Studies on the Constituents of *Chenopodium pallidicaule* (Cañihua) Seeds. Isolation and Characterization of Two New Flavonol Glycosides

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Two new flavonol triglycosides have been identified from the seeds of *Chenopodium pallidicaule*. Their structures were established as isorhamnetin 3-*O*-β-D-apiofuranosyl(1→2)-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside and quercetin 3-*O*-β-D-apiofuranosyl(1→2)-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-galactopyranoside. The known compounds quercetin 3-(2^{G}-β-D-apiosyl)rutinoside, 3-(2^{GAL}-α-L-rhamnosyl)robinobioside, 3-rutinoside, 3-robinobioside, isorhamnetin 3-(2^{GAL}-α-L-rhamnosyl) robinobioside, 3-rutinoside, 3-robinobioside, and kaempferol 3-robinobioside were also found. All structures were elucidated by chemical and spectroscopic methods.

Keywords: *Cañihua; Chenopodium pallidicaule; Chenopodiaceae; South American crop; flavonol glycosides; apiosides; ^{1}H, ^{13}C, and 2D NMR*

INTRODUCTION

Flavonoids are a class of natural products with a large number of derivatives in the plant kingdom, from fungi to angiosperme, including food plants, and are a common component in the human diet.

Flavonoids and other plant phenolics have been reported to have multiple biological effects such as antioxidant activity (Bors and Saran, 1987; Larson, 1988), anti-inflammatory action (Moroney et al., 1988; Vlaskovska et al., 1990), inhibition of platelet aggregation (Van-Wauwe and Gossenc, 1983), inhibition of mast cell histamine release (Amellal et al., 1985), and antimicrobial activities (Pratt and Hudson, 1990). Moreover, flavonoids such as the flavonols quercetin and kaempferol have antimutagenic and anticarcinogenic effects in vitro and in vivo (Kato et al., 1983; Huang et al., 1983; Fujiki et al., 1986; Verma et al., 1988; Deschner et al., 1991).

Recently, there is a growing interest in food chemistry and preventive medicine in the development of natural antioxidants from plant material because of toxic side effects from synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT), which are suspected to be carcinogenic (Branen, 1975; Ito, 1983). Natural antioxidants are useful in the food industry as ingredients added to increase the life of food products, preventing loss of their sensory and nutritional quality and improving the stability of lipids, vitamins, and flavors.

During the course of our study on food plants used by people of the Andes before the Spanish conquest, we examined the flavonol glycosides fraction present in the whole flour from the seeds of *Chenopodium pallidicaule*. This crop is still used by Andean people, but cultivation is confined to subtropical and tropical America. A higher protein content was found in the flour of cañihua than in other cereals, with an excellent amino acid pattern (Gross et al., 1989), but no investigation of secondary metabolites has been reported prior to our own work. We here report on the isolation and structure elucidation of 10 flavonol glycosides, 2 of which are new natural products.

EXPERIMENTAL PROCEDURES

Material. *C. pallidicaule* was supplied by Central Peruviane de Servicios and was collected in Ayavaca (D. of Piura), Peru, in 1991. A voucher specimen is deposited in the Herbario del Museo de Historia Natural "J. Prado" Un. H. S. Lima (Peru).

Apparatus. The FABMS spectra, in negative ion mode, were obtained by dissolving the samples in a glycerol–thioglycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of 2–6 kV energy.

The NMR spectra were obtained in CD_3OD using a Bruker WM-250 Spectrospin or a Bruker AMX-500 spectrometer. The DEPT experiments were performed using a transfer pulse of 135° to obtain positive signals for CH and CH_3 and negative ones for CH_2. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. COSY experiments were performed by employing the conventional pulse sequence, using a data set ($t_1 \times t_2$) of 1024 × 1024 points for a spectral width of 1165 Hz and a relaxation delay of 1 s. A NOESY experiment was also performed. It was conducted in the phase sensitive mode. The spectral width (t_2) was 1002 Hz; 512 experiments of 80 scans each (relaxation delay 1.5 s, mixing time 300 ms) were acquired in 2K data points.

GC–MS were run using a Hewlett-Packard 5890 apparatus, a 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 mm film).

HPLC separations were performed on a Hewlett-Packard HP 1050 series pumping system with a Varian RI-4 refractive index detector equipped with a Whatman Partisil 10 ODS-2 column.

