ISORHAMNETIN 3-O-ROBINOBIOSIDE FROM *GOMPHRENA MARTIANA*

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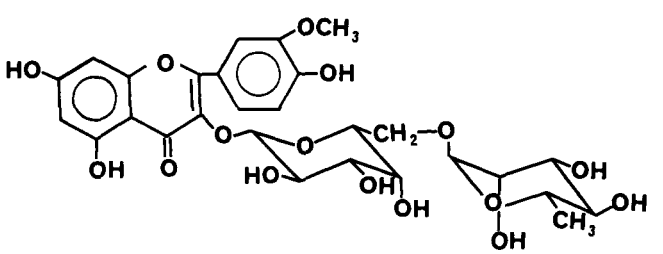
ABSTRACT.—Isorhamnetin 3-O-robinobioside (1), choline and betaine were isolated from whole plants of *Gomphrena martiana* and identified by spectral data.

Structural assignment of the disaccharide as robinobiose was mainly based upon ^{13}C-nmr and mass spectra of its trimethylsilyl derivative.

Gomphrena martiana Moquin is a medicinal plant of the Amaranthaceae family indigenous to the northwestern region of Argentine. We have recently reported (1, 2, 3) on the identification of flavones (3,5-dimethoxy-6,7-methylenedioxyflavone; 3,6-dimethoxy-5,7-dihydroxyflavone; 3,5,6,7-tetramethoxyflavone; 5,6-dimethoxy-7-hydroxyflavone; 3,5,7-trimethoxyflavone and 6-methoxy-5,7-dihydroxyflavone) isolated from its petroleum ether extract. Betacyanins (4, 5) and 3,5,4'-trihydroxy-6,7-methylenedioxyflavone (6) have been previously identified by other authors in *G. globosa*.

In continuation with our studies on this species, we now report the isolation and characterization of a flavonol glycoside, choline and betaine from the ethanolic extract.

The flavonoid showed to be isorhamnetin 3-*O*-rhamnogalactoside. A detailed spectroscopic analysis was necessary to identify the disaccharide as robinobiose. Rhamnogalactosides of isorhamnetin have been previously described (7–11) without establishing with certainty the nature of the biose. Ring size, configuration of the anomeric carbons, sequence of both sugars and interglycosidic linkage are now firmly determined. ^{13}C-nmr and ms of its trimethylsilyl derivative are, as far as we know, reported for the first time.



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RESULTS AND DISCUSSION

The defatted ethanolic extract was concentrated and percolated over polyamide with chloroform (Fraction A), water (Fraction B) and methanol (Fraction C), respectively. Fraction A was shown to be rich in flavone aglycones (1, 2, 3).

Fraction B was treated with Reinecke salt and worked up as described in the experimental section to yield choline and betaine. Comparison with authentic samples confirmed their structural assignments.

Chromatography of Fraction C on a Sephadex LH-20 column provided a flavonoid glycoside. Its uv spectrum indicated the presence of an isorhamnetin 3-*O*-glycoside. Upon acid hydrolysis, isorhamnetin, D-galactose and L-rhamnose were obtained.

Since 3-*O*-rhamnogalactoside of isorhamnetin had been previously described

(7–11), a careful analysis of its spectra was necessary to clarify the nature of the interglycosidic linkage.

The ^{1}H-nmr spectrum of its trimethylsilyl ether (TMSi) in $CDCl_3$ showed signals at δ 1.18 and 4.37 assigned to H–6 and H–1 of rhamnose, respectively. A signal at δ 5.71 (d, $J=7$ Hz) was ascribed to H–1 of galactose. The position of the latter proton as well as those of ring B of the aglycone revealed that galactose had to be directly attached to C–3 of the aglycone (7). Furthermore, a coupling constant of 7 Hz indicated a diaxial coupling of H–1" with H–2", only possible if galactose is β-linked to the aglycone. The fact that H–1'" showed a di-equatorial coupling ($J=3$ Hz) confirmed an α-linkage between the two sugars.

Fragments at *m/e* 362 (T–H) and 273 (T–TMSiOH) in the ms of its TMSi derivative further confirmed rhamnose as terminal sugar (12) (T: see footnote 1).

In order to establish the linkage between the two sugar moieties, the ^{13}C-nmr was measured. Assignments of the carbon signals of isorhamnetin were based on those previously reported for isorhamnetin 3-*O*-glycosides (13). The spectrum of the natural glycoside evidenced a 3-*O*-glycosylation and that galactose is directly attached to the aglycone since an upfield shift of C–3 is expected on 3-*O*-rhamnosylation different from that observed for 3-*O*-glycosylation (13).

Carbon sugar signals were assigned by comparison with those reported for robinin (14). ^{13}C-nmr spectrum of **1** in respect to that of isorhamnetin 3-β-D-galactoside (13) showed a remarkable down-field shift (+5.2 ppm) of C–6" and a slight upfield one of C–5" (−2.2 ppm). This effect is characteristic of glycosylation on C–6 of galactose. Therefore, C–1 of rhamnose (C–1'") had to be linked to C–6 of galactose (C–6").

Furthermore, galactose was β-linked, rhamnose α-linked, and both were in the pyranose form (15).

Therefore, the flavonol glycoside is fully identified as isorhamnetin 3-*O*-[β-D-galactopyranosyl-(1→6)-*O*-α-L-rhamnopyranoside], (isorhamnetin 3-*O*-robinobioside (**1**).

EXPERIMENTAL[1]

PLANT MATERIAL.—*G. martiana* was collected in the Province of Salta (Argentine) and a voucher specimen is on deposit at CEFAPRIN (Nr. CP 457).

