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TWO NEW KAEMPFEROL ISORHAMNINOSIDES FROM *VIGNA LUTEOLA*

ALICIA B. POMILIO,*[1]

Departamento de Química Orgánica

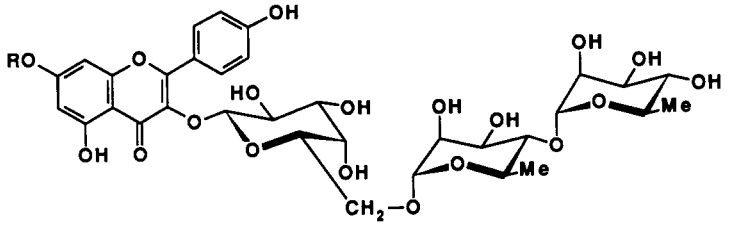
and ENRIQUE M. ZALLOCCHI

Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

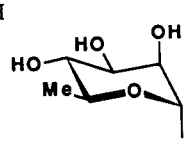
ABSTRACT.—From aerial parts of *Vigna luteola* two new flavonol glycosides were isolated and identified as kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*]-β-D-galactopyranoside [**1**] and kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*]-β-D-galactopyranoside 7-*O*-α-L-rhamnopyranoside [**2**].

Vigna luteola (Jacq.) Benth. (tribe Phaseoleae; subfamily Papilionoideae; family Leguminosae; common name "porotillo") is a climbing annual plant that grows in swampy flooding lands of northeastern Argentina up to Buenos Aires province but also spreads to North America. Its tender cooked seeds are edible but are too small to be profitable as an economic crop. Because of its abundance, *V. luteola* is considered a weed for crops, especially rice (*Oryza sativa*). However, it has a fodder value and is popularly known as "livestock fattening" (1). We describe here the isolation and identification of two novel flavonoid glycosides: kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*]-β-D-galactopyranoside [**1**] (kaempferol 3-*O*-β-D-isorhamninoside) and kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*]-β-D-galactopyranoside 7-*O*-α-L-rhamnopyranoside [**2**] (kaempferol 3-*O*-β-D-isorhamninoside 7-*O*-α-rhamnoside) from aerial parts of *V. luteola*.

This is the first report of these two kaempferol glycosides. The linear trisaccharide located at position 3 of kaempferol in both glycosides was called isorhamninose according to Riess-Maurer *et al.* (2). This trisaccharide was earlier reported as the component of the flavone glycoside xanthorhamnin A isolated from *Rhamnus petiolaris* (Rham-



1 R=H

2 R= 

[1]Research Member of the National Research Council of Argentina (CONICET).

naceae) (3). However, the synthesis (2,4) of isorhamninose and related trisaccharides and the comparison of these synthetic sugars with those of the natural glycosides showed that the trisaccharide of xanthorhamnins B and C and catharticin was not isorhamninose but rhamninose (third rhamnose moiety linked 1→3 instead of 1→4). Doubts remained on the interglycosidic bond of the third rhamnose moiety in xanthorhamnin A.

Flavonoids of four asiatic *Vigna* species have been previously described (5). However, no reports on American *Vigna* species are known. This is also the first report on the chemical components of *Vigna luteola*.

RESULTS AND DISCUSSION

The polar compounds of the MeOH extract of *V. luteola* were carefully separated and purified by repeated chromatography, yielding glycosides **1** and **2**. Uv spectra of both compounds (see Experimental) revealed the presence of two flavonol glycosides with 4′-OH [addition of NaOMe caused a bathochromic shift of Band I ($\Delta\lambda = +48$ nm) without decreasing maxima intensities]; presence of 5-OH (formation of $AlCl_3$ acid stable complexes); absence of ortho-dihydroxyls in the A and B rings (on addition of NaOAc/H_3BO_3 no shift of Band I was observed). Only **1** showed a bathochromic shift of Band II with NaOAc ($\Delta\lambda = +6$ nm) indicating a free 7-OH. These results indicated that the sugars were attached to the aglycone at C-3 in **1** and at C-3 and C-7 in **2**.

Upon acid hydrolysis both glycosides yielded the same aglycone and sugars identified as kaempferol (co-hptlc with an authentic sample), and galactose and rhamnose (co-hptlc with authentic samples), respectively. The sugars were present in a molar ratio of 1:2 in **1** and 1:3 in **2** as estimated by glc after conversion into their alditol acetates.

Both glycosides **1** and **2** decomposed in DMSO yielding kaempferol 3-*O*-β-robinobioside and kaempferol 3-*O*-β-robinobioside 7-*O*-α-rhamnoside (robinin), respectively. Robinobiose is 6-*O*-α-L-rhamnopyranosyl-D-galactopyranose. Decomposition products were characterized by co-tlc and hplc with standards.

