A NEW BIOSIDE OF QUERCETIN

O. K. Bagrii, B. V. Kurmaz, and V. I. Litvinenko

Khimiya Prirodnykh Soedinenii, Vol. 2, No. 2, pp. 85-90, 1966

Continuing an investigation of the flavonoids of plants of the genus Rumex [1], we have isolated from an aqueous extract of the fruit of Rumex maritimus L. (family Polygonaceae) hyperin, rutin, and a previously undescribed bioside $C_{26}H_{28}O_{16}$ which we have called rumarin (Table 1).

The compound isolated gives positive reactions for flavonoids: the cyanidin reaction, the boric-citric reaction, the ferric chloride reaction, and also the Bryant and Hörhammer qualitative reactions [2-4]. Solutions of alkalies are colored yellow by the addition of the substance under investigation and a black spot is formed in the spot test with an ammoniacal solution of silver nitrate.

Table 1

Physicochemical Characteristics of Rumarin and its Derivatives

Properties	Bioside	Monoglycoside	Aglycone
Mp, °C	252—253	233—234	312—315
$[\alpha]_D^{20}$ deg	−30	−59.1	—
Mol. wt.	596.5*	464.4*	302.2
Empirical formula	$C_{26}H_{28}O_{16}$	$C_{21}H_{20}O_{12}$	$C_{15}H_{10}O_7$
Carbohydrate component	D-galactose L-arabinose	D-galactose	—
	257.365	257.363	255.370
R_f in the systems			
butan-1-ol–CH_3COOH–H_2O (4:1:5)	0.37	0.54	0.76
ethyl acetate–HCOOH–H_2O (10:2:3)	0.42	0.63	0.51
30% CH_3COOH	0.66	0.72	0.20

* Molecular weight calculated from the percentage content of aglycone.

To confirm the glycosidic nature of the flavonoid, it was subjected to acid hydrolysis. The hydrolysis products were found to contain a sugar and an aglycone (see Table 1). Chromatography on paper gave two spots showing that the carbohydrate component of the glycoside rumarin consists of two sugars. The appearance of a red and a brown spot after the development of the chromatogram with aniline phthalate showed the presence of a pentose and a hexose in the flavonoid molecule [5]. Paper chromatography showed that the pentose is L-arabinose and the hexose is D-galactose.

The position of attachment of the carbohydrate component to the aglycone was determined by chemical and spec troscopic methods. The positive reactions of the aglycone with zirconyl chloride [3, 4] and the negative reactions of the glycoside show that at least one carbohydrate component is present in position 3. The position of the free hydroxyl groups in the glycoside and its aglycone were established by spectroscopic investigations both in neutral solutions and in the presence of ionizing and complex-forming reagents [6] (Table 2).

The spectroscopic results on rumarin showed that free OH groups are present in position 7 (bathochromic displacement of 13 mμ in the presence of sodium acetate), in positions 3' and 4' (22-mμ bathochromism with boric acid), and in position 5 (52-mμ bathochromism with zirconyl chloride).

The aglycone gives a pentaacetyl derivative with mp 191°-192° C. Alkaline degradation of the aglycone leads to phloroglucinol and protocatechuic acid. Consequently, the aglycone can be characterized as 3, 5, 7, 3', 4'-pentahydroxyflavone, i.e., quercetin.

Since five free hydroxy groups were found in the aglycone and spectroscopic investigations show that the glycoside had only four hydroxyls, it is most likely that the two sugars are attached in position 3 and the substance under study is a bioside.

To determine the sequence of addition of the sugars to the aglycone, it was subjected to stepwise hydrolysis with formic acid in cyclohexanol [7]. Quercetin 3-galactoside was obtained as an intermediate (see Table 1). The mono-

side is readily hydrolyzed with the β-hydrolase of emulsin, which confirms the presence of a β-glycosidic bond between the aglycone and the carbohydrate moiety. Consequently, in the molecule of the bioside the D-galactose is directly attached to the aglycone and the L-arabinose is terminal.

Table 2

UV Spectra of the Bioside, the Monoside, and the Aglycone

Medium	Absorption maxima	Bioside		Monoside		Aglycone	
		λ_{max}, mμ	$\Delta\lambda_{max}$, mμ	λ_{max}, mμ	$\Delta\lambda_{max}$, mμ	λ_{max}, mμ	$\Delta\lambda_{max}$, mμ
2×10^{-5} M solution in abs. ethanol	I	365	—	364	—	370	—
	II	257	—	257	—	255	—
The same + sodium acetate	I	378	13	379	15	385	15
	II	257	—	255	−2	258	3
The same + boric acid and sodium acetate	I	387	22	385	21	396	26
	II	267	10	268	11	265	10
The same + zirconyl chloride	I	417	52	419	55	458	88
	II	260	3	263	6	265	10
The same + zirconyl chloride and citric acid	I	360	−5	361	−3	430	60
	II	258	1	260	−3	260	5

The determination of the order of the bond between the sugars was carried out by the oxidative degradation of the glycoside with hydrogen peroxide in an ammoniacal medium [8].

