Journal of Natural Products
Vol. 52, No. 5, pp. 1132-1135, Sep-Oct 1989

ADDITIONAL TOXIC, BITTER SAPONINS FROM THE SEEDS OF *CHENOPODIUM QUINOA*

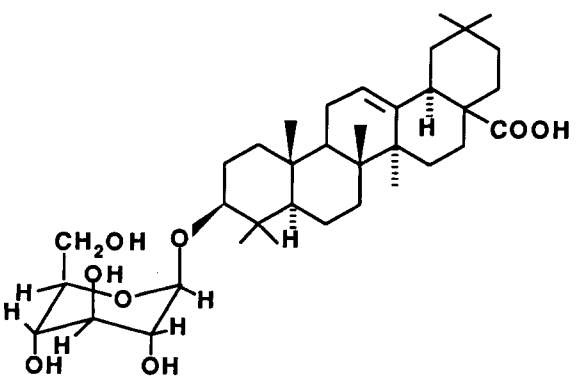
W.-W. MA, P.F. HEINSTEIN, and J.L. MCLAUGHLIN*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

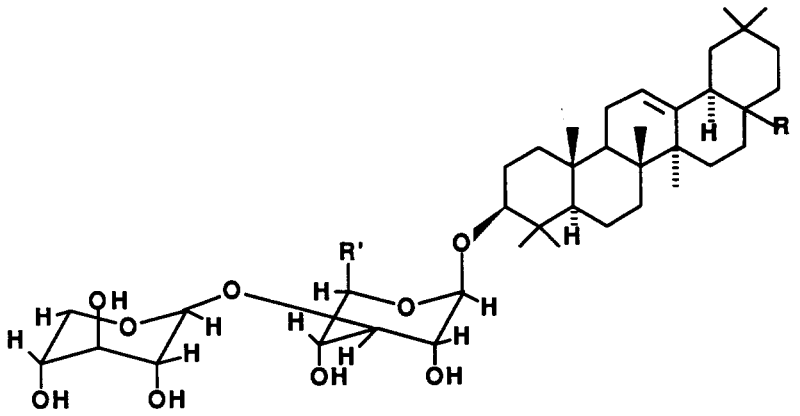
ABSTRACT.—Quinoa (*Chenopodium quinoa*) is an important Native American food grain. Prior to consumption, the seeds must be washed with H_2O to remove bitterness and improve nutritive value. From the warm-H_2O extract of quinoa seeds from Mexico, saponins **1–4** were isolated by monitoring the fractionation with brine shrimp lethality and a taste test for bitterness. By chemical, spectral, and enzymatic methods, **1–4** were identified as glycosides of oleanolic acid. Saponin **4**, 3-*O*-[(β-D-xylopyranosyl)(1↦3)]-β-D-glucuronopyranosyl-6-*O*-methyl ester]-oleanolic acid, is a new natural compound.

Chenopodium quinoa Willd. (Chenopodiaceae) has been cultivated for centuries as a grain crop in South America and south central Mexico. Its seeds are comparable in nutritional value to the cereal grasses, wheat, corn, oats, and rice (1), and have a high protein content (2). Its major disadvantage, bitterness requiring aqueous extraction prior to consumption (3), however, affects the worldwide acceptability of this grain. In our previous work (4), two saponins were detected and proven to be responsible for the bitter effects of quinoa seeds from Ecuador; one of these, quinoside A, was characterized as a hederagenin saponin, identified as olean-12-en-28-oic acid, 3,23-bis(*O*-β-D-glucopyranosyloxy)-*O*-β-D-glucopyranosyl-(1↦3)-*O*-α-L-arabinopyranosyl ester (3β,4α). From the warm-H_2O extract of quinoa seeds from Mexico, four additional saponins **1–4** have now been isolated, again via monitoring the fractionation with brine shrimp lethality and a simple taste test.

All of the four saponins **1–4** produced the same aglycone, oleanolic acid, after acid hydrolysis. This was proven by comparison with an authentic sample (tlc, ir, mmp, ms, ^{1}H nmr, ^{13}C nmr). The sugar obtained from saponin **1** by hydrolysis was glucose (tlc). Fabms of **1** gave *m/z* 641 $[M+Na]^+$ and 455 $[M-\text{hexose}]^+$. The ir spectrum showed a carboxylic group at 1680 cm^{-1}. Its methyl ester produced by CH_2N_2 gave fabms *m/z* 656. ^{1}H nmr of **1** showed an anomeric proton signal at δ 4.88 (d, J = 7.7 Hz) for the β linkage, and this configuration was confirmed by enzymatic hydrolysis. The structure of saponin **1** was, thus, identified as 3-*O*-(β-D-glucopyranosyl)-oleanolic acid, a known natural compound (5).



