Phytochemistry, 1967, Vol. 6, pp. 1449 to 1452. Pergamon Press Ltd. Printed in England

POLYPHENOLS OF *RHEUM RHAPONTICUM*—I.

FLAVONOL GLYCOSIDES

H. A. W. BLUNDSTONE

The Fruit and Vegetable Preservation Research Association, Chipping Campden, Gloucestershire

(*Received* 20 *December* 1966, *in revised form* 15 *March* 1967)

Abstract—In an analysis of the polyphenols of *Rheum rhaponticum* the flavonols identified were quercetin-3-rutinoside, quercetin-3-glucoside and quercetin-3-rhamnoside.

INTRODUCTION

RHUBARB, *Rheum rhaponticum* L., Polygonaceae, is a crop of considerable importance to the canning industry and the acids have been studied[1] with respect to the severe corrosion which this product shows towards tinplate containers. Quercetin-3-rutinoside has been reported in the leaf and flower[2] and cyanidin-3-glucoside and cyanidin-3-rutinoside have been shown to be the red pigments of the petiole.[3]

In this paper the isolation of four flavonol glycosides from the petiole is described. A complex mixture of polyphenols was extracted from the tissue by methanol and this was fractionated on a column of silicic acid by a gradient elution technique. The crude bands eluted from the silicic acid column were finally purified on polyamide columns and the components characterized by their R_f values, spectrophotometric data and hydrolysis products.

RESULTS

The eluate from the silicic acid column was divided into six bands on the basis of the compounds detected by thin-layer chromatography in 6% acetic acid. Those bands which contained more than one major component were further purified on polyamide columns and most of the compounds were separated from each other. The main constituents of bands 1 and 2 were found to be anthraquinone derivatives, while bands 4, 5 and 6 contained mainly flavonol glycosides. Band 3 contained a mixture of flavonol glycosides, stilbenes and cinnamic acid derivatives.

The flavonol glycosides isolated were designated *a*, b_1, b_2, and *d*, and on hydrolysis all gave quercetin as the aglycone residue. The R_f values in various solvents are given in Table 1. The absorption maxima were measured on the spectrophotometer and the spectral shifts obtained with various reagents were determined (Table 2).

Flavonol glycoside *a* was identified as quercetin-3-rhamnoside by its R_f values, absorption maxima, and by the spectral shifts with the reagents employed. On hydrolysis rhamnose was the only sugar residue detected.

Flavonol glycoside b_2 was identified as quercetin-3-glucoside by its R_f values, absorption maxima, and by the spectral shifts with the reagents employed. On hydrolysis glucose was the only sugar residue detected.

[1] H. A. W. BLUNDSTONE and D. DICKINSON, *J. Sci. Food Agr.* **15**, 94 (1964).
[2] L. HÖRHAMMER and K. MULLER, *Arch. Pharm.* **287**, 126 (1954).
[3] R. A. GALLOP, *Sci. Bull.* No. 5, Fruit and Veg. Canning Quick Freez. Res. Assoc. (1965).

TABLE 1. R_f VALUES AND COLOUR REACTIONS OF FLAVONOLS

Compound	R_f cellulose T.L. 6HA	BAW	BEW	Hydrolysis products
Rhubarb flavonols				
Glycoside *a*	0·20	0·78	0·86	Quercetin and rhamnose
Glycoside b_1	0·16	0·79	0·89	Quercetin and glucose
Glycoside b_2	0·16	0·68	0·80	Quercetin and glucose
Glycoside *d*	0·30	0·60	0·71	Quercetin, rhamnose, glucose, rutinose
Quercetin	0·00	0·75	0·84	Quercetin
Q-3-glucoside	0·13	0·65	0·76	Quercetin and glucose
Q-3-rhamnoside	0·22	0·83	0·87	Quercetin and rhamnose
Q-4′-glucoside	0·03		0·68	Quercetin and glucose
Q-3-rutinoside	0·31	0·63	0·68	Quercetin, rhamnose, glucose, rutinose

The key to the solvent systems is given in the experimental section.
In u.v. light alone, all except quercetin and its 4′-glucoside (yellow) fluoresced dark brown. All fluoresced yellow in u.v. + NH_3 vapour, and yellow–green in u.v. + $AlCl_3$.

TABLE 2. SPECTROPHOTOMETRIC DATA ON FLAVONOLS

Compounds	λ_{max} (nm) Solvent–methanol Band I	Band II	Inflexion	Spectral shifts on addition of various reagents (Δ nm) NaOAc (Band II)	H_3BO_3/NaOAc (Band I)	$AlCl_3$ (Band I)	$AlCl_3$ (Band II)
Rhubarb flavonols							
Glycoside *a*	348	256	262	+15	+21	+85	+17
Glycoside b_2	355	257	265	+15	+25	+65	+17
Glycoside *d*	356	257	266	+15	+25	+82	+17
Quercetin-3-glucoside*	366	260	270	+10	+14	+53	+15
Q-3-rhamnoside	352	257	265	+15	+19	+61	+15
Q-4′-glucoside	365	253	264	+20	+4	+57	+14
Q-3-rutinoside	360	258	265	+16	+20	+78	+17

* All spectra were measured except that of quercetin-3-glucoside which was taken from Nortje *et al.*[4]

Flavonol glycoside b_1 also gave quercetin and glucose on hydrolysis, but was distinct from glycoside b_2.

Flavonol glycoside *d* was identified as quercetin-3-rutinoside by its R_f values, absorption maxima, and by the spectral shifts with the reagents employed. On hydrolysis the sugar residues glucose, rhamnose and rutinose were detected.

