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Triterpene Saponins from Debittered Quinoa (*Chenopodium quinoa*) Seeds

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Twelve triterpene saponins have been isolated from the debittered seeds of quinoa (*Chenopodium quinoa)*, and their structures were characterized on the basis of hydrolysis and spectral data, especially NMR evidence. Among them, three compounds, including 3-*O*-*β*-D-glucuropyranosyl oleanolic acid (**1**), 3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin (**2**), and the new compound 3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-30-*O*-methyl spergulagenate 28-*O*-*β*-D-glucopyranosyl ester (**3**), are identified for the first time from quinoa seeds. The other isolated saponins have been previously reported in quinoa.

KEYWORDS: Quinoa seeds; *Chenopodium quinoa*; triterpene saponins; NMR

INTRODUCTION

Quinoa, *Chenopodium quinoa*, has been cultivated as a food crop for centuries in Latin America. Currently, descendants of the Inca Empire still use its seeds as an important component in their diet (*1*). The seeds are high in protein content and average 12−18% on a fresh basis. Moreover, this protein is of an exceptionally high quality and is particularly rich in essential amino acids, such as histidine and lysine, which are deficient in most grain crops but necessary for proper amino acid nutrition in humans. This fact results in a protein quality of quinoa seeds comparable to that of whole dry milk. In addition, quinoa seeds are rich in riboflavin, α-tocopherol, carotenes, Ca, Fe, K, and fiber (*2*). As a result of its high nutritional value, a 400-year decline in quinoa production that began with the Spanish conquest has been reversed since the beginning of the past decade. The seeds can be used in the same way as rice or wheat, such as boiling or grinding into flour. Now they are used to prepare pasta, puffed cereals, breads, cakes, beers, and animal feedstuffs (*2, 3*).

However, there is a big disadvantage related to the consumption of quinoa: bitterness, which was supposed to be the result of saponins (*4*). With the renewed interest in quinoa, over twenty triterpene saponins have been reported from quinoa seeds (*5*−*10*). Triterpene saponins are ubiquitous nonsteroidal secondary metabolites of terrestrial and marine flora and fauna, occurring in the form of glycoside. Although medical uses of this class of compounds have been limited, considerable work strongly indicates their great potential (*11*). For instance, substantial evidence has suggested saponins could reduce cholesterol levels, which is likely to favor the development of saponin-rich hypocholsterolemic diets. On the other hand, they have been reported as being toxic to various organisms, such as exhibiting insectidial, antibiotic, and fungicidal properties in many studies. The growth-retarding influence of certain saponins on livestock and laboratory animals also has been indicated. In addition, the improved permeability of intestinal mucosa by saponins may increase the uptake of species normally excluded by the gut, while simultaneously reducing its ability to accumulate nutrients (*12*). Fortunately, quinoa saponins seem to be free from oral toxicity in humans. It was also found that quinoa saponins do not exert any negative effect on the nutritive quality of the quinoa protein (*9*).

However, the final level of saponin content in to-be-consumed quinoa seeds is still a big concern in terms of its bitterness and possible negative biological effects. 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 (*3*). Until now, the saponin contents and composition of debittered quinoa seeds are not clear. Therefore, our study is to isolate and identify those remaining saponins in debittered commercial quinoa seeds.

Herein, we report the isolation and characterization of twelve triterpene saponins from debittered quinoa seeds. Among them, nine compounds have been identified in previous studies, and the other three are isolated for the first time from quinoa seeds.

MATERIALS AND METHODS

General. TLC was performed on Sigma-Aldrich silica gel TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 10% (v/v) H_2SO_4 ethanol solution under heat. Silica gel (130−270 mesh), Sephadex LH-20, and RP-18 (60 μm) columns (Sigma Chemical Co., St. Louis, MO) were used for column chromatography. All solvents used were purchased from Fisher Scientific (Springfield, NJ). Negative APCIMS spectra were obtained on a Micromass Platform II system (Micromass Co., Beverly, MA) equipped with a Digital DECPC XL560 computer for data analysis. 1D NMR spectra were recorded on a Varian U-200 instrument, and

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2D NMR spectra were obtained from a Varian U-400 instrument. Chemical shifts are expressed in parts per million (δ) using TMS as internal standard.