Extraction and Isolation. The whole flour from the seeds (1.0 kg) was defatted with petroleum ether and $CHCl_3$ and then extracted with MeOH to give 39 g of residue. Part of the MeOH extract (13 g) was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH soluble portion (6.0 g) which was chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as the eluent. Fractions (9 mL) were collected and checked by TLC [Si gel plates in *n*-BuOH–HOAc–H_2O

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Table 1. ^1H NMR[a] Data for the Oligosaccharide Moiety of 1 in CD_3OD

proton	glucose	apiose	rhamnose
H-1	5.55, d, $J = 7.5$	5.48, d, $J = 1.5$	4.52, d, $J = 1.5$
H-2	3.67, dd, $J = 7.5, 9.5$	4.07, d, $J = 1.5$	3.65, dd, $J = 3.5, 1.5$
H-3	3.80, t, $J = 9.5, 9.5$		3.25, dd, $J = 9.5, 3.5$
Ha-4	3.64, t, $J = 9.5, 9.5$	3.82, d, $J = 10$	3.23, t, $J = 9.5, 9.5$
Hb-4		4.05, d, $J = 10$	
Ha-5	3.60, m	3.61, s	3.42, dq, 9.5, 6.2
Hb-5		3.61, s	
Ha-6	3.69, dd, $J = 12.0, 3.5$		1.10, d, 6.0
Hb-6	3.58, dd, $J = 12.0, 5.0$		

[a] From 2D COSY. $^1H-^1H$ coupling constants in the sugar spin system were measured from COSY spectrum and are reported in hertz.

(60:15:25)]. Fractions 42–68 (800 mg) containing the crude glycosidic mixture were submitted to HPLC using MeOH–H_2O (50:50) (flow rate 3 mL/min) as eluent to yield pure compounds **3** (11 mg, Rt = 9.5 min), **4** (25 mg, Rt = 10 min), **2** (28 mg, Rt = 11 min), **5** (106 mg, Rt = 13.5 min), **6** (115 mg, Rt = 15.5 min), **7** (18 mg, Rt = 17 min), **1** (9 mg, Rt = 19.5 min), **8** (25 mg, Rt = 20.5 min), **9** (95 mg, Rt = 21 min), and **10** (10 mg, Rt = 25.5 min).

Acid Hydrolysis of Compounds 1–3. A solution of each compound (3.0 mg) in 6% HCl (3.5 mL) was refluxed for 2 h. The reaction mixture was diluted with H_2O and then extracted with EtOAc. The resulting aglycons were identified by their ^1H NMR spectra.

Methanolysis of Compounds 1–10. Each fraction (1.0 mg) was heated in a vial for 24 h at 80 °C in MeOH–2% HCl (2 mL). After MeOH and HCl distillation in a N_2 stream, Ag_2CO_3 and MeOH were added until CO_2 production stopped. The centrifugate was dried over P_2O_5. The resulting monosaccharides were treated with Trisil-Z (Pierce) and analyzed by GC–MS. Retention times were identical to those of the authentic Trisil sugars.

Compound **1.** FABMS (negative ion mode), m/z 755 $[M - H]^-$. UV λ_{max} nm in MeOH, 254, 266, 356; +NaOAc, 271, 318, 402; +NaOAc–H_3BO_3, 256, 308, 358; +$AlCl_3$, 274, 308, 360; +NaOMe, 272, 326, 409. ^1H NMR (CD_3OD), aglycon signals, δ 6.21 (1H, d, $J = 2$ Hz, H-6), 6.42 (1H, d, $J = 2$ Hz, H-8), 6.94 (1H, d, $J = 8.5$ Hz, H-5′), 7.62 (1H, dd, $J = 8.5, 2$ Hz, H-6′), 7.94 (1H, d, $J = 2$ Hz, H-2′); sugar signals, see Table 1. ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.7 (C-2), 134.5 (C-3), 179.2 (C-4), 163.1 (C-5), 99.8 (C-6), 166.7 (C-7), 94.8 (C-8), 158.4 (C-9), 105.9 (C-10), 123.5 (C-1′), 114.4 (C-2′), 150.6 (C-3′), 148.3 (C-4′), 116.1 (C-5′), 123.8 (C-6′), 56.8 (O-Me); sugar signals, see Table 2.

Compound **2.** FABMS (negative ion mode), m/z 741 $[M - H]^-$. UV λ_{max} nm in MeOH, 256, 267, 358; +NaOAc, 255, 270, 378; +NaOAc–H_3BO_3, 261, 324, 379; +$AlCl_3$, 273, 316, 430; +NaOMe, 272, 328, 412. ^1H NMR (CD_3OD), aglycon signals, δ 6.16 (1H, d, $J = 2$ Hz, H-6), 6.35 (1H, d, $J = 2$ Hz, H-8), 6.86 (1H, d, $J = 8.5$ Hz, H-5′), 7.61 (1H, dd, $J = 8.5, 2.2$ Hz, H-6′), 7.70 (1H, d, $J = 2$ Hz, H-2′); sugar signals, δ 1.17 (3H, d, $J = 6$ Hz, Me-Rha), 4.51 (1H, d, $J = 1.5$ Hz, H-1 of rhamnose), 5.41 (1H, d, $J = 7.5$ Hz, H-1 of galactose), 5.45 (1H, d, $J = 1.5$ Hz, H-1 of apiose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.3 (C-2), 133.9 (C-3), 179.4 (C-4), 163.1 (C-5), 99.8 (C-6), 165.7 (C-7), 94.6 (C-8), 158.3 (C-9), 105.8 (C-10), 123.2 (C-1′), 116.2 (C-2′), 145.8 (C-3′), 149.7 (C-4′), 117.3 (C-5′), 123.5 (C-6′); sugar signals, see Table 2.

