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Saponins from Brans of Quinoa, *Chenopodium quinoa* WILLD. I

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Grains of Quinoa, *Chenopodium quinoa* (Chenopodiaceae), have been used as a staple food in the Andes, South America. From brans of grains of this plant, five new saponins were isolated and their structures were elucidated as 28-*O*-β-glucopyranosyl esters of hederagenin 3-*O*-β-glucopyranosyl-(1→3)-α-arabinopyranoside and 3-*O*-β-glucopyranosyl-(1→3)-β-galactopyranoside, and 28-*O*-β-glucopyranosyl esters of phytolaccagenic acid 3-*O*-α-arabinopyranoside, 3-*O*-β-glucopyranosyl-(1→3)-α-arabinopyranoside and 3-*O*-β-glucopyranosyl-(1→3)-β-galactopyranoside.

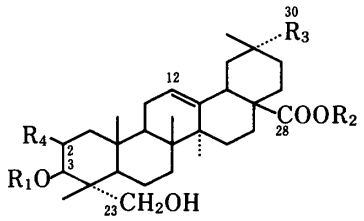
Keywords——Quinoa; *Chenopodium quinoa*; Chenopodiaceae; South-American crop; food source; saponin; hederagenin; phytolaccagenic acid

"Quinoa," *Chenopodium quinoa* WILLD. (Chenopodiaceae), grows at elevations up to 4400 m in the Andes, South America, and its grains have been used as a staple food or a feed for livestock in this district. Recently, because of the excellent nutritional quality, there has been renewed interest in quinoa as a valuable food source which can be cultivated even in dry desert at high altitudes.[1] It is known that the seed-coat of this plant contains bitter principles which must be removed as brans prior to cooking. It has also been suggested that these bitter principles are effective for protection of this plant from noxious insects. The present paper deals with the isolation and structure elucidation of saponins which account for the bitterness of the seed-coat.

The brans prepared from the grains were extracted with methanol. The methanolic extract was subjected to column chromatography on highly porous polymer by eluting with water, 50% methanol, 85% methanol, methanol and acetone, successively. The 50 and 85% methanol eluates tasted bitter and were suggested to be a complex mixture of a number of saponins, based on a thin layer chromatographic (TLC) examination. The 85% methanol eluate was separated by chromatography on silica gel followed by repeated high-performance liquid chromatography (HPLC) on a reverse-phase column, affording five bitter saponins, quinoa-saponins 1—5 (**1**—**5**), in yields of 0.7, 0.02, 0.03, 0.3 and 0.03%, respectively, along with a known saponin, HN-saponin F (**6**) isolated from *Hedera nepalensis*. The identification of **6** was established by comparison of the ^{1}H and ^{13}C nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra, melting point (mp) and optical rotation ($[\alpha]_D$) with reference data.[2]

Inspection of the ^{13}C-NMR spectra indicated that saponins **1** and **2** are 3,28-*O*-glycosylated hederagenin (**7**).[3] On acid hydrolysis, **1** yielded arabinose and glucose, while **2** afforded galactose and glucose. The ^{1}H- and ^{13}C-NMR spectra of **1** and **2** revealed the presence of three sugar units.

It is known that β-glucopyranosyl esters of acidic terpenoids give 1,6-anhydroglucose (**8**) on alkaline saponification.[4] Alkaline saponification of **1** and **2** gave the corresponding prosapogenins (**9** from **1** and **10** from **2**) and **8**, indicating the presence of a β-glucopyranosyl



	R_1	R_2	R_3	R_4
7:	-H	-H	$-CH_3$	-H
1:	-Ara[3]Glc	-Glc	$-CH_3$	-H
2:	-Gal[3]Glc	-Glc	$-CH_3$	-H
6:	-Ara	-Glc	$-CH_3$	-H
9:	-Ara[3]Glc	-H	$-CH_3$	-H
10:	-Gal[3]Glc	-H	$-CH_3$	-H
11:	-H	-H	$-COOCH_3$	-H
12:	-H	-H	$-COOCH_3$	-OH
3:	-Ara	-Glc	$-COOCH_3$	-H
4:	-Ara[3]Glc	-Glc	$-COOCH_3$	-H
5:	-Gal[3]Glc	-Glc	$-COOCH_3$	-H

Ara: α-L-arabinopyranosyl
Glc: β-D-glucopyranosyl
Gal: β-D-galactopyranosyl

Chart 1

ester at the 28-carboxyl group of **1** and **2**, and this was supported by the ^{13}C-NMR spectra (anomeric carbon signals at δ 95.7 and 95.8). Methylation analysis[5] of **9** proved the presence of terminal glucopyranoside and 3-linked arabinopyranoside, while that of **10** indicated the presence of terminal glucopyranoside and 3-linked galactopyranoside. These results together with the ^{13}C-NMR spectra and coupling constants of anomeric proton signals (see Experimental) led to the formulation of **1** (and **9**) and **2** (and **10**) as shown in Chart 1.