Plant Material. Quinoa seeds were collected and debittered in Bolivia and purchased from Quinoa Co. (Torrance, CA). A specimen of the plant also has been deposited at the herbarium of the Food Science Department, Rutgers University.

Extraction and Isolation of Quinoa Seeds. Dried seeds (4 kg) were extracted with 95% aqueous ethanol at room-temperature two times, and the extraction lasted 3 days each time. Then, the extracts were concentrated (120 g) and suspended in water, and partitioned successively by hexane (20 g), ethyl acetate (10 g), and butanol (35 g).

The butanol fraction was subjected to column chromatography on Diaion HP-20 gel to give 4 fractions (water, water/ethanol (3:7), water/ethanol (1:9), and acetone). Then the water/ethanol (3:7) fraction was separated into 7 fractions (A1−A7) by Sephadex LH-20 column using 90% ethanol aqueous solution as eluent.

Fraction A1 was subjected to a RP-18 column with methanol/water (2:1) to yield 9 mg of **3**, which was re-purified by Sephadex LH-20 column eluted with 95% aqueous ethanol.

Then, the water/ethanol (1:9) fraction was separated into 4 fractions by Sephadex LH-20 column using 90% ethanol aqueous solution as the eluent. The first fraction was repeatedly separated by column chromatography (silica gel), eluted with ethyl acetate/methanol/water/hexane (22.5:1:0.8:0.8) to give several fractions (B1−B11).

Fraction B2 was subjected to a silica gel column, eluted with ethyl acetate/methanol/water (15:1:0.8) to give five subfractions (I−V). Subfraction II was purified by a RP-18 column with methanol/water (1:2) and yielded 25 mg of **2**.

Fraction B3 was subjected to a silica gel column, eluted with ethyl acetate/methanol/water/hexane (12:1:0.8:0.4) to give one subfraction. This subfraction was purified into 14 mg of **1** by the column chromatography, using methanol/water (1.5:2.5) as eluent.

The repeated column chromatography of fraction B5 on RP-18 gel eluted with methanol/water (1:1) gave 22 mg of **5** and 7 mg of **6**.

Fraction B7 was purified by RP-18 reverse-phase column (30% methanol in H_2O), giving one subfraction, which was purified as 12 mg of **10** by repeated chromatography on a RP-18 column with methanol/water (2.7:1) as eluent.

Fraction B8 was purified into 35 mg of **8** by RP-18 column chromatography with methanol/water (3:1).

The mixture of fractions B9 and B10 was subjected to a RP-18 column and eluted with methanol/water (1.5:1) to afford 85 mg of **9** and 17 mg of **11**.

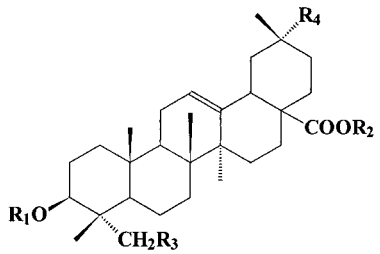
Fraction B11 was separated into 8 subfractions (I−VIII) by silica gel column chromatography, using ethyl acetate (8:1:0.8) as eluent. Subfraction III was purified as 6 mg of **4** by a RP-18 column, eluted with methanol/water (2:1.5). Subfraction V was rechromatographed on a RP-18 column with methanol/water (3:2.2) to give 65 mg of **7**. Subfraction VI was purified on a RP-18 column, eluted with methanol/water (2:1) to afford 31 mg of **12**.

Acid Hydrolysis of Saponins 1, 2, and 3. Around 2 mg of saponin (**1**, **2**, or **3**) was added into 1 mL of 1 M HCl (methanol/water 1:1) solution, individually. The mixture was heated at 80−90 °C for 2 h. After being cooled, the reaction product was extracted with ethyl acetate. Sugar composition was confirmed by R_f value comparisons with those of authentic sugar samples in TLC tests.