Compound **3.** FABMS (negative ion mode), m/z 741 $[M - H]^-$. UV λ_{max} nm, see compound **2**. ^1H NMR (CD_3OD), aglycon signals, δ 6.20 (1H, d, $J = 2$ Hz, H-6), 6.40 (1H, d, $J = 2$ Hz, H-8), 6.90 (1H, d, $J = 8.5$ Hz, H-5′), 7.62 (1H, dd, $J = 8.5, 2$ Hz, H-6′), 7.64 (1H, d, $J = 2$ Hz, H-2′); sugar signals, δ 1.11 (3H, d, $J = 6$ Hz, Me-Rha), 4.51 (1H, s, H-1 of rhamnose), 5.43 (1H, d, $J = 7.5$ Hz, H-1 of glucose), 5.50 (1H, d, $J = 1.5$ Hz, H-1 of apiose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.9 (C-2), 132.2 (C-3), 179.3 (C-4), 163.1 (C-5), 99.8 (C-6), 165.7 (C-7), 94.8 (C-8), 158.4 (C-9), 105.8 (C-10), 123.1 (C-1′), 116.2 (C-2′), 145.8 (C-3′), 149.5 (C-4′), 116.8 (C-5′), 123.5 (C-6′); sugar signals, see Table 2.

Table 2. ^{13}C NMR Chemical Shift Assignments (δ in CD_3OD) of Sugar Moieties of Compounds 1–3

carbon	DEPT	1	2	3
Glu-1	CH	102.4		102.2
Glu-2	CH	75.7		75.5
Glu-3	CH	78.7		78.6
Glu-4	CH	72.3		71.8
Glu-5	CH	78.0		78.1
Glu-6	CH_2	68.4		68.3
Rha-1	CH	100.8	101.5	100.9
Rha-2	CH	71.2	72.1[a]	72.2[a]
Rha-3	CH	72.1[a]	72.3[a]	72.3[a]
Rha-4	CH	72.3[a]	73.9	73.9
Rha-5	CH	69.7	69.7	69.7
Rha-6	CH_3	17.8	17.9	17.9
Gal-1	CH		101.8	
Gal-2	CH		78.0	
Gal-3	CH		75.3	
Gal-4	CH		70.0	
Gal-5	CH		75.5	
Gal-6	CH_2		67.1	
Api-1	CH	110.5	110.7	110.5
Api-2	CH	77.1	76.7	77.0
Api-3	C	80.1	80.8	80.9
Api-4	CH_2	75.4	75.2	75.4
Api-5	CH_2	66.5	66.2	66.7

[a] These assignments may be interchanged in each column.

Compound **4.** FABMS (negative ion mode), m/z 755 $[M - H]^-$. UV λ_{max} nm, see compound **2**. ^1H NMR (CD_3OD), aglycon signals, δ 6.19 (1H, d, $J = 2$ Hz, H-6), 6.38 (1H, d, $J = 2$ Hz, H-8), 6.90 (1H, d, $J = 8.5$ Hz, H-5′), 7.59 (1H, dd, $J = 8.5, 2$ Hz, H-6′), 7.72 (1H, d, $J = 2$ Hz, H-2′); sugar signals, δ 0.98 (3H, d, $J = 6$ Hz, Me of rhamnose linked at C-2 of galactose), 1.20 (3H, d, $J = 6$ Hz, Me of rhamnose linked at C-6 of galactose), 4.57 (1H, d, $J = 1.5$ Hz, H-1 of rhamnose linked at C-6 of galactose), 5.24 (1H, d, $J = 1.5$ Hz, H-1 of rhamnose linked at C-2 of galactose), 5.69 (1H, d, $J = 7.5$ Hz, H-1 of galactose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.2 (C-2), 134.3 (C-3), 179.0 (C-4), 163.4 (C-5), 99.7 (C-6), 165.8 (C-7), 94.4 (C-8), 158.2 (C-9), 105.6 (C-10), 123.1 (C-1′), 115.9 (C-2′), 145.6 (C-3′), 149.4 (C-4′), 117.1 (C-5′), 122.8 (C-6′); sugar signals, Gal ppm 102.4 (C-1), 77.3 (C-2), 75.1 (C-3), 70.7 (C-4), 75.5 (C-5), 66.8 (C-6); Rha (1→2) ppm 101.6 (C-1), 71.9 (C-2), 72.1 (C-3), 73.7 (C-4), 69.6 (C-5), 17.1 (C-6); Rha (1→6) ppm 100.8 (C-1), 72.1 (C-2), 72.2 (C-3), 73.9 (C-4), 69.6 (C-5), 17.8 (C-6).

