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FLAVONOL GLYCOSIDES FROM *ANTHYLLIS ONOBRYCHIOIDES*

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Key Word Index—*Anthyllis onobrychioides*; Leguminosae; structure elucidation; flavonoids; flavonol glycosides.

Abstract—A new flavonol glycoside, rhamnocitrin 3-*O*-β-D-galactopyranoside, has been isolated from aerial parts of *Anthyllis onobrychioides*, together with the known 3-*O*-β-D-galactopyranosides of quercetin, isorhamnetin and kaempferol.

INTRODUCTION

Anthyllis onobrychioides Cav. is a dwarf shrub, very commonly found on sandy terrains and rock clefts in south-east Spain. Most papers on the genus *Anthyllis* are related to its flavonoid content [1, 2] and many of them refer to the species *A. vulneraria*. A chemotaxonomic study of the tribe Loteae [3] also includes this genus. *A. vulneraria* has attracted the attention of many investigators because of its curative properties and has been the object of several pharmacological and biological studies [4]. Its flavonoid content is especially rich and includes various glycosides of quercetin, kaempferol, isorhamnetin and rhamnocitrin. In the present investigation of *A. onobrychioides* we now report a new flavonol glycoside, rhamnocitrin 3-galactoside and the corresponding quercetin-, kaempferol- and isorhamnetin 3-galactosides which are known already.

RESULTS AND DISCUSSION

The flavonoids were isolated as described in the Experimental by standard techniques [5]. Their identification was achieved using routine spectroscopic methods and, in the case of quercetin 3-*O*-galactoside, also by direct comparison with an authentic sample. The nature of the carbohydrate residue was ascertained by acid hydrolysis and GC of the silylated derivative [5, 6]. Two of the aglycones (quercetin and kaempferol) could also be compared with authentic samples. The β-stereochemistry at the anomeric centre was evident from the value of the coupling constant, $J_{1'',2''} = 7.6$ Hz [5, 6]. Finally, the ^{13}C NMR spectra of quercetin 3-galactoside (hyperin), isorhamnetin 3-galactoside (cacticin) and kaempferol 3-galactoside (trifolin) were identical with the published data [7].

The structure of rhamnocitrin 3-*O*-galactoside was evidenced from the following data: the UV spectrum and its changes, after addition of several shifts reagents [5, 6], pointed to the presence of free hydroxyl functions at C-5 and C-4′ on a 3-*O*-substituted flavonol framework. After acid hydrolysis and separation of sugar and aglycone, the latter showed typical flavonol UV absorptions [5, 6]. The ^{1}H NMR spectrum clearly displayed signals of a *p*-substituted B-ring and a 5,7-disubstituted A-ring, the 7-substituent being a methoxyl group [6]. Further support was provided by the ^{13}C NMR spectrum which fitted well with a superposition of the expected signals for a rhamnocitrin (kaempferol 7-methyl ether) and a galactose moiety [7]. The mass spectrum (field desorption technique) was consistent with the proposed structure as it showed a distinct peak at *m/z* 463 [M + 1]. Although numerous glycosides of quercetin, isorhamnetin and kaempferol are known, only six glycosides of rhamnocitrin have been described [8].

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were run at 200.1 and 50.3 MHz, respectively, in DMSO-d_6 soln, using the solvent signal as reference. FDMS were measured on a Varian MAT 731 apparatus, and required the addition of NaI as a cationizing agent [9, 10].

Plant material. Anthyllis onobrychioides was collected in May 1983 at La Cañada (Valencia) and authenticated by Professor J. Mansanet, of the Botany Department at the Faculty of Biology of this institute. A voucher specimen has been deposited in the herbarium of the above-mentioned department.

Extraction and chromatography. The aerial parts of the plant (4 kg) were air-dried, ground and extracted with MeOH (10 l.) at room temp. (10 days). The MeOH extract was concd to 2 l., diluted with H_2O (6 l.) and continuously extracted with Et_2O (2 days) and EtOAc (3 days). The Et_2O extract (7.5 g) showed the absence of any flavonoid aglycones. The EtOAc extract (5.4 g) was fractionated by CC on Polyamide Macherey–Nagel SC 6, 0.05–0.16 mm (elution with $CHCl_3$–MeOH–MeCOEt, 12:2:1). In this way, crude fractions containing rhamnocitrin 3-galactoside, trifolin, cacticin and hyperin were successively eluted. These fractions were further purified by PC (Macherey–Nagel MN-218) and column percolation through Sephadex LH-20, to give 50, 30, 80 and 100 mg, respectively, of the above-mentioned compounds.

Rhamnocitrin 3-galactoside. Crystallized from 80% aq. MeOH as a pale yellow powder, which melts without decomposition at 184–187°. For R_f-values, see Table 1. UV λ_{max}^{MeOH} nm: 266, 348; $\lambda_{max}^{MeOH+NaOMe}$ nm: 267, 400; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 274, 303, 354, 400; $\lambda_{max}^{AlCl_3+HCl}$ nm: 274, 303, 349, 398; $\lambda_{max}^{MeOH+NaOAc}$ nm: 266, 401;

Table 1. R_f-values for rhamnocitrin 3-galactoside

Solid phase	Solvent system	R_f (×100) Absolute	R_f (×100) Relative to cacticin
Silica gel (Merck)	EtOAc–MeCOEt–HCOOH–H_2O (5:3:3:1)	67	103
	$CHCl_3$–MeOH–H_2O (7:3:0.5)	63	124
Polyamide (Macherey–Nagel TLC-11)	$CHCl_3$–MeOH–MeCOEt–2,4-pentandione (20:10:5:1)	82	119
Cellulose	H_2O	10	53
(Merck)	PhOH–H_2O (4:1)	92	106
Paper	15% HOAc	43	107
(Macherey–Nagel 218)	*t*-BuOH–HOAc–H_2O (3:1:1)	78	104

$\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 266, 355. ^{1}H NMR (Me_2SO-d_6, 50°): δ3.2–3.8 (*m*, sugar H-2″–H-6″), 3.87 (3H, *s*, OMe), 5.42 (*d*, J = 7.6 Hz, anomeric H-1″), 6.37 (*d*, J = 2 Hz, H-6), 6.74 (*d*, J = 2 Hz, H-8), 6.86 (*d*, J = 8.9 Hz, H-3′, H-5′), 8.11 (*d*, J = 8.9 Hz, H-2′, H-6′), 12.60 (*br s*, OH-5). ^{13}C NMR (Me_2CO-d_6, 90°): δ55.94 (OMe), 60.09, 67.82, 71.20, 73.13, 75.70 (C-2″–C-6″, galactose moiety), 92.22 (C-8), 97.77 (C-6), 101.78 (anomeric C-1″), 104.95 (C-10), 115.03 (C-3′, C-5′), 120.72 (C-1′), 130.90 (C-2′, C-6′), 133.59 (C-3), 156.31, 156.72 (C-2/C-9), 160.11 (C-4′), 160.95 (C-5), 165.15 (C-7), 177.67 (C-4). FDMS (probe), m/z (rel. int.): 531 $[M+3Na]^+$ (25), 485 $[M+Na]^+$ (60), 463 $[M+1]^+$ (100), 369 $[M+3Na-C_6H_{10}O_5]^+$ (10), 300 $[M-C_6H_{10}O_5]^+$ (5).

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