ISOLATION PROCEDURE.—Dried ground whole plants (2.2 kg) were defatted with petroleum ether in a Soxhlet (2 days) and then extracted with ethanol to exhaustion. The ethanolic extract was evaporated on polyamide (Woelm, 70 g), and the residue was successively percolated with chloroform (1 liter) and water (700 ml) until a negative Dragendorff reaction occurred, and methanol (500 ml). Organic solvents were removed *in vacuo* and aqueous eluates lyophilized to give Fraction A (chloroform, 9.9 g), Fraction B (water, 13.8 g) and Fraction C (methanol, 1.1 g).

A portion (7 g) of Fraction B was dissolved in water and basified to pH 12 with potassium carbonate. The basic solution was treated with an excess of Reinecke salt until precipitation ceased. The crude complex was filtered by suction, washed with water, dissolved in acetone, and passed over an anion exchange resin column (Amberlite IRA–400 [HO$^-$] packed in methanol. The eluate, when evaporated, afforded 60 mg of free bases that were chromatographed over a column of neutral alumina (Woelm, 6 g) in chloroform-methanol-acetic acid (100:10:1). The main component was identified by spectral data as choline (26 mg) and confirmed by comparison with a standard.

The alkaline solution remaining after precipitation of the Reinecke complex at pH 12 was acidified to pH 1 with conc HCl. Treatment of the acidic solution with Reinecke reagent gave a precipitate that was dissolved in acetone and chromatographed over a silica gel H column. Elution with methanol-ammonium hydroxide solution (10:0.5) afforded betaine (180 mg) identical to an authentic sample by direct comparison.

A portion of Fraction C (517 mg) was chromatographed over a Sephadex LH–20 column (33 g, 20 x 400 mm). Elution with methanol yielded four main fractions. The second one (207 g; positive Shinoda test) was purified by chromatography over a silica gel H column (21 g, 20 x 180 mm) in ethyl acetate-methanol-water (15:1.5:1) to yield isorhamnetin 3-*O*-robinobioside (**1**) (67 mg), mp 178–181° (MeOH-Bc); uv λmax (MeOH): 255, 267.5, 356 nm; MeOH+$AlCl_3$: 269, 304, 360 (sh), 400; +$AlCl_3$/HCl: 269, 304, 360 (sh), 400; +NaOMe: 273, 327 (sh), 412;

[1]Mass spectra were recorded with a Hewlett Packard 5995 A, ^{1}H-nmr spectra with a Varian XL–100 apparatus (TMS as internal reference), and ^{13}C-nmr with a Varian FT–80.

A, T, and OS correspond to the ms fragments described in reference 12.

+NaOAc: 274.5, 317, 375; +NaOAc/H_3BO_3: 255, 267.5, 356; ^{1}H-nmr (TMSi derivative, 100 MHz, $CDCl_3$): δ 1.18 (m, 3H, Me-6'''), 3.10-3.80 (complex signal, 10H, sugar protons), 3.88 (s, 3H, OMe-3'), 4.37 (d, 1H, J=3 Hz, H-1'''), 5.71 (d, 1H, J_{aa}=7 Hz, H-1"), 6.19 (d, 1H, J=2 Hz, H-6); 6.48 (d, 1H, J=2 Hz, H-8), 6.84 (d, 1H, J_o=8 Hz, H-5'), 7.47 (dd, 1H, J_m=2 Hz, J_o=8 Hz, H-6'), 7.76 (d, 1H, J_m=2 Hz, H-2'); ^{13}C-nmr (20 MHz, DMSO-d_6): 17.8 (C-6'''), 56.0 (OMe-3'), 65.6 (C-6"), 68.2 (C-5''' and C-4"), 70.4 (C-2''')[a], 70.7 (C-3''')[a], 71.2 (C-2"), 72.0 (C-4'''), 73.1 (C-3")[b], 73.7 (C-5")[b], 93.8 (C-8), 98.8 (C-6), 100.2 (C-1'''), 101.9 (C-1"), 104.1 (C-10), 113.6 (C-2'), 115.2 (C-5'), 121.1 (C-1'), 122.1 (C-6'), 133.2 (C-3), 147.0 (C-4'), 149.4 (C-3'), 156.5 (C-2 and C-9), 161.2 (C-5), 164.1 (C-7), 177.4 (C-4). (Assignments bearing the same superscript may be interchanged); ms (TMSi derivative, 70 eV): *m/e* (rel int, %) 742 (OS+H, 1.9), 741 (OS, 3.2), 740 (OS-H, 4.6), 590 (A+TMSi-Me+H, 14.0), 589 (A+TMSi-Me, 26.1), 532 (A+H, 47.2), 517 (A+H-Me, 100.0), 460 (532-TMSi+H, 16.5), 362 (T-H, 20.9), 273 (T-TMSiOH, 5.9), 217 (15.2), 204 (10.9), 147 (13.2), 73 (90.7).

HYDROLYSIS OF THE FLAVONOID GLYCOSIDE.—A solution of the glycoside (4 mg) in 1 ml o 10% aq HCl was heated in a sealed tube at 100° for 1 hr and, on cooling, the aglycone crystallized. Its spectral data were the same as those of isorhamnetin (16). The hydrolysis filtrate was evaporated to dryness and the residue examined by tlc on cellulose. Rf values of the sugars were coincident with those of standards of D-galactose and L-rhamnose.

TRIMETHYLSILYLATION OF THE FLAVONOID.—It was carried out with HMDS and TMCS (1:1) in pyridine in the usual manner (16).

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