0 | 4.38 |
| | 5 | 78.8 | 4.28 | 79.8 | 4.28 | 78.8 | 4.28 | 78.8 | 4.28 | | | 78.8 | 4.23 |
| | 6 | 62.2 | 4.44, 2H | 63.0 | 4.44, 2H | 62.1 | 4.41, 2H | 61.7 | 4.41, 2H | | | 62.1 | 4.39, 2H |

[a] The multiplicities and $J$ values of all signals except those of the anomeric protons were obscure owing to overlapping with other signal(s).

charide moieties were first inferred from the hydrolysis experiment. Basic hydrolysis of **1** gave a prosapogenin and glucose. The acid hydrolysis of this prosapogenin then gave the aglycon and glucuronic acid. This was indicative of C-28 esterification of the glucose moiety and the C-3 linkage of glucuronic acid. Confirmation of this came from the HMBC spectrum by which $^3J_{CH}$ correlations were observed between C-1 ($\delta$ 107.2) and H-3 ($\delta$ 3.36) of glucuronic acid and between C-28 ($\delta$ 176.4) and H-1 ($\delta$ 6.31) of glucose. The relative stereochemistry of both monosaccharides was determined as $\beta$-D-glucuronopyranose and $\beta$-D-glucopyranose on the basis of the characteristic $J_{1,2}$ coupling constants (*11*). **1** was thus identified as 3-*O*-$\beta$-D-glucuronopyranosyl oleanolic acid 28-*O*-$\beta$-D-glucopyranosyl ester.

Compound **2** was obtained as an amorphous powder after HPLC purification. Its positive ion high-resolution FAB MS displayed $[M + Na]^+$ at $m/z$ 789.5417 and the

negative ion mode spectrum FAB MS $[M - H]^-$ at *m/z* 765. This gave the molecular formula $C_{41}H_{66}O_{13}$ for **2**. The aglycon region in the $^1H$ and $^{13}C$ NMR spectra showed great similarity to that of **1** except for the significant downfield shift (+4.8 ppm) exhibited by C-4 ($\delta$ 44.3) and the high-field shifts (−6.3, −7.3, and −2.4 ppm) exhibited, respectively, by C-3 ($\delta$ 82.7), C-5 ($\delta$ 48.4), and C-24 ($\delta$ 14.5). This together with the shift by 16 mass units in the $M^+$ peak in the EI MS of the hydrolyzed aglycon and comparison with literature $^{13}C$ data (*13*) confirmed it to be hederagenin.

Basic hydrolysis of **2** gave rise to glucose and a prosapogenin. Acid hydrolysis of the resulting prosapogenin then yielded arabinose. Eleven $^{13}C$ resonances, not assignable to the aglycon, and two resonances of anomeric carbons at $\delta$ 107.6 and 95.7, which were correlated to two anomeric protons at $\delta$ 4.98 (1H, *d*, $J$ = 7.2 Hz) and 6.34 (1H, *d*, $J$ = 7.9 Hz), respectively, using the HMQC spectrum, confirmed the presence of the two monosaccharide moieties. In addition to indications obtained from the hydrolysis experiments on the intact saponin regarding the sites of attachments of the monosaccharides, chemical shifts of the anomeric carbons suggested that the arabinose unit might be attached at the C-3 position and that the glucose moiety might be found at C-28. This was later confirmed by the HMBC spectrum, which showed cross-peaks due to $^3J_{CH}$ correlations between C-3 ($\delta$ 82.7) and H-1 of arabinose ($\delta$ 4.98, *d*, $J$ = 7.2 Hz) and between C-28 ($\delta$ 177.3) and H-1 of glucose ($\delta$ 6.34, *d*, $J$ = 7.9 Hz). The coupling constants of both anomeric protons confirmed the α- and β-configurations of the monosaccharides, respectively. Thus, the structure of **2** was identified as 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester.

Compound **3** was found to have the molecular formula $C_{47}H_{76}O_{18}$, as deduced from its high-resolution positive ion FAB mass spectrum (*m/z* 951.4934 $[M + Na]^+$; *m/z* 929.5123 $[M + H]^+$), which differs from that of **2** by 162 mass units. Alkaline hydrolysis liberated glucose, and consequent acid hydrolysis of the prosapogenin gave rise to arabinose, glucose, and the aglycon hederagenin. The sugars were identified by comparison with authentic samples on TLC and through GC-MS analysis of their trimethylsilyl derivatives. The aglycon, on the other hand, was identified through comparison on TLC and $^1H$ and $^{13}C$ NMR spectra of that from **2**.

