Journal of Natural Products
Vol. 49, No. 3, pp. 528-529 May-Jun 1986

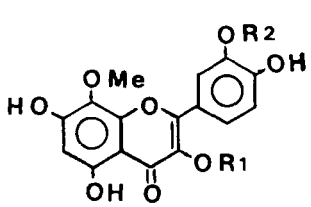
TWO NEW FLAVONOIDS FROM *PROSOPIDASTRUM GLOBOSUM*

A.M. AGNESE, C.A. CHIALE, J.L. CABRERA, and H.R. JULIANI*

Instituto Multidisciplinario de Biología Vegetal (CONICET) Cátedra de Farmacognosia, Facultad de Ciencias Químicas, Casilla de Correo 61, Sucursal 16, Córdoba 5016, República Argentina

Prosopidastrum globosum (Gill. ex Hook. et Arn.) Burk. (Leguminosae) is the only species of this genus that grows in Argentina, and it is known by the common names of "manca caballo" and "manca potrillo." The oil composition of its seeds has been studied (1).

From its aerial parts, two flavonoids were isolated that have not been previously cited in the literature. They were identified as gossypetin-8-methylether-3-β-*O*-robinobioside (**1**) and gossypetin-8,3′-dimethylether-3-β-*O*-galactoside (**2**) by the usual analytical methods; uv, ^{1}H nmr, eims, and acid hydrolysis, as well as enzymatic and chemical transformations. Compounds very closely related to **1** and **2** have been isolated from other genera of Leguminosae (2,3). Also identified was a dihydroflavonol already known: dihydrofisetin (fustin) (4).



1 R^1=robinobiose, R^2=H
2 R^1=galactose, R^2=Me

The EtOH-H_2O (50%) extract from *P. globosum,* previously concentrated and defatted, was fractionated through a Sephadex LH 20 column, yielding two fractions that produced the compounds **1** and **2**.

By means of hydrolysis, the aglycone of **1** and the sugars galactose and rhamnose were obtained and identified by pc and gc. The spectral properties in uv of the aglycone and shifts with the usual reagents revealed that it was a gossypetin derivative substituted at C-8 (5,6). By comparison with the spectral data of **1**, the glycosidic linkage through position 3 was concluded, and the other hydroxyl groups were free.

The eims of the aglycone indicated the presence of a -OCH_3 at C-8 determined by the signal for M-15 (100%) and the absence of peaks corresponding to M-18 and M-1 (7). The fragmentation pattern, mp and Rf of the aglycone in the indicated solvent systems were also in accordance with those of the gossypetin-8-methylether (corniculatusin) (6,8,9). The aromatic region of the ^{1}H-nmr spectrum showed a similar pattern of protons to those of corniculatusin (9). The beta configuration of the linkage between the disaccharide and the aglycone, as well as the structure of robinobioside, was determined by the ^{1}H-nmr signals of the C-1″ proton of galactose δ 5.67 (J=7 Hz) and the C-1‴ proton 4.34 (J=2 Hz) and C-6‴ (methyl group), 0.82 (J=6 Hz) of rhamnose. The robinobioside was confirmed by treatment of **1** with H_2O_2 compared chromatographically with an authentic sample of robinobiose which was obtained by the same procedure from robinin.

The structure of **2** was determined by the same procedures as for **1**. The acid hydrolysis yield galactose, identified by standard chromatographic procedures. The aglycone was identified by its uv, eims, mp, and Rf in several solvent systems (9,10). The ^{1}H nmr showed a signal at 5.67 (J=7 Hz) that corresponded with a beta linkage between galactose and the aglycone. The enzymatic hydrolysis with β-galactosidase was positive.

Dihydrofisetin, also isolated from *P. globosum,* was identified by usual uv and pc procedures. This dihydroflavonol was converted to fisetin by Pew's method.

EXPERIMENTAL

PLANT MATERIALS.—*P. globosum* was collected in Dpto. Luján (Mendoza-República Argentina) and was identified by Ing. Agr. Luis Del Vitto (IADIZA, CONICET). A voucher specimen was deposited in the Ruiz Leal Herbarium (Merl) as L.A. Del Vitto and J.L. Cabrera No 818.

EXTRACTION AND ISOLATION.—Dry aerial parts of *P. globosum* (500 g) were extracted with EtOH-H_2O (50%) for 48 h twice, concentrated to ¼ of the initial volume, filtered, and defatted with petrol. An aliquot was put on a Sephadex LH 20 column, equilibrated previously with H_2O, and eluted with EtOH-H_2O (25:75). Two fractions were isolated, containing **1** and **2**.

The Rf values by pc on Whatman 3 MM with H_2O as developing solvent were 0.15 for **2** and 0.29 for **1**. In order to achieve a pure product, both fractions were rechromatographed on Sephadex LH 20 column, developed with H_2O and EtOH-H_2O (1:1).