These results indicate that galactose is linked by its hemiacetalic group to the aglycone at C-3, while the other two rhamnose moieties must be bound to this galactose in **1** and **2**, and in **2** a third rhamnose unit is linked to kaempferol at C-7.

^{1}H nmr of the glycosides led to the sugar configuration and the sequence bond of galactose to the aglycone. The ^{1}H-nmr spectra of **1** and **2** showed the same signals for the aglycone: two doublets centered at δ 6.86 and δ 8.08 with a coupling constant of $J = 8$ Hz were assigned to a para substitution in ring B, the upfield doublet (δ 6.86) to H-3′ and H-5′, and the other doublet to H-2′ and H-6′. Two additional doublets at δ 6.42 and δ 6.78 with a meta coupling (2 Hz) were assigned to H-6 and H-8, respectively, supporting the 5,7-disubstitution in ring A. These signals were in agreement with those of kaempferol.

Three signals derived from anomeric protons were observed in the ^{1}H-nmr spectrum of **1**. The doublet at δ 5.16 with a diaxial coupling (7 Hz) between H-1″ and H-2″ was assigned to the anomeric proton of D-galactopyranose (H-1″) β-linked to the 3-OH of kaempferol. The other two signals at δ 4.40 and δ 4.90 with a diequatorial coupling constant of 2 Hz were assigned to the anomeric protons of the L-rhamnopyranose moieties (H-1‴ and H-1⁗) α-linked to galactose and to rhamnose, respectively. This was supported by the shifts of the carbon signals.

The pyranose sugar form and the interglycosidic bonds were determined by ^{13}C nmr. The presence of a signal at δ 131.7 in the ^{13}C-nmr spectrum of **1** agreed with the glycosylation at the C-3 hydroxyl in accordance with the expected upfield shift ($\Delta\delta \sim -4.0$ ppm) when compared with C-3 of kaempferol (135.6 ppm). The adjacent

positions, C-2 and C-4, were consequently downfield shifted ($\Delta\delta$ +8.6 ppm and +0.6 ppm, respectively; see Experimental). Obviously, the large ortho effect on C-2 is due to a 3-glycosylation (6,7).

Three anomeric carbon signals were observed at 101.1, 100.5, and 100.1 ppm. The remaining sugar signals were characteristic of pyranoses (8,9) and appeared in the range 79.5 to 18.3 ppm.

Upon comparison of the carbon signals of the galactose moiety with those of methyl β-D-galactopyranoside, an upfield shift ($\Delta\delta$ −3.8 ppm) of C-1 of the galactose (C-1″) was observed, indicating that this carbon is linked to the aglycone. It has been previously reported (10) that the anomeric carbons of arylglycosides are more shielded ($\sim$ −2 ppm) than those of methyl glycosides. On the contrary, C-6″ was downfield shifted ($\Delta\delta$ +4.1 ppm) suggesting a 1↦6 linkage to the rhamnose. This deshielding due to an interglycosidic linkage has been previously reported (11). Simultaneously, C-5″ was shielded (−2.8 ppm).

The chemical shift values of this galactorhamnoside were in agreement with those of a robinobiose moiety (12), except by a downfield shift of C-4 of rhamnose (C-4‴). This fact suggested that the terminal rhamnose was attached at C-4 of the middle rhamnose unity (C-4‴).

The downfield shift value of C-4‴ was $\Delta\delta$ +6.4 ppm in relation to that of C-4 of methyl α-L-rhamnopyranoside (73.1 ppm). This signal at δ 79.5 could be well distinguished from the other sugar carbons. Moreover, the carbon signals of the middle rhamnose moiety were in agreement with those reported by Riess-Maurer *et al.* (2). On the basis of these data, **1** was identified as kaempferol 3-*O*-[α]-L-rhamnopyranosyl-(1↦4)-*O*-α-L-rhamnopyranosyl-(1↦6)-*O*]-β-D-galactopyranoside (kaempferol 3-*O*-β-D-isorhamninoside).

Four signals of anomeric protons were observed in the ^{1}H-nmr spectrum of **2**. The signal at δ 5.34 was assigned to the H-1 of galactopyranose (H-1″) attached to the aglycone, and the diaxial coupling (J = 7 Hz) between H-1″ and H-2″ indicated the β configuration. Similarly, the signals at δ 4.56 (J = 2 Hz) and δ 5.54 (J = 2 Hz) were assigned to H-1 of the rhamnopyranoses (H-1‴ and H-1⁗) (α configuration). A fourth signal at δ 5.18 (J = 2 Hz) was due to the rhamnose α-linked to C-7 of kaempferol.