Analysis of the $^1H$ and $^{13}C$ NMR spectra revealed that **2** and **3** had similar spectra. However, unlike in **2**, **3** displayed three anomeric carbon resonances at $\delta$ 106.5, 106.2, and 95.7 and their corresponding anomeric protons at $\delta$ 4.96 (1H, *d*, $J$ = 7.4 Hz), 5.30 (1H, *d*, $J$ = 7.8 Hz), and 6.31 (1H, *d*, $J$ = 7.9 Hz). The $^{13}C$ NMR signal of arabinose C-3 (at $\delta$ 84.1) showed a downfield shift (+8.5 ppm) indicating glycosidic substitution at this point. This was confirmed by correlation observed in the HMBC spectrum between the anomeric carbon of the additional glucose unit at $\delta$ 106.2 and H-3 of arabinose at $\delta$ 4.00. The determination of the anomeric configuration for individual monosaccharides is based on the magnitude of the coupling constants of the anomeric protons. Consequently, the structure of **3** was characterized as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester.

Compound **4** was obtained as a white amorphous powder. Its $[M + 2Na]^+$ and $[M + Na]^+$ ions appeared at *m/z* 855.4194 and 833.4358, respectively, in the high-resolution FAB mass spectrum, whereas the $[M - H]^-$ ion appeared at *m/z* 809 in the negative ion mode. This was consistent with the molecular formula $C_{42}H_{66}O_{15}$. Hydrolysis of **4** and analysis of the aglycon obtained confirmed its identity as phytolaccagenic acid. This was achieved through EI mass spectrometry of the aglycon and comparison of the $^1H$ and $^{13}C$ NMR spectra with that of **3** and $^{13}C$ data in the literature (*12*). Its triterpene nature and the presence of an additional carboxy methyl ester group was easily inferred from the typical retro Diels−Alder type of fragmentation it undergoes and the shifting of the main fragments including the molecular ion by 44 mass units. Its position at C-29 was inferred from the downfield shift (+13.2 ppm) observed for C-20 ($\delta$ 43.9) compared to that in **3**. Confirmation of this came from the $^1H$ and HMBC spectra. The $^1H$ NMR spectrum gave the chemical shift for the methyl protons in the carboxy methyl ester group at $\delta$ 3.66. In the HMBC spectrum, this showed a cross-peak with C-30 ($\delta$ 176.0). Furthermore, cross-peaks were observed between C-30 and H-29, H-19, and H-21.

**Basic Hydrolysis of the Saponin-Liberated Glucose.** Consequent acid hydrolysis of the prosapogenin yielded arabinose. The presence of two monosaccharide moieties was confirmed from the observation of signals for two anomeric carbons at $\delta$ 95.7 and 106.7 attached to proton doublets at $\delta$ 6.31 ($J$ = 7.9 Hz) and 4.99 ($J$ = 7.2 Hz), respectively. Seven methine resonances between $\delta$ 69.6 and 78.8, one oxymethylene at $\delta$ 61.7, and one methylene resonance at $\delta$ 67.0 supported the existence of a hexopyranose and a pentopyranose residue. The HMBC spectrum showed, among others, coupling between aglycon C-3 ($\delta$ 81.8) and arabinose H-1 ($\delta$ 4.99), confirming its location at C-3. Also observed in the HMBC spectrum was a cross-peak between C-28 ($\delta$ 176.9) and the anomeric proton of the glucose unit at $\delta$ 6.31, locating the glucose unit at the C-28 position. The stereochemistry of the monosaccharides was determined as having a β-configuration for glucose and an α-configuration for arabinose on the basis of the coupling constants of their anomeric protons. Consequently, the structure of **4** was determined to be 3-*O*-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester.

Saponin **5**, a novel natural product, exhibited an $[M + Na]^+$ quasimolecular ion at *m/z* 833.4241 in the positive ion mode and an $[M - H]^-$ ion at *m/z* 809 in the negative ion FAB mass spectrum, giving the molecular formula $C_{42}H_{66}O_{15}$. Although having a similar molecular weight as **4**, its retention time on HPLC analysis was found to be different. Analysis of the intact saponin as well as the hydrolysis products revealed that it comprised phytolaccagenic acid, arabinose, and glucose but was different from **4** in that alkaline hydrolysis failed to liberate a monosaccharide. Acid hydrolysis, on the other hand, gave rise to arabinose and glucose, indicating that both monosaccharide units might be found at the C-3 position. The $^{13}C$ NMR spectrum showed two anomeric carbons at $\delta$ 106.4 and 106.6, which were correlated to the proton resonances at $\delta$ 5.33 (1H, *d*, $J$ = 7.8 Hz) and 4.98 (1H, *d*, $J$ = 7.4 Hz) in the HMQC spectrum. The spin systems of each monosaccharide were identified by walking through the easily identifiable anomeric protons and identifying the vicinal connectivities in the COSY spectrum. The disaccharide sequence at C-3 was then determined using the HMBC

spectrum. This showed correlations between arabinose H-1 ($\delta$ 4.98) and C-3 of the aglycon ($\delta$ 81.8). Similarly, a correlation was observed between the downfield-shifted C-3 of arabinose at $\delta$ 84.3 and H-1 of glucose at $\delta$ 5.33. On the basis of the above findings the structure of **5** was concluded to be 3-*O*-$\beta$-D-glucopyranosyl-(1→3)-*O*-$\alpha$-L-arabinopyranosyl phytolaccagenic acid.