CORNICULATUSIN-3-β-*O*-ROBINOBIOSIDE (**1**).—Uv λ max (MeOH) nm 355, 270, 258; (NaOMe) 417, 349, 278; ($AlCl_3$) 431, 363, 304 sh, and 274; ($AlCl_3$+HCl) 407, 359, 304 sh, 274; (NaOAc) 403, 342, 280; (NaOAc+H_3BO_3) 377, 304 sh, 265; ^{1}H nmr (trimethylsilyl derivative) (80 MHz, $CDCl_3$) δ 0.82 (br. 3H, OCH_3 from rhamnose); multiplet between 3.5 to 3.8 (C-H sugars, 10H); 3.89 (s, 3H, OCH_3, C-8); 4.34 (d, 1H, J=2 Hz, C-1‴); 5.67 (d, 1H, J=7 Hz, C-1″); 6.23 (s, 1H, C-6); 6.87 (d, 1H, J=8 Hz, C-5′); 7.63 and 7.73 (br., 2H, C-2′ and C-6′).

AGLYCONE OF **1**.—eims *m/z* 332 (M^+), 317 (M-15) (100%), 303 (M-29), 289 (M-43), 167 (A-15), 139 (A-43), 137 (B), 109 (B-28); mp 273° [lit. (5) 273°]; uv and Rf in pc accordance with Harborne *et al.* (6).

HYDROLYSIS OF **1** (6% HCl).—The sugars rhamnose and galactose were identified by comparison with authentic samples by pc and gc. Robinobioside was obtained from **1** by treatment with H_2O_2 and was compared with the same structure obtained from robinin. Rf on Whatman 3 MM paper: HOAc 15%, 0.57; H_2O, 0.29; and *t*-BuOH-HOAc-H_2O (3:1:1), 0.73.

LIMOCITRIN-3-β-*O*-GALACTOSIDE (**2**).—Uv λ max (MeOH) nm 361, 274, 262; (NaOMe) 413, 342, 278; ($AlCl_3$) 411, 361, 310 sh, 278; ($AlCl_3$+HCl) idem; (NaOAc) 395, 338, 282; ^{1}H nmr (trimethylsilyl derivative) (80 MHz, $CDCl_3$) δ 3.4 to 4 (m, 6H, C-H galactose), 3.85 and 3.92 (s, 3H each one, O-CH_3, C-8 and C-3′), 5.67 (d, 1H, J=7 Hz, C-1″), the signals corresponding to the aromatic protons showed a similar pattern to that of **1**.

AGLYCONE OF **2**.—Eims *m/z* 346 (M^+), 331 (M-15) (100%), 303 (M-43), 151 (c), in accordance with Jay *et al.* (9), mp: 274° [lit. (11) 274-275°].

HYDROLYSIS OF **2**.—The aglycone and galactose were obtained by means of treatment with 6% HCl. The sugar was identified by gc. The enzymatic hydrolysis with beta galactosidase (Miles Lab. Inc.) was positive, identifying both components as in the above mentioned case.

DIHYDROFISETIN.—Isolated by Sephadex LH 20 column eluted with EtOH. The uv spectral properties and Rf in pc were coincident with literature data (11). By Pew's method (12) fisetin was obtained and identified by the usual procedures (11).

ACKNOWLEDGMENTS

The authors thank Ing. Agr. Luis Del Vitto (IADIZA, CONICET) for the botanical advice, Dr. M. González Sierra (IQUIOS, CONICET) for nmr spectra and Mr. Mario Carvajal (Lab. de Espectrometría de Masas, Fac. de Ciencias Químicas) for ms. Part of this work was carried out with funds provided by CONICOR and CONICET.

LITERATURE CITED

1. C. Madriñan Polo, J.H. Hunziker y P. Cattaneo, *An. Asoc. Quím. Argent.*, **64**, 127 (1976).
2. J.B. Harborne, *Phytochemistry*, **20**, 1117 (1981).
3. J. Reynaud, M. Jay, and J. Raynaud, *Phytochemistry*, **21**, 2604 (1982).
4. J.B. Harborne, T.J. Mabry, and H. Mabry, "The Flavonoids," Chapman and Hall, New York, 1975, p. 585.
5. J.B. Harborne and T.J. Mabry, "The Flavonoids Advances in Research," Chapman and Hall, New York, 1982, p. 241.
6. J.B. Harborne, N.A.M. Saleh, and D.M. Smith, *Phytochemistry*, **17**, 589 (1978).
7. M. Goudard, J. Favre-Bouvin, P. Lebreton, and J. Chopin, *Phytochemistry*, **17**, 145 (1978).
8. J.G. Nielsen, *Tetraedron Lett.*, **11**, 803 (1970).
9. M. Jay, A. Hasan, B. Voirin, and M.R. Viricel, *Phytochemistry*, **17**, 827 (1978).
10. R.M. Horowitz and B. Gentilli, *J. Org. Chem.*, **26**, 2899 (1961).
11. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970.
12. J.C. Pew, *J. Am. Chem. Soc.*, **70**, 3031 (1948).

Received 31 October 1985