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FLAVONOL TETRAGLYCOSIDES FROM THE LEAVES OF *PRUNUS CERASUS* AND *P. AVIUM*

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Key Word Index—*Prunus cerasus*; *P. avium*; Rosaceae; flavonol tetraglycosides; kaempferol- and quercetin 3-*O*-rutinosyl-4′-di-*O*-glucosides; quercetin 3-*O*-rutinosyl-7,3′-*O*-bisglucoside.

Abstract—Four new flavonol glycosides have been identified from fresh leaves and fruits of sweet and sour cherries (*Prunus avium* and *P. cerasus*) as minor flavonoids: quercetin 3-*O*-rutinosyl-7,3′-*O*-bisglucoside; two quercetin 3-*O*-rutinosyl-4′-di-*O*-glucosides; kaempferol 3-*O*-rutinosyl-4′-di-*O*-glucoside.

INTRODUCTION

A number of species of the genus *Prunus* (Rosaceae) have been examined for flavonoids. In both sweet and sour cherries the 3-glucosides, 3-galactosides and 3-rutinosides of kaempferol and quercetin are common constituents [1–5]. More recently, Melin [6] has found kaempferol 7-glucoside, isorhamnetin 3-rutinoside, (quercetin- and kaempferol 4′-glucosides and kaempferol 3-(2^G-glucosyl-rutinoside)) from a sweet cherry, cv Bigarreau Napoléon. In sour cherries kaempferol 3-rhamnosyl-4′-galactoside has been identified [7].

As part of a continuation of our chemical investigation of leaves and fruits of the genus *Prunus* [8–10] we now report the isolation and characterization of four flavonol tetraglycosides which we have found in small concentrations in the leaf tissue of *P. cerasus* and *P. avium*.

RESULTS AND DISCUSSION

First analyses indicated that four of the flavonols isolated from sour cherries were tetraglycosides. Standard methods were used for identification. Acid hydrolyses with 1 N methanolic HCl gave quercetin from compounds **1**, **2**, **3** and kaempferol from compound **4**; the sugars were identified as glucose and rhamnose in each case. Enzymic hydrolyses with β-D-glucosidase yielded glucose and a selective cleavage of sugars, attached to the C_3 of the flavonoid nucleus. Treatment with hydrogen peroxide gave rutinose from all four glycosides identified by co-TLC with an authentic marker prepared from rutin. The results of the quantitative determinations of aglycones after hydrolyses with an unspecific glycosidase (EL-1-77, Fa. Roehm, Darmstadt) and the quantitative determinations of sugars with anthrone–H_2SO_4 [11] are given in Table 1. In each case the ratio of aglycone to sugar was found to be 1:4.

The positions of the sugars were identified by UV spectral analyses using standard procedures [12] (see Table 2). After hydrolyses the free aglycones gave the following UV spectral data (in MeOH): aglycone from compounds **1**, **2**, **3** (quercetin) 256, 269 sh, 301 sh, 371 nm, and from compound **4** (kaempferol) 267, 296 sh, 325 sh, 368 nm. Comparison of MeOH spectral data of the aglycones with the glycosides gave hypsochromic shifts of

Table 1. Quantitative hydrolyses of the flavonol tetraglycosides

Glycosides		% aglycone	% sugar	mol aglycone:mol sugar
Quercetin 3-rutinosyl-7,3′-bisglucoside	**1**	30.4	—	1:4
Quercetin 3-rutinosyl-4′-diglucoside	**2**	30.9(±1.5)	70.6(±1.1)	1:4
Quercetin 3-rutinosyl-4′-diglucoside	**3**	30.6(±0.7)	68.9(±1.6)	1:4
Kaempferol 3-rutinosyl-4′-diglucoside	**4**	29.8	68.7	1:4