Hydrolysis of **3**, **4** and **5** with crude hesperidinase[6] afforded a sapogenin (**11**). It was found that the ^{13}C-NMR spectrum of **11** was composed of signals due to the carbons of the A, B, C and D rings of **7**[3] and those due to the carbons of the B, C, D and E rings of phytolaccagenin (**12**),[7] leading to the identification of **11** as phytolaccagenic acid,[8,9] a sapogenin previously isolated from *Phytolacca americana*. This was further confirmed by comparison of the melting point and the ^1H-NMR spectrum of **11** with those of an authentic specimen. The glycosylation shifts observed in the ^{13}C-NMR spectra of **3**, **4** and **5** indicated that these compounds were 3,28-di-*O*-glycosylated phytolaccagenic acid. On acid hydrolysis, **3** and **4** yielded arabinose and glucose, while **5** gave galactose and glucose. Alkaline saponification of **3**, **4** and **5** afforded **8**. Further, the carbon signals due to the sugar moieties of **3**, **4** and **5** were observed at almost the same positions as those of **6**, **1** and **2**, respectively. Based on these results, **3**, **4** and **5** can be formulated as shown in Chart 1.

Investigation of the other saponins in the 85% methanol eluate as well as saponins in the 50% methanol eluate is in progress.

Experimental

General Procedure——Melting points were determined on a Yanaco micro hot stage and are uncorrected. Optical rotations were measured with Union PM-101 automatic digital polarimeter. Infrared (IR) spectra were taken on a Shimadzu IR-408 spectrometer. NMR spectra were recorded on JEOL FX-100 and GX-270 instruments in C_5D_5N, using tetramethylsilane (TMS) as an internal standard. For gas liquid chromatography (GLC), a Shimadzu GC-6A apparatus was used. Mass spectra (MS) were taken on a JEOL JMS-01-SG-2 spectrometer by the direct inlet method; ionization voltage 75 eV. HPLC was carried out on a column of TSK-gel ODS-120T (21.5 mm × 30 cm) with a Toyo Soda HLC 803D pump and a Toyo Soda RI-8 differential refractometer as a detector. For column chromatography, Kieselgel 60 (70—230 mesh, Merck), LiChroprep RP-8 (40—63 μm, reversed-phase, Merck) and DIAION HP-20 (highly porous polymer, Mitsubishi Chem. Ind. Co., Ltd., Tokyo) were used.

Alkaline saponification and acid hydrolysis of saponins followed by identification of the resulting monosac-

