A New Flavonol Diglycoside from Anthyllis onobrychioides

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JANUARY 1986

Impact Factor: 3.8 · DOI: 10.1021/np50043a022

4 AUTHORS, INCLUDING:

Óscar Barberá
University of Valencia
44 PUBLICATIONS **321** CITATIONS

Journal of Natural Products
Vol. 49, No. 1, pp. 151-152, Jan-Feb 1986

A NEW FLAVONOL DIGLYCOSIDE FROM *ANTHYLLIS ONOBRYCHIOIDES*

J.A. MARCO,* O. BARBERÁ, J.F. SANZ, and J. SÁNCHEZ-PARAREDA

Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad de Valencia, Burjasot, Valencia, Spain

Anthyllis onobrychioides Cav. (Leguminosae) is a dwarf shrub, very commonly found on sandy terrains and rock clefts in southeast Spain. Most papers on the genus *Anthyllis* are related to flavonoid content (1-3), and this genus has been the object of several pharmacological and biological studies (4); we recently reported (5) the isolation of a new flavonol monoglycoside, rhamnocitrin-3-*O*-β-D-galactopyranoside, from *A. onobrychioides*. In contrast to other aglycones, such as kaempferol or quercetin, rhamnocitrin (7-*O*-methylkaempferol) and its glycosides are rather uncommon in nature (6). We now wish to report the isolation of another new rhamnocitrin glycoside, rhamnocitrin-3-*O*-β-D-galactopyranoside-4′-*O*-β-D-glucopyranoside, from *A. onobrychioides*.

The new compound was isolated from an aqueous extract as described in the experimental section. Acid hydrolysis enabled the isolation of rhamnocitrin, identified by comparison with an authentic sample, D-glucose, and D-galactose, identified by gc (as silylated derivatives) and paper chromatography. On the other hand, oxidative degradation of the glycoside (7) enabled the identification of galactose, a fact which assures the placement of this sugar at the 3-position. The uv spectrum of the compound and its changes after addition of shift reagents (8) indicated the existence of a free OH group at C-5. Since galactose was located at the C-3 hydroxyl group, this left only the 4′-OH for binding to the glucose moiety. The ^{1}H-nmr spectrum (200 MHz, DMSO-d_6) supported this conclusion and revealed the stereochemistry at the two anomeric centers. Two doublets (J=9 Hz) at δ 8.16 and δ 7.16 corresponded to the protons H-2′/6′ and H-3′/5′, respectively, which form an AA′XX′ system. The AX system (J=2.2 Hz) at δ 6.69 and δ 6.36 arises from H-8 and H-6, respectively, while the two doublets at δ 5.36 (J=7.6 Hz) and δ 5.00 (J=7.3 Hz) are originated by the anomeric protons from the galactose and glucose moieties, respectively. The values of the coupling constants pointed to a *trans*-diaxial relationship with the neighbor protons and, hence, to a β-stereochemistry at the anomeric center in a pyranoside ring (8). The OMe group could be seen as a sharp singlet at δ 3.86 while the sugar non-anomeric protons formed a broad unresolved absorption at δ 3.2-3.8. The ^{13}C-nmr spectrum (50 MHz, DMSO-d_6) also supported this conclusion as it fit satisfactorily with the superimposition of the expected signals for the rhamnocitrin, glucose, and galactose fragments. Glycosylation at C-4′ is a not very usual feature, and only one case has been studied by ^{13}C-nmr spectroscopy (9). This produced a distinctive downfield shift of about 3-4 ppm in the signal of C-1′, which appeared at about δ 123-125. In our case, a signal at δ 123.65 could be attributed to C-1′. All other signals have been attributed according to literature data (9). Finally, the molecular weight, determined by fdms (10), was consistent with the proposed structure.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were measured with a Perkin-Elmer uv spectrophotometer model 575. ^{1}H- and ^{13}C-nmr spectra were measured on a Bruker AC-200 spectrometer at 200.1 and 50.3 MHz, respectively, in DMSO-d_6 solution at 60°, using the solvent signal as reference. Mass spectra were re-

corded on a Varian MAT-731 spectrometer with the field desorption technique, with addition of NaI as a cationizing agent (10,11).