Alkaline Hydrolysis of Saponin 3. Compound **3** (1−2 mg) was heated in 0.5 M aqueous KOH (1 mL) at 105 °C in a stoppered reaction flask for 2 h. The reaction mixture was adjusted to pH 7 with HCl solution and then extracted with ethyl acetate for TLC tests.

RESULTS AND DISCUSSION

Detailed study on the ethanol extract of debittered quinoa seeds led to the isolation and characterization of twelve triterpene saponins (**Figure 1**). Nine of them have been isolated in previous studies. By the comparison of NMR and MS spectral data (*5*, *6*, *10*), their structures were determined as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**4**), 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester (**5**), 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester (**6**), 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid (**7**), 3-*O*-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**8**), 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**9**), 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**10**), 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**11**), and 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-30-*O*-methyl spergulagenate 28-*O*-β-D-glucopyranosyl ester (**12**). Of the other three compounds (**1**−**3**), their structures were determined by a detailed analysis of their MS and NMR (1D and 2D) spectra, as well as comparison with those of published data in the literature.



1:	R_1 = Glucuronoic acid	R_2 = H	R_3 = H	R_4 = CH_3
4:	R_1 = Ara(3-1)-Glc-(2-1)-Glc	R_2 = Glc	R_3 = H	R_4 = CH_3
2:	R_1 = Ara(3-1)-Glc	R_2 = H	R_3 = OH	R_4 = CH_3
5:	R_1 =Ara(3-1)-Glc	R_2 = Glc	R_3 = OH	R_4 = CH_3
6:	R_1 = Gal(3-1)-Glc	R_2 = Glc	R_3 = OH	R_4 = CH_3
7:	R_1 = Ara(3-1)-Glc	R_2 = H	R_3 = OH	R_4 = $COOCH_3$
8:	R_1 = Ara	R_2 = Glc	R_3 = OH	R_4 = $COOCH_3$
9:	R_1 =Ara(3-1)-Glc	R_2 = Glc	R_3 = OH	R_4 = $COOCH_3$
10:	R_1 = Gal(3-1)-Glc	R_2 = Glc	R_3 = OH	R_4 = $COOCH_3$
11:	R_1 = Ara(3-1)-Glc-(2-1)-Glc	R_2 = Glc	R_3 = OH	R_4 = $COOCH_3$
3:	R_1 = Ara(3-1)-Glc	R_2 = Glc	R_3 = H	R_4 = $COOCH_3$
12:	R_1 = Ara(3-1)-Glc-(2-1)-Glc	R_2 = Glc	R_3 = H	R_4 = $COOCH_3$

Figure 1. Saponins identified in quinoa seeds.

Compound **1** was obtained as a white powder which showed a pseudo-molecular ion $[M - H]^-$ at *m/z* 631 in the negative APCIMS. From this, together with its ^{13}C NMR data, the molecular formula was determined as $C_{36}H_{56}O_9$.

Acid hydrolysis of **1** afforded oleanolic acid as the aglycon and glucuronic acid as the sugar unit, which were suggested by the comparison of TLC results with those of authentic samples. The linkage site of the monosaccharide moiety was indicated by the anomeric signal at δ 106.9 and its corresponding proton at δ 4.38 (1H, d, $J = 7.6$ Hz), as well as the hydrolysis behavior (*5*). The relative stereochemistry of the glucuronic acid moiety was determined as β-D-glucuronopyranose on the basis of the characteristic $J_{1,2}$ coupling constant ($J = 7.6$ Hz) (*5*). With the help of literature data (*10*), compound **1** was thus confirmed as 3-*O*-β-D-glucuropyranosyl oleanolic acid.