Compound **5.** FABMS (negative ion mode), m/z 609 $[M - H]^-$. UV λ_{max} nm, see compound **2**. ^1H NMR (CD_3OD), aglycon signals, δ 6.22 (1H, d, $J = 2$ Hz, H-6), 6.41 (1H, d, $J = 2$ Hz, H-8), 6.89 (1H, d, $J = 8.5$ Hz, H-5′), 7.64 (1H, dd, $J = 8.5, 2$ Hz, H-6′), 7.69 (1H, d, $J = 2$ Hz, H-2′); sugar signals, δ 1.16 (3H, d, $J = 6$ Hz, Me-Rha), 4.58 (1H, d, $J = 1.5$ Hz, H-1 of rhamnose), 5.14 (1H, d, $J = 7.5$ Hz, H-1 of glucose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 159.3 (C-2), 135.6 (C-3), 179.2 (C-4), 162.9 (C-5), 98.6 (C-6), 166.2 (C-7), 94.2 (C-8), 158.5 (C-9), 105.5 (C-10), 123.1 (C-1′), 116.4 (C-2′), 145.8 (C-3′), 149.8 (C-4′), 117.3 (C-5′), 122.3 (C-6′); sugar signals, Glu ppm 103.7 (C-1), 75.1 (C-2), 79.3 (C-3), 72.5 (C-4), 78.3 (C-5), 68.5 (C-6); Rha ppm 101.2 (C-1), 71.0 (C-2), 71.0 (C-3), 73.2 (C-4), 70.2 (C-5), 17.0 (C-6).

Compound **6.** FABMS (negative ion mode), m/z 609 $[M - H]^-$. UV λ_{max} nm, see compound **2**. ^1H NMR (CD_3OD), aglycon signals, δ 6.22 (1H, d, $J = 2$ Hz, H-6), 6.41 (1H, d, $J = 2$ Hz, H-8), 6.90 (1H, d, $J = 8.5$ Hz, H-5′), 7.62 (1H, dd, $J = 8.5, 2$ Hz, H-6′), 7.91 (1H, d, $J = 2$ Hz, H-2′); sugar signals, δ 1.20 (3H, d, $J = 6$ Hz, Me-Rha), 4.55 (1H, d, $J = 1.5$ Hz, H-1 of rhamnose), 5.09 (1H, d, $J = 7.5$ Hz, H-1 of galactose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 159.0 (C-2), 135.3 (C-3), 179.4 (C-4), 162.9 (C-5), 99.5 (C-6), 166.4 (C-7), 94.9 (C-8), 158.5 (C-9), 105.9 (C-10), 123.1 (C-1′), 116.1 (C-2′), 145.7 (C-3′), 149.9 (C-4′), 117.9 (C-5′), 122.8 (C-6′); sugar signals, Gal ppm 101.9 (C-1), 73.9 (C-2), 75.2 (C-3), 70.2 (C-4), 75.4 (C-5), 67.6 (C-6); Rha ppm 100.1 (C-1), 72.1 (C-2), 72.4 (C-3), 73.2 (C-4), 69.7 (C-5), 17.9 (C-6).

Compound **7.** FABMS (negative ion mode), m/z 769 [M − H]$^-$. UV λ_{max} nm, see compound **1**. ^{1}H NMR (CD_3OD), aglycon signals, δ 4.02 (3H, s, 3-OMe), 6.20 (1H, d, J = 2 Hz, H-6), 6.41 (1H, d, J = 2 Hz, H-8), 6.93 (1H, d, J = 8.5 Hz, H-5′), 7.55 (1H, dd, J = 8.5, 2 Hz, H-6′), 8.10 (1H, d, J = 2 Hz, H-2′); sugar signals, δ 0.89 (3H, d, J = 6 Hz, Me of rhamnose linked at C-2 of galactose),1.20 (3H, d, J = 6 Hz, Me of rhamnose linked at C-6 of galactose), 4.58 (1H, d, J = 1.5 Hz, H-1 of rhamnose linked at C-6 of galactose), 5.18 (1H, d, J = 1.5 Hz, H-1 of rhamnose linked at C-2 of galactose), 5.82 (1H, d, J = 7.5 Hz, H-1 of galactose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.4 (C-2), 134.3 (C-3), 179.3 (C-4), 163.1 (C-5), 99.8 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 105.9 (C-10), 123.3 (C-1′), 114.7 (C-2′), 150.5 (C-3′), 148.4 (C-4′), 115.9 (C-5′), 123.2 (C-6′), 57.2 (−OMe); sugar signals, Gal ppm 102.7 (C-1), 77.8 (C-2), 75.4 (C-3), 70.5 (C-4), 75.6 (C-5), 67.2 (C-6); Rha (1→2) ppm 101.8 (C-1), 72.1 (C-2), 72.4 (C-3), 73.8 (C-4), 69.8 (C-5), 17.3 (C-6); Rha (1→6) ppm 100.3 (C-1), 72.3 (C-2), 72.4 (C-3), 73.9 (C-4), 69.7 (C-5), 17.9 (C-6).