1



2 R=R′=COOH
3 R=COO-Glucose, R′=COOH

4 R=COOH. R′=COOMe

The sugars obtained from saponin **2** by acid hydrolysis were D-glucuronic acid and D-xylose (tlc). Fabms of **2** showed *m/z* 803 $[M+K]^+$, 787 $[M+Na]^+$, 764 $[M]^+$, 637 $[M+Na-\text{pentose}]^+$, and 455 $[M-\text{pentose}-\text{hexose}]^+$. The ir spectrum showed two carbonyl groups at 1660 and 1680 cm^{-1}. These two signals were shifted to 1720 and 1750 cm^{-1}, respectively, when **2** was methylated with CH_2N_2. Fabms of the dimethyl ester of **2** gave *m/z* 792 $[M]^+$. Saponin **2** was permethylated by the Hakomori method (6) and then reduced by $LiAlH_4$. The product was hydrolyzed by acid and gave an aglycone that was identified as erythrodiol (tlc, ms) and two glycones which were identified as α,β-methyl-2,4-dimethylglucoside and α,β-methyl-2,3,4,-trimethylxyloside (gc and gc-ms). ^{13}C nmr of the dimethyl ester of **2** showed that the C-3 signal of the glucuronic methyl ester was shifted to 86.09 ppm. These results indicated the interglycosidic linkage was 1↦3. The *J* values of the anomeric protons at δ 4.43 (1H, d, $J=7.8$ Hz) and δ 4.52 (1H, d, $J=7.7$ Hz) and results of the enzymatic hydrolysis showed that the linkages were of the β configuration. Therefore, saponin **2** was identified as 3-*O*-[(β-D-xylopyranosyl)(1↦3)]-[β-D-glucuronopyranosyl]-oleanolic acid, a previously reported natural compound (7).

The sugars obtained from saponin **3** by acid hydrolysis were glucuronic acid, glucose, and xylose (tlc). Basic hydrolysis of **3** yielded D-glucose and saponin **2**. Fabms of **3** gave *m/z* 965 $[M+K]^+$, 949 $[M+Na]^+$, 929 $[M+H]^+$, 817 $[M+Na-\text{hexose}]^+$, 787 $[M+Na-\text{pentose}]^+$, and 757, which indicated that glucose was connected with the -COOH of oleanolic acid. Saponin **3** was methylated with CH_2N_2, and the methyl ester fabms peaks at *m/z* 979 and 803 also showed that only the -COOH of glucuronic acid was free. After acid and enzymatic hydrolysis, the methyl ester yielded oleanolic acid, glucose, xylose, and the methyl ester of glucuronic acid (tlc). Thus, the structure of saponin **3** was determined as 3-*O*-[(β-D-xylopyranosyl)(1↦3)]-[β-D-glucuronopyranosyl]-oleanolic acid-[β-D-glucopyranosyl] ester; this is also a previously reported natural compound (7).

The sugars obtained from saponin **4** by acid hydrolysis were xylose and glucuronic methyl ester. Fabms of **4** gave peaks at *m/z* 801 $[M+Na]^+$, 817 $[M+K]^+$, 779 $[M+H]^+$, and 647. Ir of saponin **4** showed two carbonyl groups at 1700 and 1750 cm^{-1}. ^{13}C nmr showed signals at 171.2 and 52.9 ppm for the -COOMe group of glucuronic acid methyl ester. The methyl ester of **4** obtained by treating

with CH_2N_2 showed the same physical and spectral data as the dimethyl ester of **2** obtained by treating with CH_2N_2. ^1H nmr of **4** showed an -OMe signal at δ 3.7. These results demonstrated that saponin **4** is 3-*O*-[(β-D-xylopyranosyl)(1→3)]-[β-D-glucuronopyranosyl-methyl-ester]-oleanolic acid, which is a new natural compound.