The $^1$H and $^{13}$C NMR spectra of saponin **6** closely resembled that of **4**, but an additional signal at $\delta$ 106.2 in the $^{13}$C and at $\delta$ 5.30 in the $^1$H NMR spectra together with the shift in the quasimolecular ions $[M + Na]^+$ and $[M - H]^-$ by 162 mass units at *m*/*z* 995.4982 and 971, respectively, in FAB indicated the presence of an additional glucose unit while establishing the molecular formula as $C_{48}H_{76}O_{20}$. The position of this additional glucose moiety was inferred from the downfield shift observed for C-3 of arabinose ($\delta$ 84.1), implying substitution at this position. Correlation between arabinose C-3 and H-1 of this additional glucose was observed in the HMBC spectrum, confirming its site of attachment as C-3 of the arabinose unit. Thus, saponin **6** was 3-*O*-$\beta$-D-glucopyranosyl-(1→3)-*O*-$\alpha$-L-arabinopyranosyl phytolaccagenic acid-28-*O*-$\beta$-D-glucopyranosyl ester.

The other 10 saponins detected (on TLC with Lieberman−Burchard reagent) in the methanolic extract of the quinoa seed powder were not present in sufficient quantity to enable their isolation and structural elucidation. However, acid hydrolysis of their mixture and analysis of the resulting products as their trimethylsilyl derivatives using GLC-MS showed that the saponins possessed hederagenin, phyatolaccagenic acid, or oleanolic acid as their aglycons.

**Biological Activities of Bidesmoside and Monodesmoside Saponins.** The crude saponin fraction and individual saponins were examined for antifungal activity as outlined under Experimental Procedures. The total saponin fraction of *C. quinoa* was found to inhibit the growth of *Candida albicans* at 50 $\mu$g/mL. However, individual saponins did not exhibit any comparable effect. Compounds **1**, **1a**, **2**, **2a**, **3**, **3a**, **4a**, and **6** had MIC values >500 $\mu$g/mL, compound **4** had an MIC ≤500 $\mu$g/mL, and compound **5** had an MIC ≤100 $\mu$g/mL.

In this study, concentrations of saponins up to 500 $\mu$g/mL were incorporated into the growth medium according to test methods commonly used to evaluate the antimicrobial susceptibility of fungi. The broth microdilution test showed that among the tested mono- and bidesmoside saponins, only **5** exhibited some activity. *C. albicans* was not found to be inhibited by most saponins even at concentrations of 500 $\mu$g/mL. On the other hand, the control fungal agent, amphotericin B, showed a significantly low MIC at 1 $\mu$g/mL. These results are in agreement with the findings of Anisimov and Chirva (*14*), who observed lack of antifungal activity in glycosides of oleanolic acid and hederagenin (normally highly inhibitory saponins) with shorter carbohydrate chains. Because *Candida* is a rather peculiar fungi of medicinal interest, it is probable that these compounds might be active against other fungi.

Results of the hemolysis test (Table 3) show that although most bidesmoside saponins were hemolytically inactive, most monodesmoside saponins were active. The only bidesmoside to be active, **1**, showed activity at 260 $\mu$g/mL, which can only be described as weak. However, the most active saponin **1a** was its monodesmoside form. Hederagenin monodesmosides, **2a** and **3a**, also showed strong activity. This has also been reported by other groups, which found that monodesmoside saponins having oleanolic acid and hederagenin as aglycon were highly hemolytic (*15*). Comparison of the $HC_{50}$ of **1a** with those of **2a** and **3a** implies that although the addition of a hydroxyl group at C-24 may not dramatically alter the degree of hemolysis, the type and number of monosaccharides do play a role. It suggests that addition of a glucose unit on the C-3 chain decreases activity. Comparison between **2a** and **4a** shows that introduction of an additional carboxy group on ring E dramatically reduces hemolytic activity because hydrophobic interactions with membrane lipids become reduced. The mechanism of hemolysis itself may involve the interaction of the saponins with membrane cholesterol creating porelike structures leading to rupture of the membrane and leakage of hemoglobin into the surrounding medium (*16*).

**Table 3. Hemolytic Activity of Saponins 1−6 and Their Derivatives on Sheep Erythrocytes**

| | saponin | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **1a** | **2** | **2a** | **3** | **3a** | **4** | **4a** | **5** | **6** |
| $HC_{50}$ ($\mu$g/mL) | 260 | 6.2 | *a* | 8 | *a* | 15 | *a* | 625 | *a* | *a* |

[a] $HC_{50}$ values were not in the concentration range tested.

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