Compound **8.** FABMS (negative ion mode), m/z 593 [M − H]$^-$. UV λ_{max} nm in MeOH, 265, 286, 347, +NaOAc, 271, 301, 362, +$AlCl_3$, 272, 305, 357, +NaOMe, 273, 321, 391. ^{1}H NMR (CD_3OD), aglycon signals, δ 6.25 (1H, d, J = 2 Hz, H-6), 6.44 (1H, d, J = 2 Hz, H-8), 6.90 (1H, d, J = 8.5 Hz, H-3′, H-5′), 8.12 (1H, d, J = 8.5 Hz, H-2′, H-6′); sugar signals, δ 1.21 (3H, d, J = 6 Hz, Me-rhamnose), 4.54 (1H, d, J = 1.5 Hz, H-1 of rhamnose), 5.06 (1H, d, J = 7.5 Hz, H-1 of galactose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.7 (C-2), 135.4 (C-3), 178.8 (C-4), 163.1 (C-5), 100.0 (C-6), 165.5 (C-7), 94.8 (C-8), 158.7 (C-9), 105.4 (C-10), 124.0 (C-1′), 132.3 (C-2′, C-6′), 116.0 (C-3′, C-5′), 159.2 (C-4′); sugar signals, Gal ppm 105.4 (C-1), 72.8 (C-2), 75.0 (C-3), 70.1 (C-4), 75.3 (C-5), 67.3 (C-6); Rha ppm 101.8 (C-1), 72.0 (C-2), 72.2 (C-3), 73.7 (C-4), 69.6 (C-5), 17.8 (C-6).

Compound **9.** FABMS (negative ion mode), m/z 623 [M − H]$^-$. UV λ_{max} nm, see compound **1**. ^{1}H NMR (CD_3OD), aglycon signals, δ 3.99 (3H, s, −OMe), 6.22 (1H, d, J = 2 Hz, H-6), 6.41 (1H, d, J = 2 Hz, H-8), 6.92 (1H, d, J = 8.5 Hz, H-5′), 7.61 (1H, dd, J = 8.5, 2 Hz, H-6′), 8.04 (1H, d, J = 2 Hz, H-2′); sugar signals, δ 1.20 (3H, d, J = 6 Hz, Me-rhamnose), 4.55 (1H, d, J = 1.5 Hz, H-1 of rhamnose), 5.24 (1H, d, J = 7.5 Hz, H-1 of galactose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.8 (C-2), 135.4 (C-3), 179.4 (C-4), 162.9 (C-5), 98.8 (C-6), 166.2 (C-7), 93.6 (C-8), 158.4 (C-9), 105.5 (C-10), 123.0 (C-1′), 114.6 (C-2′), 150.8 (C-3′), 148.3 (C-4′), 115.8 (C-5′), 122.8 (C-6′), 55.6 (−OMe); sugar signals, Gal ppm 101.3 (C-1), 73.2 (C-2), 74.3 (C-3), 70.4 (C-4), 75.0 (C-5), 68.8 (C-6); Rha ppm 100.5 (C-1), 70.8 (C-2), 71.1 (C-3), 72.7 (C-4), 68.8 (C-5), 18.1 (C-6).

Compound **10.** FABMS (negative ion mode), m/z 623 [M − H]$^-$. UV λ_{max} nm, see compound **1**. ^{1}H NMR (CD_3OD), aglycon signals, δ 3.93 (3H, s, −OMe), 6.17 (1H, d, J = 2 Hz, H-6), 6.35 (1H, d, J = 2 Hz, H-8), 6.88 (1H, d, J = 8.5 Hz, H-5′), 7.59 (1H, dd, J = 8.5, 2 Hz, H-6′), 7.93 (1H, d, J = 2 Hz, H-2′); sugar signals, δ 1.09 (3H, d, J = 6 Hz, Me-rhamnose), 4.51 (1H, d, J = 1.5 Hz, H-1 of rhamnose), 5.22 (1H, d, J = 7.5 Hz, H-1 of glucose). ^{13}C NMR (CD_3OD), aglycon signals, ppm 158.8 (C-2), 135.5 (C-3), 179.3 (C-4), 162.9 (C-5), 100.0 (C-6), 166.2 (C-7), 95.0 (C-8), 158.8 (C-9), 105.6 (C-10), 124.0 (C-1′), 114.6 (C-2′), 150.8 (C-3′), 148.3 (C-4′), 116.1 (C-5′), 124.0 (C-6′), 56.8 (−OMe); sugar signals, Glu ppm 104.5 (C-1), 75.9 (C-2), 78.2 (C-3), 71.6 (C-4), 77.3 (C-5), 68.5 (C-6); Rha ppm 102.4 (C-1), 72.1(C-2), 72.3 (C-3), 73.9 (C-4), 69.7 (C-5), 17.9 (C-6).