TABLE I. ^{13}C-NMR Chemical Shifts of Aglycone Moieties in C_5D_5N

Carbon No.	7[3]	6	1	2	12[7]	11	3	4	5
1	38.9	38.8	38.7	38.9	44.8	38.7	38.6	38.8	38.7
2	27.6	26.1	26.1	26.2	71.5	27.6	26.1	26.0	26.1
3	73.7	81.8	81.8	82.6	73.0	73.5	81.9	81.8	82.1
4	42.9	43.5	43.5	43.5	42.4	42.7	43.5	43.5	43.4
5	48.8	47.5	47.5	47.9	48.1	48.6	47.6	47.5	47.5
6	18.7	18.1	18.1	18.4	18.2	18.6	18.1	18.1	18.1
7	33.0	32.9	32.9	32.7	33.0	32.9	32.8	32.8	32.8
8	39.8	39.9	39.9	40.1	39.8	39.7	39.9	39.9	39.9
9	48.2	48.2	48.2	48.3	48.5	48.1	48.2	48.1	48.1
10	37.3	36.9	36.9	37.1	37.2	37.2	36.9	36.8	36.8
11	23.8	23.6	23.6[a]	23.9[a]	23.8	23.8	23.7	23.8[a]	23.7
12	122.7	123.5	123.5	123.0	123.3	123.2	123.5	123.5	123.5
13	145.0	144.1	144.1	144.3	144.4	144.4	143.7	143.7	143.8
14	42.2	42.1	42.1	42.3	42.2	42.1	42.0	42.0	42.0
15	28.4	28.2	28.2	28.4	28.4	28.4	28.3	28.5	28.3
16	23.8	23.5	23.5[a]	23.9[a]	23.8	23.8	23.7	23.7[a]	23.7
17	46.7	46.9	46.9	47.1	46.1	46.1	46.5	46.4	46.4
18	42.0	41.7	41.7	41.9	43.3	43.3	43.2	43.2	43.1
19	46.5	46.1	46.1	46.4	42.7	42.7	42.5	42.5	42.3
20	31.0	30.8	30.8	30.8	44.1	44.2	44.0	43.9	43.9
21	34.3	34.0	34.0	34.1	30.8	30.8	30.5	30.5	30.6
22	33.3	33.1	33.1	33.1	34.5	34.5	33.9	34.0	33.9
23	68.2	64.1	64.1	65.0	67.7	68.0	64.5	64.1	64.4
24	13.1	13.6	13.6	13.6	14.5	13.1	13.5	13.6	13.6
25	16.0	16.2	16.2	16.2	17.4[a]	15.9	16.2	16.1	16.1
26	17.5	17.5	17.5	17.7	17.2[a]	17.4	17.5	17.4	17.4
27	26.2	26.1	26.1	26.2	26.2	26.1	26.1	26.0	26.1
28	180.4	176.4	176.4	176.4	179.7	179.1	176.9	176.9	176.0
29	33.3	33.1	33.1	33.1	28.4	28.4	28.3	28.3	28.3
30	23.8	23.3	23.3	23.8	177.1	177.1	175.9	175.9	176.9
OCH_3					51.6	51.7	51.7	51.7	51.7

a) These assignments may be interchanged in each column.

charides and determination of sugar sequence by methylation analysis were carried out as described in the previous paper.[4,5]

Extraction and Separation of Glycosides——The brans (200 g) were defatted with Et_2O and then extracted with MeOH. The MeOH extract (40.0 g) was chromatographed on a column of DIAION HP-20 using H_2O, 50% MeOH, 85% MeOH, MeOH and Me_2CO, successively. The 85% MeOH eluate was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (40 : 10 : 1, 10 : 5 : 1, 6 : 4 : 1 and 4 : 6 : 1, successively) to give five fractions (Fr. I—V) in order of elution. Fr. I was applied to combination columns of silica gel [$CHCl_3$–MeOH–H_2O (40 : 10 : 1)] and RP-8 (73% MeOH), and finally purified by preparative HPLC (68% or 73% MeOH) to give **3** and **6** in yields of 0.03 and 0.05%, respectively, Fr. III was applied to a column of RP-8 (65% MeOH) to afford **1** and **4**. Fr. IV was repeatedly chromatographed on a column of RP-8 (57%, 62% and 67% MeOH) and finally purified by preparative HPLC to give **1**, **2**, **4** and **5**. Total yields of **1**, **2**, **4** and **5** were 0.7, 0.02, 0.3 and 0.03%, respectively. Compound **1**: A white powder, $[\alpha]_D^{17}+31.1°$ ($c=1.00$, MeOH). *Anal.* Calcd for $C_{47}H_{76}O_{18}\cdot H_2O$: C, 59.60; H, 8.30. Found: C, 59.35; H, 8.47. ^{1}H-NMR δ: 4.93 (1H, d, $J=7.0$ Hz, H-1 of Ara), 5.27 (1H, d, $J=7.4$ Hz, H-1 of Glc), 6.29 (1H, d, $J=7.0$ Hz, H-1 of 28-*O*-Glc). Compound **2**: A white powder, $[\alpha]_D^{24}+46.9°$ ($c=0.97$, C_5H_5N). *Anal.* Calcd for $C_{48}H_{78}O_{19}\cdot 3H_2O$: C, 56.90; H, 8.36. Found: C, 56.85; H, 8.18. ^{1}H-NMR δ: 4.98 (1H, d, $J=7.2$ Hz, H-1 of Gal), 5.25 (1H, d, $J=7.7$ Hz, H-1 of Glc), 6.24 (1H, d, $J=6.8$ Hz, H-1 of 28-*O*-Glc). Compound **3**: A white powder, $[\alpha]_D^{19}+53.8°$ ($c=1.00$, MeOH). *Anal.* Calcd for $C_{42}H_{66}O_{15}\cdot 3H_2O$: C, 58.31; H, 8.39. Found: C, 58.37; H, 8.08. ^{1}H-NMR δ: 4.94 (1H, d, $J=6.6$ Hz, H-1 of Ara), 6.27 (1H, d, $J=6.8$ Hz, H-1 of Glc). Compound **4**: A white powder, $[\alpha]_D^{17}+48.1°$ ($c=1.00$, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{20}\cdot 2H_2O$: C, 57.13; H, 7.99. Found: C, 57.26; H, 8.14. ^{1}H-NMR δ: 4.96 (1H, d, $J=7.6$ Hz, H-1 of Ara), 5.30 (1H, d, $J=7.6$ Hz, H-1 of Glc), 6.27 (1H, d, $J=6.6$ Hz, H-1 of 28-*O*-Glc). Compound **5**: A white powder, $[\alpha]_D^{24}+53.8°$ ($c=1.05$, C_5H_5N). *Anal.* Calcd for $C_{49}H_{78}O_{21}\cdot 5/2H_2O$: C, 56.15; H, 7.98. Found: C, 56.05; H, 7.88. ^{1}H-NMR δ: 4.91 (1H, d, $J=7.4$ Hz, H-1 of Gal), 5.16 (1H, d, $J=7.1$ Hz, H-1 or Glc), 6.12 (1H, d, $J=7.1$ Hz, H-1 of 28-