EXTRACTION AND FRACTIONATION.—*A. onobrychioides* (4 kg of aerial parts) was collected, authenticated, and extracted as described elsewhere (5). The aqueous solution that remained after extraction of the main extract with Et_2O and EtOAc was concentrated and chromatographed through Sephadex G-50, using H_2O as eluent, to separate gross sugar contaminants. The obtained product still contained sugar impurities and was submitted to purification by paper chromatography (Macherey-Nagel MN 218, elution with *t*-BuOH-HOAc-H_2O (3:1:1). After extraction, the crude product was rechromatographed through Sephadex LH-20, using MeOH-H_2O (80:20) as eluent, and then further purified by hplc (μ-Bondapak C_{18} column, L=30 cm, Ø=7.8 mm, with 8% HOAc-MeOH, 1:1, as eluent) (12).

The compound crystallized from H_2O as pale yellow needles (40 mg, 0.001% of dry plant weight), mp 226-228°, uv max (MeOH) nm (log ε) 268 (4.3), 328 (3.6); (+NaOMe) 284, 318sh, 390; (+NaOAc) 253, 272, 283, 320sh, 360sh; (+NaOAc+H_3BO_3) 267, 330; (+$AlCl_3$) 276, 297sh, 345, 397, not altered by addition of HCl; ^1H nmr δ 8.16 (d, J=9 Hz, H-2′ and 6′), 7.16 (d, J=9 Hz, H-3′ and 5′), 6.69 (d, J=2.2 Hz, H-8), 6.36 (d, J=2.2 Hz, H-6), 5.36 (d, J=7.6 Hz, anomeric H of gal), 5.00 (d, J=7.3 Hz, anomeric H of gluc), 3.86 (s, 3H, OMe), 3.8-3.2 (m, 12H nonanomeric sugar protons); ^{13}C nmr δ 177.64 (C-4), 165.20 (C-7), 160.85 (C-5), 159.25 (C-4′), 156.30, 156.04 (C-2/C-9), 134.19 (C-3), 130.50 (C-2′ and 6′), 123.65 (C-1′), 115.92 (C-3′ and 5′), 105.04 (C-10), 101.93 (anomeric C of galactose), 100.29 (anomeric C of glucose), 97.79 (C-6), 92.26 (C-8), 76.96 (C-5 of glucose), 76.52 (C-3 of glucose), 75.70 (C-5 of galactose), 73.21 (C-3 of galactose and C-2 of glucose), 71.27 (C-2 of galactose), 69.84 (C-4 of glucose), 67.89 (C-4 of galactose), 60.80 (C-6 of glucose), 60.16 (C-6 of galactose), 55.92 (OMe); fdms *m/z* (rel. int.) 648 (28, M+1+Na^+), 647 (54, M+Na^+), 485 (100, M+Na^+-$C_6H_{10}O_5$), 463 (5, M+1-$C_6H_{10}O_5$), 323 (12, M+Na^+-$C_{12}H_{20}O_{10}$), 300 (6, M-$C_{12}H_{20}O_{10}$). Calcd for $C_{28}H_{32}O_{16}$: M=624.

LITERATURE CITED

1. J.F. Gonnet, *Phytochemistry*, **17**, 1319 (1978).
2. J.A. Marco, J. Sánchez-Parareda, E. Seoane, B. Abarca, and J.M. Sendra, *Phytochemistry*, **17**, 1438 (1978) and references 1 to 9 therein.
3. M. Jay, B. Voirin, A. Hasan, J.F. Gonnet, and M.R. Viricel, *Biochem. Syst. Ecol.*, **8**, 127 (1980).
4. D. Le Rudulier, G. Goas, and F. Larher, *Z. Pflanzenphysiol.*, **105**, 417 (1982).
5. J.A. Marco, O. Barberá, J.F. Sanz, and J. Sánchez-Parareda, *Phytochemistry*, **24**, 2471 (1985).
6. J.B. Harborne and T.J. Mabry, *The Flavonoids: Advances in Research*, Chapman & Hall, New York, 1982, p. 307.
7. B.V. Chandler and K.A. Harper, *Aust. J. Chem.*, **14**, 586 (1961).
8. T.J. Mabry, K.R. Markham, and M.B. Thomas, *The Systematic Identification of Flavonoids*, Springer, New York, 1970, Chapts. 4,8.
9. K.R. Markham, B. Ternai, R. Stanley, H. Geiger, and T.J. Mabry, *Tetrahedron*, **34**, 1384 (1978).
10. K.M. Biswas, M.E. Ali, A.H. Jackson, and D.E. Games, *J. Indian Chem. Soc.*, **55**, 1240 (1978).
11. G.W. Wood, *Tetrahedron*, **38**, 1125 (1982).
12. D.G.I. Kingston, *J. Nat. Prod.*, **42**, 237 (1979).

Received 7 June 1985