The ^{13}C-nmr spectrum of **2** showed that all sugars were in the pyranose form and that kaempferol was glycosylated at C-3 and C-7. Thus, an upfield shift of C-3 ($\Delta\delta$ −4.4 ppm) was observed in comparison with that of kaempferol (C-3 135.6 ppm), indicating that a sugar different from rhamnose was attached at this carbon. Also C-2 ($\Delta\delta$ +9.1 ppm = ortho effect) and C-4 ($\Delta\delta$ +0.6 ppm) were affected in agreement with reported shifts in other kaempferol 3-glycosides (7). An upfield shift of C-7 ($\Delta\delta$ −2.4 ppm) was of diagnostic value for a 7-rhamnoside. Shifts in ortho and para carbon signals (C-6, C-8, and C-10) were also observed; the para effect on C-10 was of +0.4 ppm. B-ring carbons, C-1′ (−0.9 ppm), C-2′, and C-6′ (+0.7 ppm), were also shifted.

Four anomeric carbon signals were observed at δ 101.0, 100.5, 100.1, and 99.5. The signal most upfield shifted (99.5 ppm) was assigned to C-1 of the rhamnose attached at C-7. The signals of C-1 and C-6 of galactose (C-1″ and C-6″) differed from those of methyl β-*O*-D-galactopyranoside because of the presence of the aglycone instead of the methyl group ($\Delta\delta$ C-1″ −3.9 ppm) and of the attachment of the rhamnose at C-6″ ($\Delta\delta$ +4.1 ppm).

Finally, C-4‴ is downfield shifted (+6.4 ppm) indicating that the terminal rhamnose moiety is linked to this carbon as mentioned for compound **1**. Compound **2** is therefore identified as kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1↦4)-*O*-α-L-rhamnopyranosyl-(1↦6)-*O*]-β-D-galactopyranoside 7-*O*-α-L-rhamnopyranoside (kaempferol

3-*O*-β-D-isorhamninoside 7-*O*-α-L-rhamnoside). The fact that **2** was decomposed in DMSO yielding robinin supported part of the proposed structure.

EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *V. luteola* were collected in Ceibas, Departamento de Gualeguaychú, Province of Entre Ríos, Argentina. Voucher specimens were deposited under Nr. RAP 1378 (BAFC).

EXTRACTION.—Dried ground plants (2.3 kg) were defatted with petroleum ether (60–80°) and extracted with MeOH in a Soxhlet. The MeOH extract (358.3 g, 15.6% dry wt) was further percolated on polyamide with CH_2Cl_2, H_2O, and finally MeOH. The CH_2Cl_2 (120 g), aqueous (210 g), and MeOH (18 g) percolates were obtained.

ISOLATION OF THE FLAVONOIDS.—The aqueous percolate was chromatographed on a Si gel H column using gradients of CH_2Cl_2-MeOH (4:1, 39:11, 3:1, 2:1, 0:100). Three main groups of flavonoids were obtained. Chromatography on Sephadex LH-20 (MeOH, approx. 0.3 ml/min) led to separation of mono-, di-, and triglycosides. The latter were further chromatographed on a Si gel H column yielding compounds **1** and **2**. Each was purified by preparative hplc: μBondapak RP-C18, i.d. 7.8 mm, length 30 cm, uv detector 291 nm, MeOH-H_2O (55:45), 1.5 ml/min, Rt **1** 10 min 50 sec, Rt **2** 7 min 36 sec.

COMPOUND **1**.—λ max MeOH nm 243 (sh), 266, 352; +NaOMe 244 (sh), 269, 400; +NaOAc 272, 301 (sh), 353; +NaOAc/H_3BO_3 272, 301, 353; +$AlCl_3$ 262, 354, 400; +$AlCl_3$/HCl 262, 354, 400; ^{1}H nmr (100 MHz, MeOH-d_4) δ 1.10 (6H, m, H-6 rham = H-6‴ and H-6⁗); 3.00–3.90 (m, other sugar protons); 4.40 (1H, d, J_{ee} = 2 Hz, H-1 rham = H-1‴); 4.90 (1H, d, J_e = 2 Hz, H-1 rham = H-1⁗); 5.16 (1H, d, J_{aa} = 7 Hz, H-1 gal = H-1″), 6.42 (1H, d, J_m = 2 Hz; H-6); 6.78 (1H, d, J_m = 2 Hz, H-8); 6.86 (2H, d, J = 8 Hz, H-3′ and H-5′); 8.08 (2H, d, J = 8 Hz, H-2′ and H-6′); ^{13}C nmr (25.2 MHz, DMSO-d_6) ppm 176.5 (C-4), 163.4 (C-7), 160.3 (C-5), 159.1 (C-4′), 156.2 (C-9), 155.4 (C-2), 131.7 (C-3), 130.2 (C-2′), 120.6 (C-1′), 115.4 (C-3′ and C-5′), 103.3 (C-10), 101.1 (C-1″), 100.5 (C-1⁗), 100.1 (C-1‴), 98.4 (C-6), 92.9 (C-8), 79.5 (C-4‴), 73.4[a] (C-5″), 73.2[a] (C-3″), 72.3 (C-4⁗), 71.5 (C-2″), 71.1[b] (C-3‴), 70.8[b] (C-3⁗), 70.7[b] (C-2⁗), 70.1 (C-2‴), 68.9[c] (C-5⁗), 68.6[c] (C-4″), 68.3 (C-5‴), 66.2 (C-6″), 18.6 (C-6‴), 18.3 (C-6⁗). Signals with the same superscript may be interchanged. (TMS as internal standard).