Table 2. UV spectral data for flavonol tetraglycosides

Glycoside	No.	MeOH	MeOH + NaOMe	MeOH + $AlCl_3$	MeOH + $AlCl_3$ HCl	MeOH + NaOAc	MeOH + NaOAc H_3BO_3
Quercetin	1	257	272	274	270	258	260
3-rutinosyl-		269 sh	305 sh	302 sh	302 sh	267 sh	298 sh
7,3'-bisglucoside		296 sh	412	370 sh	368 sh	294 sh	364
		362		418	404	378	
Quercetin	2	256	274	273	276	274	254
3-rutinosyl-		268	302 sh	300 sh	302 sh	300 sh	268
4'-diglucoside		298 sh	384	356	352	380	296 sh
		348		398	396		350
Quercetin	3	254	280	276	278	278	253
3-rutinosyl-		268	315 sh	304 sh	304 sh	310 sh	270
4'-diglucoside		348	380	354	350	378	350
				398	396		
Kaempferol	4	268	277	275	276	277	268
3-rutinosyl-		296 sh	306 sh	304 sh	304 sh	306 sh	296 sh
4'-diglucoside		346	378	347	344	375	348
				396	396		

band I (11–23 nm), indicating linkage of sugar to the C_3 hydroxyls.

Addition of NaOMe to glycoside **1** gave a bathochromic shift of band I (50 nm) and to glycosides **2**, **3** and **4** gave bathochromic shifts of band I (32–36 nm) in each case with a decrease in intensity. All NaOMe spectra were stable (second recording after 10 min). Bathochromic shifts in this range coupled with decrease in intensity indicated sugars at the C_3 and $C_{3'}$ hydroxyls in **1** and at the C_3 and C_4 hydroxyls in **2**, **3** and **4**. $C_{3'}$ $C_{4'}$ Di-hydroxy-borate complexes in NaOAc–H_3BO_3 spectra were not detected and there were no bathochromic shifts of band I (12–30 nm) when compared with the MeOH spectra. Evaluation of $AlCl_3$ and $AlCl_3$/HCl spectra comfirmed these results, no change of absorbance was measured. Therefore only complexes between the free C_5 hydroxyl and C_4 keto function were formed. The presence of free C_7 hydroxyls was indicated in **2**, **3** and **4** by a bathochromic shift of band II (11–22 nm) after addition of NaOAc; the absence of a band II shift in **1** suggested that the C_7 hydroxyl was glycosylated.

^{1}H NMR analysis of peracetylated **1**, **2** and **3** confirmed their identities as quercetin glycosides. Thus aromatic proton signals appear at 7.3 (J = 2 Hz) and 6.82 ppm (J = 2 Hz) for the benzoyl protons $H_{6'}$ and H_{8}. The $H_{6'}$ proton of the cinnamoyl nucleus gives two doublets at 7.9 ($J_{1',2'}$ = 8, J = 2 Hz); the $H_{2'}$ a doublet at 7.78 (J = 2 Hz) and $H_{5'}$ at 7.17 ppm (J = 8 Hz). Similarly the ^{1}H NMR of peracetylated **4** confirmed its structure as a kaempferol glycoside. Benzoyl protons: H_8 doublet at 7.1 (J = 2 Hz) and H_6 doublet at 6,8 ppm (J = 2 Hz); cinnamoyl protons $H_{2'}$,$H_{6'}$ and $H_{3'}$,$H_{5'}$ as *ortho*-coupled system (A_2B_2 system) at 7.96 (J = 10 Hz) and 7,22 ppm (J = 10 Hz).

The glycosylation pattern postulated from UV spectral data was confirmed by NMR analyses. The acetyl resonances of the flavonoid nucleus and sugar hydroxyls appear in the range 1.9–2.45 ppm. For interpretation of acetyl resonances the ^{1}H NMR spectra of peracetylated kaempferol, quercetin and its 3-, 7-, 4'-, 3,7- and 3,4'-glycosides were recorded. Acetyl resonances of the derivated hydroxyls at the flavonoid nucleus appear at: C_3 2.35, C_5 2.45, C_7 2.35, $C_{3'}$ 2.30, $C_{4'}$ 2.30 ppm. In **1** resonances of acetylated hydroxyls could only be determined from the C_5 and $C_{4'}$ or $C_{3'}$ position. Therefore in **1** sugars are present at the 3, 7 and 3' or 4' positions. In **2** and **3** signals from C_3 and $C_{3'}$ or $C_{4'}$ were absent, therefore **2** and **3** are glycosylated at the 3, 4' or 3' positions. In **4** signals from C_3 and $C_{4'}$ were absent, so this is a kaempferol 3,4'-glycoside.