RESULTS AND DISCUSSION

Compound **1** showed in a FAB mass spectrum an [M − H]$^-$ ion at m/z 755. From the mass and ^{13}C NMR and ^{13}C DEPT NMR data, a $C_{33}H_{40}O_{20}$ molecular formula was deduced. On acid hydrolysis, it gave isorhamnetin, identified by direct comparison with UV and ^{1}H NMR spectra. The chromatographic analysis of the methanolysis products showed the presence of glucose, rhamnose, and apiose in the ratio 1:1:1.

The ^{1}H NMR spectrum of **1** (CD_3OD, Table 1) indicated the presence of a rhamnosyl methyl signal at δ 1.10 (3H, J = 6 Hz), a rhamnosyl anomeric proton at δ 4.52 (1H, d, J = 1.5 Hz), an apiosyl anomeric proton at δ 5.48 (1H, d, J = 1.5 Hz), and a signal at δ 5.55 (1H, d, J = 7.5 Hz) well suited to an anomeric proton of a β-D-glucopyranose linked to an γ-pyran ring with an O-glycosidic bond. The aromatic side exhibited proton signals at δ 7.62 (dd, J = 8.5 Hz, J = 2 Hz, H-6′), 6.94 (d, J = 8.5 Hz, H-5′), and 7.94 (d, J = 2 Hz, H-2′) in agreement with a 3′,4′-disubstitution. The 5,7-disubstitution was demonstrated by the presence of a pair of meta-coupled 1H doublets at δ 6.21 (J = 2 Hz) and 6.42 (J = 2 Hz), assignable to the H-6 and H-8 protons from literature data; in fact, for 5,7-dihydroxyflavones and flavonols the H-6 resonance was reported at higher field than the H-8 resonance (Shen et al., 1993). The singlet at δ 3.99 confirmed the presence of an aromatic methoxy group. In the ^{13}C NMR spectrum the shift at 134.5 ppm suggested O-glycosidic substitution at C-3; the carbon resonances confirmed the presence of a glucosyl, a rhamnosyl, and a pentose unit. The DEPT spectrum showed signals for CH_2 at δ 75.4 and 66.5 and lack of a signal at δ 80.1 in agreement with values for C-4, C-5, and C-3 of a β-apiosyl unit (Table 2) (De Simone et al., 1990).

The interglycosidic linkage and the sequential arrangement of the oligosaccharide moiety were determined using a combination of two-dimensional ^{1}H correlation (COSY) and two-dimensional nuclear Overhauser effect (NOESY) spectroscopy. The COSY experiment allowed the sequential assignment (Table 1) of most resonances for each sugar ring starting from the anomeric proton signals. Diagnostic NOE correlations were observed between G-2 (δ 3.67, H-2 glu) and A-1 (δ 5.48, H-1 api) and between G-6 (δ 3.69, H-6 glu) and R-1 (δ 4.52, H-1 rha), indicating a glycosidic linkage at C-2 and C-6 of glucose of the apiosyl and the rhamnosyl units, respectively.

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 (Table 2) indicated the β configuration at the anomeric positions for glucopyranosyl (J_{H1-H2} = 7.5 Hz) and apiofuranosyl (J_{H1-H2} = 1.5 Hz) units and the α configuration for the rhamnopyranosyl unit (J_{H1-H2} = 1.5 Hz).

From all of these data the structure of **1** was concluded to be isorhamnetin 3-O-β-D-apiofuranosyl(1→2)-O-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside (Figure 1).

The mass FAB spectrum of compound **2** in negative ion mode showed an [M − H]$^-$ ion at m/z 741. From the mass and the ^{13}C and ^{13}C DEPT NMR data a $C_{32}H_{38}O_{20}$ molecular formula was deduced. On acid hydrolysis, **2** afforded quercetin, identified by ^{1}H NMR. Acid methanolysis yielded methyl galactoside, methyl rhamnoside, and methyl apioside in the ratio 1:1:1.