Saponins **1**, **2**, and **3** showed lethality to brine shrimp and bitterness in the taste tests, but saponin **4** was inactive. This suggests that the free -COOH group of glucuronic acid in saponins **2** and **3** may facilitate the bioactivity of these saponins. We conclude that oleanolic acid saponins **1–3**, with the previously described hederagenin saponin, quinoside A (4), are the major bioactive principles removed by H_2O-washing of quinoa seeds. They are responsible for the bitter taste, and their deleterious effects may explain the low nutritive value reported for unwashed quinoa in weanling rats (8) and in malnourished human infants (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were measured on the Meltemp apparatus and were uncorrected. Ir spectra were determined with a Beckman IR-33 in KBr. ^1H-nmr and ^{13}C-nmr spectra were obtained with a Nicolet-200 in CD_3OD and C_5D_5N. Fabms were measured on VG ZAB and Kratos 50 instruments. Cims were obtained on a Finnigan 4023. Gc was carried out on an SP-2330, 68% cyanopropyl column at 130–240° on a Varian 1400 with a flame ionization detector. Tlc analyses of saponins were performed on Si gel plates using EtOAc-MeOH-H_2O (8:2:1) for development and the Lieberman-Burchard spray agent for visualization.

BIOASSAYS.—As a taste test for bitterness, the sample was dissolved or suspended in H_2O (500 μg/ml), and about 0.1 ml of the solution was dropped onto the tongue and expelled after 3 sec. Samples were scored subjectively according to the following scale: B− not bitter, B± slightly bitter, B+ definitely bitter, and B++ intensely bitter. Brine shrimp lethality was determined as LC_{50} values in ppm (μg/ml) as previously described (10).

PLANT MATERIALS.—Seeds of "huazontle," Santa Elena 7, *C. quinoa*, were donated by Dr. Francisco Cardenas Ramos, Instituto Nacional de Investigaciones Agricolas, Mexico, through the cooperation of Dr. G.A. White, Plant Introduction Office, USDA, ARS, Northeastern Region, Beltsville, Maryland (accession no. 476820). A voucher specimen is maintained in the Department of Medicinal Chemistry and Pharmacognosy, Purdue University.

EXTRACTION AND ISOLATION.—The dry seeds of the Mexican quinoa (3 kg) were extracted three times with H_2O at 60°. After freeze-drying, the aqueous extract (108 g) was partitioned between *n*-BuOH and H_2O. The crude saponins (60 g) from the residue of the *n*-BuOH layer were chromatographed over a Si gel column (1.5 kg) and eluted with a gradient of MeOH in $CHCl_3$. The fractions which showed activities in the brine shrimp and bitterness tests were chromatographed over C-18 columns eluted with H_2O, MeOH, and $CHCl_3$ in gradients and on Si gel Chromatotron plates eluted with gradients of MeOH in EtOH. After final purification by preparative tlc, the four saponins **1–4** were obtained. Quinosides A and B (4) were not found in this Mexican variety.

SAPONIN **1**.—Compound **1** (14 mg): white crystals; mp 250° [lit. (5) mp for $C_{36}H_{58}O_8 \cdot H_2O$, 231.5–233°]; brine shrimp LC_{50} 274 ppm; B+; ir (KBr) 3400, 2900, 1680, 1450, 1440, 1370 cm^{-1}; fabms *m/z* 641, 455, 439, 248, 203, 163; ^1H nmr (pyridine-d_5) δ 5.47 (1H, t, J = 3.02, H-12), 4.88 (1H, d, J = 7.7, anomeric proton), 4.18 (1H, dd, J = 3.3, 9.5, H-3), 3.38 (1H, dd, J = 4.2, 11.84, H-18), 3.29 (1H, dd, J = 3.0, 13.77, H-5), 0.3, 1.0, 1.3, 1.3 (21H, s, 7 Me).

SAPONIN **2**.—Compound **2** (14 mg): white powder; mp 236° [lit. (7) 235–240°]; brine shrimp LC_{50} 138 ppm; B+; ir (KBr) 3360, 2900, 1670, 1440, 1350, 1050 cm^{-1}; fabms *m/z* 803, 787, 764, 637, 455, 439, 203; ^1H nmr (pyridine-d_5) δ 4.43 (1H, d, J = 7.8, anomeric proton of xylose), 4.52 (1H, d, J = 7.7, anomeric proton of glucuronic acid); ^{13}C nmr of the dimethyl ester of **2** (pyridine-d_5) (glucuronic acid methyl ester moiety) δ 106.24 (C-1), 75.35 (C-2), 86.09 (C-3), 74.47 (C-4), 79.75 (C-5), 170.24 (C-6), 52.13 (-OMe), (xylose moiety) δ 106.81 (C-1), 76.67 (C-2), 78.12 (C-3), 71.08 (C-4), 67.33 (C-5).