A count of all the acetyl resonances (acetylated phenolic and sugar hydroxyls of the flavonol glycoside) in the NMR spectra was consistent with the number postulated for quercetin and kaempferol tetraglycosides, namely 16 for **1**, 15 for **2** and **3** and 14 for **4**. Apart from a rhamnosyl Me at 1.26 ppm (J = 8 Hz) we could not determine the other sugar protons exactly; they give multiplets in the range 3.6–5.9 ppm. Further characterization was accomplished by TLC and HPLC (Table 3).

From the above data we postulate the structure of the flavonol glycosides as: **1** quercetin 3-*O*-rutinosyl-7,3'-*O*-bisglucoside, **2** quercetin 3-*O*-rutinosyl-4'-di-*O*-glucoside, **3** quercetin 3-*O*-rutinosyl-4'-di-*O*-glucoside (not identical with glycoside **2**) and **4** kaempferol 3-*O*-rutinosyl-4'-di-*O*-glucoside. This is the first report of flavonol tetraglycosides in the Rosaceae.

EXPERIMENTAL

Isolation of flavonoids. Fresh frozen leaves (*P. cerasus* cv Schattenmorelle, 1150 g; *P. avium*, cv Große Prinzessin, 1090 g) or fruits (skins) were lyophilized, homogenized and extracted with boiling MeOH. The final soln was extracted with petrol to remove chlorophyll, waxes and similar substances and afterwards conc hot MeOH was added to ppt. impurities. The final conc aq. soln was percolated through a MN-SC 6 polyamide (Macherey, Nagel & Co., Duren) column without traces of iron (5 × 25 cm), the column was washed with H_2O and eluated with MeOH [13].

Separation of flavonoids. The glycosides were separated by (1) polyamide column chromatography (5 × 60 cm), solvent

Table 3. HPLC and melting point values for the kaempferol and quercetin tetraglycosides

Glycoside	No.	R_fs (× 100) in: A*	B*	C*	D*	E*	F†	G‡	Mp	HPLC§ k' values	Spot colour UV/UV + NH_3	Flavognost‖
Quercetin 3-rutinosyl 7,3'-bisglucoside	**1**	61	16	65	05	34	06	80		9.05	dark/yellow-green	yellow
Quercetin 3-rutinosyl-4'-diglucoside	**2**	67	25	73	07	43	06	72	199 202	7.90	dark/dark	yellow-green
Quercetin 3-rutinosyl-4'-diglucoside	**3**	73	28	78	12	46	07	77	186–188	8.15	dark/dark	yellow-green
Kaempferol 3-rutinosyl-4'-diglucoside	**4**	70	33	76	11	48	06	77	176–180	7.12	dark/dark	yellow-green

Key: A = PrOH–MeOH–H_2O, 1:2:8; B = EtOAc–HCOOH–H_2O, 10:2:3; C = 15% HOAc; D = $CHCl_3$–HOAc–H_2O, 10:9:1; E = *n*-BuOH–HOAc–H_2O, 4:1:2,2; F = EtOAc–butan-2-one–HOAc–H_2O, 5:8:1:1; G = H_2O–EtOH–butan-2-one–pentan-2,4-dione, 13:3:3:1.

* On Cellulose (Avicel, Merck, Darmstadt).

† On Si gel (Merck, Darmstadt, No. 5721).

‡ On Polyamide 6 (Macherey, Nagel & Co., Duren).

§ Values for paracetylated glycosides.

‖ Spot colour after spraying with Flavognost (diphenylborsaureaminoethylester complex, Heyl, Berlin) in UV light.

H_2O–butan-2-one–EtOH–pentan-2,4-dione, 13:3:3:1 and (2) PPC, as previously described [8].

Identification of the flavonol glycosides was carried out as described in earlier papers (e.g. [14]). Details of TLC adsorbents and solvents are given in Table 3. Mps are uncor. HPLC was performed with a Siemens S 200 chromatograph, UV detector PYE-Unicam LC-3 and Spectra Physics integrator System I using peracetylated [15] compounds. Chromatographic parameters: stainless steel column 600 × 3 mm ϕ, Sorbens LiChrosorb Si 60 (5 μm) (Merck, Darmstadt), detection UV 300 nm, flow 0.9 ml/min, pres. 170 bar, temp. 50°, eluents C_6H_6–acetonitrile (4:1), isocratic procedure. Column dead-time was determined with *n*-hexane. ^{1}H NMR spectra were measured employing deuterochloroform as solvent and TMS (=0 ppm) as int. standard.

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