The ^{1}H NMR spectrum of **2** showed a three proton doublet at δ 1.17 (J = 6 Hz) typical for the rhamnosyl methyl group and three anomeric proton signals at δ 4.51 (d, J = 1.5 Hz), assignable to the H-1 α-rhamnosyl proton, δ 5.41 (d, J = 7.5 Hz) assignable to the H-1 β-galactoside proton, and δ 5.45 (d, J = 1.5 Hz), assignable to H-1 β-apiosyl proton. For the aromatic side, two signals at δ 6.16 and 6.35 (both d, J = 2 Hz) could be assigned to H-6 and H-8, respectively, and signals at δ 6.86 (d, J = 8.5 Hz, H-5′), 7.61 (dd, J = 8.5, 2 Hz, H-6′), and 7.70 (d, J = 2 Hz, H-2′) could be ascribed to H-2′, H-5′, and H-6′, respectively. The ^{13}C

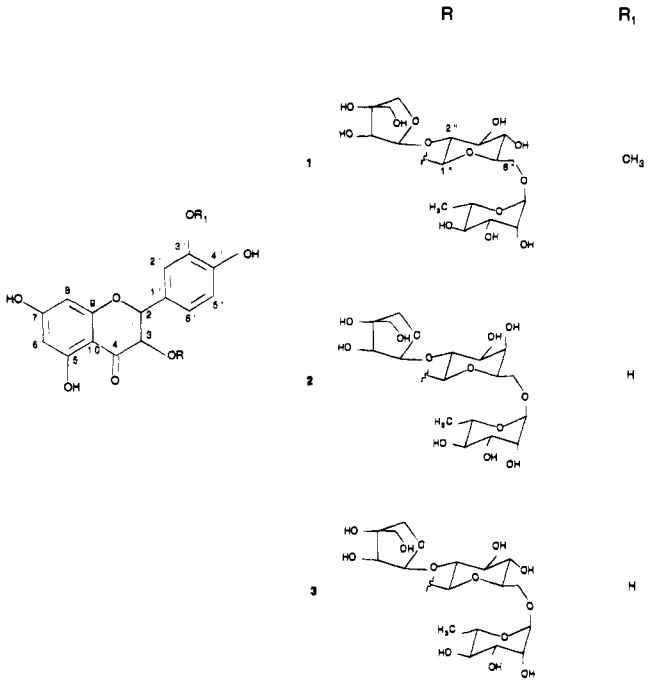
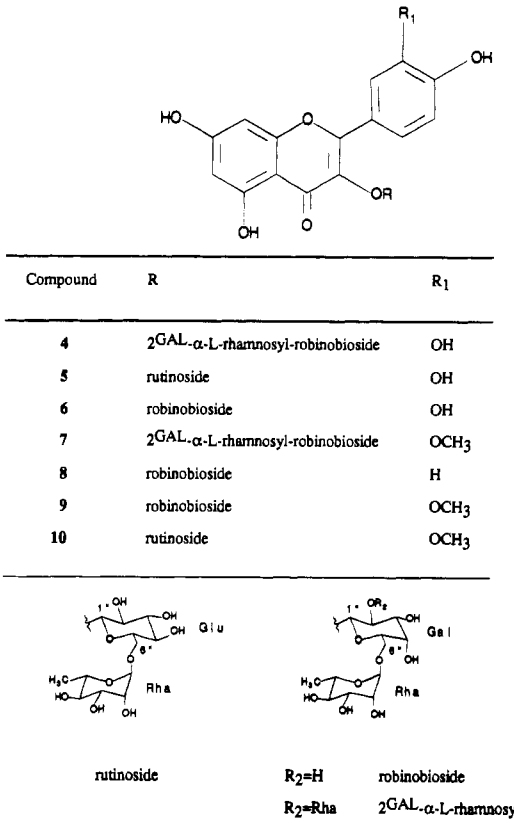


Figure 1. Structure of flavonol apiosides isolated from *C. pallidicaule* seeds: (**1**) isorhamnetin 3-*O*-β-D-apiofuranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside; (**2**) quercetin 3-*O*-β-D-apiofuranosyl(1→2)-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-galactopyranoside; (**3**) quercetin 3-*O*-β-D-apiofuranosyl(1→2)-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside.



Compound	R	R_1
4	2^{GAL}-α-L-rhamnosyl-robinobioside	OH
5	rutinoside	OH
6	robinobioside	OH
7	2^{GAL}-α-L-rhamnosyl-robinobioside	OCH_3
8	robinobioside	H
9	robinobioside	OCH_3
10	rutinoside	OCH_3

Figure 2. Flavonol glycosides **4**–**10** isolated from *C. pallidicaule* seeds.

NMR shifts of the aglycon part of **2** corresponded well with the shifts for quercetin, the only significant difference being an upfield shift of 1.6 ppm for the C-3. This shift is analogous to that reported when the 3-hydroxy group is glycosylated in a flavonol glycoside (Markham et al., 1976). The ^{13}C NMR also showed that **2** had galactose, rhamnose, and apiose in its structure (Table 2). The substitution pattern on galactose was deduced by comparison of the ^{13}C NMR chemical shifts with the data for the quercetin 3-*O*-α-rhamnosyl(1→2)-β-galactoside and quercetin 3-*O*-β-glucosyl(1→6)-β-galactoside (Nawwar et al., 1989). The downfield shifts of 6.7 ppm for C-2 and of 7.1 ppm for C-6 indicated a glycosidic linkage at C-2 and C-6. The (1→2) and (1→6) attachment on the galactose of the apiosyl and rhamnosyl units respectively, was elucidated by decoupling experiments and by two-dimensional nuclear Overhauser effect (NOESY) spectroscopy. Diagnostic NOE correlation was observed between Gal-2 (δ 3.94, H-2 gal) and A-1 (δ 5.45, H-1 api) and led to the formulation of **2** as quercetin 3-*O*-β-D-apiofuranosyl(1→2)-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-galactopyranoside (Figure 1).

