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SHORT COMMUNICATION

FLAVONOL GLUCURONIDES FROM RED RASPBERRY, *RUBUS IDAEUS* (ROSACEAE)

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Abstract—Quercetin-3-β-glucuronide and kaempferol-3-β-glucuronide were isolated from the fruit of raspberry. In addition, two other glycosides were isolated by TLC.

INTRODUCTION

THE FLAVONOL glycosides of the Rosaceae have been well documented,[1(a,b)] particularly *Prunus*, but those in *Rubus* have received little attention (e.g. raspberry, loganberry, boysenberry). In connection with our interest in the phenolics[2] of food plants, we report our work on the flavonoids of the red raspberry, *Rubus idaeus*.

Both kaempferol and quercetin were isolated in about equal yield from *Fragaria ananassa* by Williams and Wender.[3] Co and Markakis[4] reported the occurrence of quercetin-3-glucoside and kaempferol-3-glucoside in American strawberries. The related anthocyanins have been studied in *Rubus* by Harborne and Hall[5] who found that cyanidin is the main pigment and occurs both as monosides and branched triglycosides. Pelargonidin is present in trace amounts in some varieties. No other flavonoid component of raspberry has been identified.

RESULTS AND DISCUSSION

Two flavonol glycosides were isolated from the fruit by extraction and TLC on cellulose in yields from 1 to 10 ppm. The major component was identified as quercetin-3-β-glucuronide from its UV spectra,[6] chromatography, hydrolysis under acid and enzymatic[7] conditions, and H_2O_2 oxidation.[8] However, an authentic sample was not available for comparison.

The second component was identified as kaempferol-3-β-glucuronide. Additionally, a third glycoside fraction was purified in small amount. Its TLC (5 solvent systems) indicated a mixture of quercetin-3-glucoside and -3-galactoside but too little material was available for full identification.

[1] (a) E. C. BATE-SMITH, *Advan. Food Res. 5, 261* (1954).
(b) T. SWAIN, in The *Chemistry of Flavonoid Compounds* (edited by T. GEISSMAN), MacMillan, New York (1962).
[2] D. E. COFFIN, *J. Agri. Food Chem.* (in press).
[3] B. L. WILLIAMS and S. H. WENDER, *J. Am. Chem. Soc.* 74, 5919 (1952).
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[6] L. JURD, in *The Chemistry of Flavonoid Compounds* (edited by T. GEISSMAN), MacMillan, New York (1962).
[7] B. V. CHANDLER and K. A. HARPER, *Australian J. Chem.* 14,586 (1961).
[8] MILES LABORATORIES, Inc., Research Product Dept., Elkhart, Indiana 46514.

These glucuronides have been identified previously in *Phaseolus vulgaris*,[9] *Gaultheria migueliana*,[10] *Populus grandiden tata*,[11] *Euphorbia lathyris*,[12] and *Vitis vinifera*.[13]

EXPERIMENTAL

Extraction

The fruit of locally grown 1969 summer crop raspberries (2.3 kg) which had been stored at -10" for 3 months, was thawed, macerated with hot MeOH (2 1. and 1 1.) and the solvent evaporated. The aqueous portion remaining (1.5 l., pH 3-4) was extracted 2 x 1 1. benzene, NaCl (40 g) added, and extracted 3 x 1 1. EtOAc. The latter was removed to produce a red oil (5·0 g).

This residue was triturated with H_2O (150 ml) to remove anthocyanins and extracted again into EtOAc (250 ml). After removal of solvent, Na_2CO_3 (50 ml, 5 %) was added to the residue (1.4 g) and neutral and basic material removed by extraction with EtOAc. The aqueous solution was then acidified (10% HCl) and flavonols extracted into EtOAc. Removal of EtOAc produced a brown-yellow oil (0·5 g) which was suitable for TLC without further purification. The use of polyamide as absorbent is not recommended[12] because of the presence of the glucuronic acid residue.

Chromatography

TLC was performed using Avicel microcrystalline cellulose coated (0·5 mm thickness) glass plates for both analytical and preparative work. The solvent systems particular suited for separation of flavonol glycosides on cellulose are: 30% HOAc (A), 3 % PhOH in H_2O (B), 20% H_2O in PhOH (C), H_2O (D), *n*-BuOH–HOAc–H_2O (6: 1: 2) (E) and Forrestal (F). Visualization was effected by $AlCl_3$ spray and by the UV absorption at 254 nm.

The brown-yellow oil above (0·5 g) was purified by TLC in solvent A, B and then C until all fractions were demonstrated as pure by individual spottings (3-4 elutions necessary). R_f values are given in Table 1 along with reference compounds. This method produced two glycosides in quantity suitable for identification: glycoside 1 (major)—10 mg, 5 ppm; and glycoside 2 (minor)-l-2 mg, 1 ppm; as well as traces of a third glycoside which was still a mixture. Trial separations with quercitrin (quercetin-3-rhamnose) showed that at least 80% of the flavonoid could be recovered by extraction of the cellulose with MeOH.

TABLE 1. R_f VALUES OF FLAVONOL GLYCOSIDES ON AVICEL CELLUWSE TLC PLATES

Compound*	R_f in solvent A	B	C	D	E	F
Q		<0·03	0·34	<0.03	0.61	0.30
K		<0.03	0·66	<0.03	0·82	0·50
M		<0.03	0·09		0·30	0.20
Q-3-gal	0·48	0.22	0·59	<0.03 0.05	0·47	0.66
Q-3-gl		0.21	0.53	0.05	0.48	0·65
Q-3-rh	0·58	0.35	0.58	0·13	0.66	0·71
Q-3-rut	0·59	0·39	0.41	0·23	0.35	0.70
Q-7-gl	0·14	0·04	0·32	<0·03	0.17	0·42
K-3-rh	0.63	0.42	0.75	0·19	0.80	0.78
Robinin	0.76	0.66	0.68	0·43	0.28	0.83
Glycoside 1=	0·48	0.78	0·30	0.49	0·43	0.60
	0.57	0·82	0·45	0·59	0.57	0·67
	0.48	0·21	0·55	0.05	—	0.63

* Abbreviations: Q = quercetin, K = kaempferol, M = myricetin, gl = glucose, gal = galactose, rh = rhamnose, ga = glucuronic acid, rut = rutinose.

[9] (a) C. ENDRES, R. HUTTEL and L. KAUFMANN, *Ann. Chem. 537, 205 (1939);* (b) C. A. MARSH, *Nature 176, 176 (1955).*
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Identification

UV spectra were taken using the standard reagents[6] for detection of substitution of flavonol glycosides. Hydrolysis of glycosides was performed with 5 % HCl, Dowex 50W-X12 and β-glucuronidase.[8,14] Chandler and Harper's method[7] was used for the H_2O_2 oxidation.

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