DISCUSSION

Rhubarb is interesting in that both the anthocyanin and flavonol glycosides which have been found are derived from one aglycone residue only in each case, namely cyanidin and quercetin which both contain the same hydroxylation pattern. The occurrence of structurally

[4] B. K. NORTJÉ and B. H. KOEPPEN, *Biochem. J.* **97**, 209 (1965).

related anthocyanins and flavonols has been observed in other plants[5] and it is suggested that this indicates a common biosynthetic pathway. Only two anthocyanins have been detected,[3] although four quercetin glycosides were isolated. Three of these glycosides, the -3-rhamnoside, the -3-glucoside and the -3-rutinoside have been isolated from many plants.

Quercetin glycoside b_1 was not positively identified although it was shown to be a quercetin glucoside. It was distinct from the -3-glucoside and the -4′-glucoside. However the R_f value on Whatman No. 1 paper in BAW (0·65) is in agreement with that quoted for a quercetin glucoside isolated from canned apricots (0·64).[6]

The spectral data are mainly in accordance with those of other workers.[7] The spectral shifts obtained with sodium acetate and boric acid in sodium acetate indicate that a free -7-hydroxy group, and, except in the case of the -4′-glucoside, orthodihydroxy groups were present in the quercetin glycosides studied. The spectral shifts obtained with aluminium chloride were considerably greater than those reported by Jurd, although good agreement was obtained between reference compounds and rhubarb flavonols. The reliability of the magnitude of shift obtained with aluminium chloride as a method of locating free -3-hydroxyl groups must be regarded with suspicion.

EXPERIMENTAL

Preparation of material. The rhubarb petioles were wiped clean and trimmed before being cut into inch lengths. These were then frozen at −18° and dried in a Vickers Accelerated Freeze Drying Cabinet. After drying the samples were packed under N_2 and stored in the dark.

Extraction of polyphenols. A quantity of freeze-dried rhubarb equivalent to about 200 g of fresh material was macerated with approx. 150 ml of 80% methanol containing 1% acetic acid. The mixture was simmered for 2 min over a steam-bath, and then cooled quickly in water, and the contents transferred to a 250 ml stoppered flask. The flask was then shaken on a mechanical shaker for 6 hr.

After filtration under vacuum the extracted material was washed several times with aqueous 80% methanol. The combined washings and extract were then shaken four or five times with petroleum spirit (60°–80° b.r. analytical grade). The layers were separated and the methanol layer was concentrated and made up to 25 ml in 80% methanol.

Fractionation of Methanol Extract on Silicic Acid Column

The chromatographic technique was adapted from that used in these laboratories for the separation of organic acids.[1] An analysis column 18 × 300 mm was packed with a slurry of 12 g silicic acid, Malinckrodt A.R. 100 mesh (washed free of finer particles as described in the original paper), mixed with 6 ml of 10% v/v H_3PO_4. The upper equilibration column was packed with 7 g silicic acid containing 3·5 ml of 0·75 M Na_2HPO_4 in 10% v/v H_3PO_4. This solution was used as the saturation layer above the column.

The sample for separation was taken to dryness on a waterbath and 2 ml 10% v/v H_3PO_4 was added. This was thoroughly mixed and 4 g silicic acid were added and mixed until a smooth powder resulted. This mixture was slurried in chloroform and added to the top of the analysis column.

Gradient elution with chloroform and tertiary butanol was carried out. The initial solvents were 340 ml of chloroform and 400 ml of a 50% v/v solution of tertiary butanol in chloroform.

120 fractions of 5 ml each were collected over 6 hr and the flavonols were detected by examination of the fractions by TLC.

Thin-layer chromatography. Air-dried cellulose layers were found most useful for the identification of the polyphenols isolated. The solvents used with their abbreviations were as follows: 6HA, acetic acid–water (6:94 v/v); BAW, butan-1-ol–acetic acid–water (4:1:5 v/v); BEW, butan-1-ol–ethanol–water (4:1:2·2 v/v).

Detection of the compounds was by u.v. light of 350 nm with and without ammonia vapour. Aluminium chloride in ethanol (2% wt./v) was used with u.v. light to detect flavonols.

Hydrolysis of glycosides. A sample containing 1–2 mg of glycoside was hydrolysed for 6 hr with 5 ml of 10% acetic acid. The hydrolysate was evaporated to dryness, dissolved in 50% aqueous methanol and the products examined by TLC.

[5] J. B. Harborne, *Biochemistry of Phenolic Compounds*, p. 129. Academic Press, New York (1964).
[6] A. S. El-Sayed and B. S. Luh, *J. Food. Sci.* **30**, 1016 (1965).
[7] L. Jurd, *Chemistry of the Flavonoid Compounds* (Edited by T. A. Geissman), p. 107. Pergamon Press, Oxford (1962).

The sugars were identified on silica layers by the technique of Adachi.[8]

Spectrophotometry. Measurements were carried out on a Perkin Elmer model 137 u.v. spectrophotometer. All spectral measurements were carried out using methanol as the solvent.

Spectral shifts were measured after the addition of aluminium chloride, sodium acetate, and boric acid with sodium acetate.[4]

Acknowledgement—This work was supported by grants from the British Sugar Refiners on the advice of the Sugar Research Foundation. The author wishes to thank Mr. A. H. Williams for a gift of quercetin-3-glucoside.

[8] S. ADACHI, *J. Chromatog.* **17**, 295 (1965).