Compound **2** was obtained as a white powder with the molecular formula $C_{41}H_{66}O_{13}$, determined on the basis of the ^{13}C NMR spectrum and negative APCIMS, which showed the quasimolecular ion $[M - H]^-$ at *m/z* 865. Analysis of its hydrolysis products indicated the presence of hederagenine, arabinose, and glucose, which suggested that the linkage site of saccharide moiety was at the C-3 position, rather than the C-28 position (*5*). This finding was also supported by the anomeric signals at δ 106.2 and 105.6, which were correlated

Table 1. ^{13}C NMR Spectra Data of Compound **3**[a]

position	aglycon	position	sugar moiety
1	39.8 t	Ara-1	105.9[a]d
2	27.2 t	-2	72.0 d
3	89.9 d	-3	84.2 d
4	40.5[a]s	-4	69.7 d
5	56.7 d	-5	66.9 d
6	19.4 t	Glc-1	105.2[a]d
7	33.8 t	-2	75.1 d
8	40.6[a]s	-3	78.4[b]d
9	49.1 d	-4	71.1 d
10	37.8 s	-5	77.7[b]d
11	24.5 t	-6	62.5 t
12	123.6 d	C-28 sugar	
13	144.6 s	Glc-1	96.2 d
14	42.8 s	-2	74.7 d
15	28.8[b]t	-3	78.8[b]d
16	24.5 t	-4	71.6 d
17	47.4 s	-5	78.4[b]d
18	43.8 d	-6	62.6 t
19	44.0 t		
20	45.0 s		
21	30.8 t		
22	34.9 t		
23	28.7[b]q		
24	16.5 q		
25	13.6 q		
26	17.8 q		
26	26.3 q		
28	178.8 s		
29	28.7 q		
30	177.6 s		
OCH_3	52.4 q		

[a] Spectra were recorded in CD_3OD. Superscript letters ([a, b]) refer to the chemical shifts that are interchangeable in each compound.

to the proton resonance at δ 4.55 (1H, d, $J = 7.9$ Hz) and 4.38 (1H, d, $J = 7.4$ Hz), respectively. Similarly, the stereochemistry of monosaccharide 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. With the help of literature data (*5*, *13*), the structure of **2** was confirmed to be 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin.

Compound **3**, a new saponin, was obtained as a white powder. The molecular formula of $C_{48}H_{76}O_{19}$ was deduced from its negative APCIMS (*m*/*z* 955) and ^{13}C NMR spectrum (**Table 1**). Alkaline hydrolysis afforded glucose, whereas the acid hydrolysis liberated glucose and arabinose. The sugars were identified by comparison of the TLC behavior with those of authentic samples. Except for the signals belonging to those sugar units, the NMR data comparison between **3** with **12**, which was reported by Mizui et al. (*6*), indicated that either ^1H or ^{13}C NMR signals were in agreement with those of 30-*O*-methyl spergulagenate. This fact was further confirmed by its negative APCIMS spectrum, which exhibited $[M - H]^+$ at *m*/*z* 519.

Further analysis of the NMR data indicated that **3** and **10** were very similar, especially those signals belonging to the sugar moiety. This evidence suggested that **3** had the same sugar unit with a different aglycon, which was identified as 30-*O*-methyl spergulagenate. Moreover, the disaccharide sequence at C-3 was confirmed by the exhibited HMBC correlations of arabinose H-1 with C-3 of the aglycon, and glucose H-1 with C-3 of the aglycon. Similarly, the HMBC correlation between H-1 of second glucose and C-28 proved that the glucose moiety was attached at the C-28 position. On the basis of the above facts, the structure of **3** was determined as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-30-*O*-methyl spergulagenate 28-*O*-β-D-glucopyranosyl ester.

In summary, twelve triterpene saponins were isolated from the debittered quinoa seeds. Among them, three compounds, including a new compound **3**, have never been reported in previous studies on this foodstuff. Further more, our study conformed the presence of the fourth aglycon, 30-*O*-methyl spergulagenate, whereas only three aglycons, oleanolic acid, hederagenin, and phytolaccagenic acid, were indicated in previous reports (*2*, *3*). Our study also confirmed the result of Dini et al. (*9*): the debittering process was not particularly efficient at removing those saponins with phytolaccagenic acid as the aglycon. To our knowledge, low acidity is associated with these remaining saponins, especially those with phytolaccagenic acid as the aglycon. Therefore, it would be beneficial to use slightly alkaline water to debitter quinoa seeds rather than to use neutral water.

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