TABLE II. ^{13}C-NMR Chemical Shifts of Sugar Moieties in C_5D_5N

Carbon No.	6	1	2	3	4	5
C-3-sugar						
Ara-1	106.5	106.5[a]		106.6	106.4[a]	
Ara-2	73.0	71.9		73.1	71.9	
Ara-3	74.6	84.2		74.7	84.1	
Ara-4	69.5	69.3		69.6	69.3	
Ara-5	66.9	67.1		66.9	67.1	
Glc-1		106.3[a]	106.4[a]		106.2[a]	106.4[a]
Glc-2		75.6	75.8		75.6	75.7
Glc-3		78.3[b]	78.5[b]		78.3[b]	78.3[b]
Glc-4		71.5	71.4		71.4	70.9
Glc-5		78.7[b]	78.4[b]		78.6[b]	78.6[b]
Glc-6		62.6	62.8		62.6	62.5
Gal-1			106.0[a]			106.1[a]
Gal-2			72.2			72.2
Gal-3			85.2			85.0
Gal-4			69.9			69.7
Gal-5			76.5			76.4
Gal-6			62.4			61.8
C-28-sugar						
Glc-1	95.7	95.7	95.8	95.7	95.7	95.7
Glc-2	74.0	74.1	74.2	74.1	74.0	74.0
Glc-3	79.2[a]	79.2[b]	79.1[b]	79.2[a]	79.2[b]	79.2[b]
Glc-4	71.0	71.1	71.7	70.9	70.8	71.4
Glc-5	78.8[a]	78.8[b]	78.9[b]	78.9[a]	78.8[b]	78.8[b]
Glc-6	62.2	62.2	62.5	62.2	61.9	62.2

a, b) These assignments may be interchanged in each column.

O-Glc). ^{13}C-NMR data of **1**—**6** were given in Tables I and II.

Prosapogenins of 1 and 2——Alkaline saponification[4] of **1** and **2** afforded **9** and **10**, respectively, along with **8**. Compound **9**: A white powder, $[\alpha]_D^{17}+38.8°$ ($c=1.00$, MeOH). *Anal.* Calcd for $C_{41}H_{66}O_{13}$: C, 64.20; H, 8.67. Found: C, 64.21; H, 9.01. ^{1}H-NMR δ: 4.98 (1H, d, $J=6.6$ Hz, H-1 of Ara), 5.29 (1H, d, $J=7.1$ Hz, H-1 of Glc). Compound **10**: A white powder, $[\alpha]_D^{24}+50.4°$ ($c=0.68$, C_5H_5N). *Anal.* Calcd for $C_{42}H_{68}O_{14}\cdot 3H_2O$: C, 59.27; H, 8.77. Found: C, 59.21; H, 8.68. ^{1}H-NMR δ 5.06 (1H, d, $J=7.8$ Hz, H-1 of Gal), 5.33 (1H, d, $J=7.7$ Hz, H-1 of Glc).

Aglycone of 3, 4 and 5——According to the reported method,[6] hydrolysis of **3**, **4** and **5** with crude hesperidinase afforded a common aglycone **11**, which was identified as phytolaccagenic acid by comparison of the melting point and the ^{1}H-NMR spectrum with those of an authentic sample.

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