SAPONIN **3**.—Compound **3** (29.6 mg): white powder; mp 258° [lit. (7) 270–275°]; brine shrimp LC_{50} 479 ppm; B+; ir (KBr) 3400, 2900, 1725, 1680 cm^{-1}; fabms *m/z* 965, 949, 927, 817, 787, 757, 455, 439, 248.

SAPONIN **4**.—Compound **4** (19.5 mg): white powder; mp 194°; brine shrimp LC_{50} >1000 ppm; B−; ir (KBr) 3400, 2900, 1730, 1700

cm^{-1}; fabms *m/z* 817, 801, 779, 647, 439, 173; ^1H nmr (CD_3OD) δ 4.52 (1H, d, $J=7.5$, anomeric proton of xylose), 4.43 (1H, d, $J=7.8$, anomeric proton of methyl ester of glucuronic acid), 3.7 (3H, s, -Me); ^{13}C nmr (CD_3OD) (glucuronic acid methyl ester moiety) δ 171.11 (C-6), 105.8 (C-1), 86.5 (C-3), 77.56 (C-5), 75.20 (C-2), 74.7 (C-4), 52.89 (-OMe).

PERMETHYLATION.—A mixture of 300 mg NaH and 5 ml DMSO was stirred at 70–80° under N_2 for 1 h. A solution of 5–10 mg saponin in DMSO was added to the above mixture and stirred at 60° under N_2 for another hour. After cooling the solution, 0.5 ml of MeI was added, and the mixture was stirred overnight at room temperature. The mixture was poured into ice-H_2O and extracted with EtOAc to yield the permethylated products.

$LiAlH_4$ REDUCTION.—$LiAlH_4$ (3 mg) was added to a solution of saponin (a few mg) in dry Et_2O. The mixture was stirred at room temperature for 1 h. A few drops of hydrated Et_2O were then added. The solution was adjusted to pH 7, and the product was precipitated.

ENZYMATIC HYDROLYSIS.—A given saponin (a few mg) was mixed with β-glucosidase (emulsin, Sigma Type II) in 2 ml of pH 4 buffer and 1 ml of H_2O. The mixture was kept at 28° for 1–2 days. The progress of hydrolysis was monitored with tlc. Also, another portion of saponin was mixed with α-glucosidase (maltase, Sigma Type III) and pH 7 buffer and maintained at room temperature for 4–5 days, and the result was then checked with tlc. Only the β-glucosidase cleaved the saponins.

ACKNOWLEDGMENTS

This research was supported by the U.S. Department of State through AID grant no. DAN-5542-G-SS-2127-00 and the NIH through RO1 grant no. CA-30909 from NCI. The authors thank Dr. J.L. Occolowitz at the Lilly Research Labs for the fabms analyses and Dr. C.-J. Chang and Dr. M. Antoun for helpful discussions. The cooperation of Dr. G.A. White at the USDA, Beltsville, and Dr. F.C. Ramos was essential in obtaining the plant material.

LITERATURE CITED

1. A.J. de Bruin, *Food Sci.*, **29**, 892 (1964).
2. D.W. Grade, *Econ. Bot.*, **24**, 55 (1970).
3. M.L. Telleria Rios, V.C. Sgarbieri, and J. Amaya-F., *Arch. Latinoam. Nutr.*, **28**, 253 (1978).
4. B.N. Meyer, P.F. Heinstein, M. Burnouf-Radosevich, N.E. Delfel, and J.L. McLaughlin, accepted for publication in *J. Agric. Food Chem.*
5. Nanking Institute of Materia Medica (Nanking, China), *Chung Ts'ao Yao*, **55**, 11 (1980).
6. S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
7. F. Gafner, J.D. Msonthi, and K. Hostettmann, *Helv. Chim. Acta*, **68**, 555 (1985).
8. P. Mazzocco, *Rev. Soc. Argent. Biol.*, **10**, nos. 6 and 7 (1934).
9. G. Lopez de Romaña, H.M. Creed, and G.G. Graham, *Arch. Latinoam. Nutr.*, **28**, 419 (1978).
10. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).

Received 22 June 1988