Compound **3** showed in the FAB mass spectrum an $[M - H]^-$ ion at *m/z* 741. The mass and ^{13}C and ^{13}C NMR DEPT data suggested a molecular formula of $C_{32}H_{38}O_{20}$. The signals in ^1H NMR, ^{13}C, and ^{13}C DEPT NMR were superimposable on those of **1** except for the aglycon moiety identified as quercetin (see Experimental Procedures). These data suggest that compound **3** is identical with quercetin 3-*O*-β-D-apiofuranosyl(1→2)-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside reported from *Solanum glaucophyllum* (Rappaport et al., 1977) without NMR data (Figure 1). Thus, we report the NMR data here for compound **3**.

The known compounds **4**–**10** (Figure 2) showed chromatographic, UV absorption, and hydrolytic data identical with those of quercetin 3-(2^{GAL}-α-L-rhamnosyl)-robinobioside (**4**), 3-rutinoside (**5**), 3-robinobioside (**6**), isorhamnetin 3-(2^{GAL}-α-L-rhamnosyl)robinobioside (**7**), 3-robinobioside (**9**), 3-rutinoside (**10**), and kaempferol 3-robinobioside (**8**), respectively. These structures were confirmed by FABMS and ^1H and ^{13}C NMR spectral analysis (see Experimental Procedures).

In the ^1H NMR spectra of **4** the 3H doublets at δ 0.98 and 1.20 (J = 6 Hz) indicated two rhamnosyl methyl groups, and these data clearly showed that **4** has 2 mol of rhamnose. The substitution pattern on galactose was deduced by comparison of the carbon chemical shifts observed in **4** with those of the corresponding methyl pyranosides and taking into account the known effects of glycosidation (Breitmaier et al., 1987). Thus, the observed carbon resonances of C-2 (77.3 ppm) and C-6 (66.8 ppm) of galactose were consistent with a glycosidic linkage at these positions. These observations led to the formulation of the sugar moiety of **4** as 2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranoside; the nature of sugars was confirmed by acid methanolysis. The same trisaccharide was found in compound **7**, but in this case the aglycon was identified as isorhamnetin (see Experimental Procedures).

The ^1H NMR spectrum of **5** gave a 3H doublet at δ 1.16 (J = 6 Hz) typical for the rhamnosyl methyl group and two anomeric proton signals at δ 4.58 (d, J = 1.5 Hz), assignable to the H-1 α-rhamnosyl proton, and δ 5.14 (d, J = 7.5 Hz), assignable to the H-1 β-glucosyl proton. In the ^{13}C NMR of **5**, the C-6 signal (δ 68.5) of glucose showed a downfield shift of 7.2 ppm in comparison with the corresponding C-6 signal (δ 61.3) of quercetin 3-glucoside (Markham et al., 1982), thus indicating a 1→6 linkage between the C_3-glucose and the rhamnose. The same disaccharide was found in compound **10**, but in this case the aglycon was identified as isorhamnetin (see Experimental Procedures).

Compound **6** was identified as quercetin 3-robinobioside by acid methanolysis, UV spectral analysis, and ^{1}H and ^{13}C NMR. The ^{13}C NMR signal of C-6 of the galactopyranosyl group appeared at δ 67.6 as expected for a 6-*O*-substituted galactose; these spectral data indicated a 1→6 linkage between the galactose and the terminal rhamnose.

The signals in ^{1}H and ^{13}C NMR spectra of compounds **8** and **9** were superimposable on those of **6** except for the aglycon moieties identified as kaempferol and isorhamnetin, respectively (see Experimental Procedures).

C. pallidicaule presents a rich flavonol glycosides fraction that includes these 10 compounds. They contain the aglycons of quercetin, isorhamnetin, and kaempferol and oligosaccharide moieties as disaccharides and trisaccharides linked at the C-3 position. The quantitative content (1.33 g/kg) and the structural variability appear to be very interesting for the alimentary and taxonomic properties ascribed to flavonol glycosides. Moreover, flavonol apiosides **1** and **2** are new natural products; the novelty, as far as we know, resides in the not yet described combination of known aglycons with sugar moieties, rarely reported for natural products. Apiose appears to occur frequently in glycosides of the Chenopodiaceae, as it has also been found in flavonol glycosides of *Chenopodium quinoa* seeds (De Simone et al., 1990).

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