COMPOUND **2**.—λ max MeOH nm 257 (sh), 266, 352; +NaOMe 244 (sh), 269, 400; +$AlCl_3$ 262, 354, 400; +$AlCl_3$/HCl 262, 354, 400; +NaOAc 266, 352; +NaOAc/H_3BO_3 266, 352; ^{1}H nmr (100 MHz, MeOH-d_4) δ 1.04 (3H, d, J = 7 Hz, H-6 rham), 1.17 (3H, d, J = 7 Hz, H-6 rham); 3.00–3.90 (m, other sugar protons), 4.56 (1H, d, J_{ee} = 2 Hz, H-1 rham = H-1‴); 5.18 (1H, d, J_e = 2 Hz, H-1 rham = H-1 R7); 5.34 (1H, d, J_{aa} = 7 Hz, H-1 gal = H-1″), 5.54 (1H, d, J_{ee} = 2 Hz, H-1 rham = H-1⁗); 6.43 (1H, d, J_m = 2 Hz, H-6); 6.78 (1H, d, J_m = 2 Hz, H-8); 6.85 (2H, d, J = 8 Hz, H-3′ and H-5′); 8.08 (2H, d, J = 8 Hz, H-2′ and H-6′); ^{13}C nmr (25.2 MHz, DMSO-d_6) ppm 176.5 (C-4), 161.5 (C-7), 160.7 (C-5), 159.1 (C-4′), 157.3 (C-9), 155.9 (C-2), 131.2 (C-3), 130.2 (C-2′ and C-6′), 120.8 (C-1′), 115.5 (C-3′ and C-5′), 103.5 (C-10), 101.0 (C-1″), 100.5 (C-1⁗), 100.1 (C-1‴), 99.5 (C-1 R7), 98.4 (C-6), 93.2 (C-8), 79.5 (C-4‴), 73.4 (C-5″), 73.2 (C-3″), 72.3 (C-4⁗), 71.9 (C-4 R7), 71.5 (C-2″), 71.1[a] (C-3‴), 71.0[a] (C-3 R7), 70.8[a] (C-3⁗), 70.7[a] (C-2⁗), 70.5[b] (C-2 R7), 70.2[b] (C-5 R7), 70.1[b] (C-2‴), 68.9[c] (C-5⁗), 68.6[c] (C-4″), 68.3[c] (C-5‴), 66.2 (C-6″), 18.6 (C-6‴); 18.3 (C-6⁗), 16.8 (C-6 R7). Signals with the same superscript may be interchanged. (R7 = rhamnose moiety attached at C-7; TMS as internal standard).

ACID HYDROLYSIS.—Compounds **1** and **2** were separately hydrolyzed with 10% aqueous HCl at 85° for 1 h. Sugars were chromatographed in hptlc (cellulose) using *n*-BuOH–pyridine–H_2O (6:4:3) (two runs); spots were visualized with (a) $AgNO_3$ in Me_2CO (b) 15% KOH in MeOH, (c) 5 min at 120°.

Compounds **1** and **2** each gave spots with R_f values coincident with those of standards of D-galactose and L-rhamnose. Acetate alditols were also prepared in the usual manner and analyzed by glc.

Aglycones were chromatographed in hptlc (Si gel). Only kaempferol was detected.

DECOMPOSITION IN DMSO.—Compounds **1** and **2** were dissolved in DMSO-d_6 for recording the ^{13}C-nmr spectra. The evaporation of the solvent under vacuum gave rise to bond cleavage yielding kaempferol 3-*O*-β-robinobioside from **1** and robinin from **2**.

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