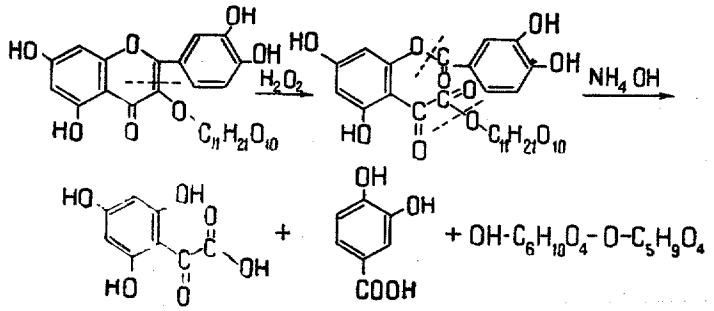

Table 3

Analysis of the Structural Features of the Sugars in Rumarin

Glycoside	$[M]_D$	K_f	$[M]_D K_f$	ΔC	K_m	Δ C. K_m
Rumarin	−179.0	0.65	−116.0	+35.0	0.73	+26.0
Quercetin 3-galactoside	−274.0	0.57	−151.0	—	—	—
Phenyl-β-D-galactopyranoside	−110.0	1.0	−110.0	—	—	—
Methyl-α-L-arabinopyranoside [14]	+30.0	—	—	—	1.0	+30.0

As can be seen from the reaction scheme given, hydrogen peroxide oxidizes the aglycone at the double bond between carbon atoms 2 and 3 with the formation of a sugar ester at position 3. On mild saponification of the glycoside in ammonia, the biose is split off without decomposition into monosaccharides.

A paper-chromatographic investigation of the carbohydrate component obtained by oxidative degradation showed that its mobility was similar to that of the bioses. The brown color of the spot after the development of the chromatogram with aniline phthalate confirmed that the hexose is a reducing sugar (galactose). In repeat analyses, the chromatograms were developed with diphenylamine reagents [9]. On development with a mixture of diphenylamine and p-anisidine, the spot of the sugar assumed a blue-green color which changed to a deep blue. Development with diphenyl-

amine and urea was accompanied by the formation of a red-purple spot. This gave a basis for assuming that the sugars in the biose are connected in the 1-6 position.

To confirm this particular position of the linkage of the sugars, the glycoside was hydrolyzed with the enzyme rhamnodiastase, which is a stereospecific hydrolase splitting a β-biose with a 1-6 position of the linkage between the monoses [10, 11]. By paper chromatography the hydrolyzate was found to contain a biose similar to that obtained by the oxidative degradation of rumarin.

The nature of the glycosidic bonds and the sizes of the oxide rings in the carbohydrate part of the glycoside were investigated by means of polarimetry [12, 13]. It can be seen from Table 3 that both sugars are present in the pyranose form, and the D-galactose is attached to the aglycone by a β-glycosidic bond and the L-arabinose, in its turn, is connected to the galactose by an α bond.

Thus, on the basis of the results obtained the flavonoid $C_{26}H_{28}O_{16}$ isolated may be characterized as quercetin 3-(β-D-galactopyranosyl-6-α-L-arabinopyranoside).

Experimental

Isolation of the flavonoids. 1 kg of the fruit and perianths of R. maritimus previously treated with petroleum ether was exhaustively extracted by boiling with 90% methyl alcohol. The alcoholic extracts were evaporated under vacuum to small bulk, diluted with 10 *l* of hot water, and left for 12 hr. The filtered solution was concentrated to a volume of 300 ml and extracted with ethyl acetate (eight 700-ml portions).

The chromatographic separation on columns of polyamide sorbent (Kapron) of the ethyl acetate extracts gave the known flavonoid hyperin, and chromatography of the aqueous residue gave rutin.

During the extraction of the aqueous extract with ethyl acetate, 1.2 g of the pale yellow crystalline substance rumarin with mp 252°-253° C (from water) separated out at the phase boundary.

Found, %: C 52.61, 52.68; H 5.08, 5.16. Calculated for $C_{26}H_{28}O_{16}$, %: C 52.34; H 4.73.

Aglycone. The acid hydrolysis of rumarin with 7% sulfuric acid gave the aglycone quercetin with mp 312°-315° C (50% methanol).

Found, %: C 59.63, 59.72; H 3.34, 3.25. Calculated for $C_{15}H_{10}O_7$, %: C 59.76; H 3.31.

Alkaline degradation. A solution of 200 mg of the aglycone in 150 ml of 30% caustic potash was heated at 100° C for 2 hr. The solution was acidified with sulfuric acid to an acid reaction (pH 4) and was extracted with ethyl ether (four 30-ml portions). The ethereal extracts were washed with water to neutrality, the ether was distilled off to dryness, and the residue was dissolved in alcohol and analyzed by paper chromatography. The decomposition products were identified as phloroglucinol and protocatechuic acid.

Stepwise hydrolysis of rumarin. With heating, 50 mg of the glycoside was dissolved in 5 ml of cyclohexanol and the solution was treated with 1.2 ml of 90% formic acid. Hydrolysis was carried out at 102°-105° C for 10 hr. The course of the hydrolysis was followed by paper chromatography. The mixture, consisting of the initial bioside, a monoglycoside, and the aglycone, was diluted with 25 ml of ethyl acetate and transferred to a column of polyamide sorbent. Elution was carried out with ethyl acetate. The first fractions of the eluate contained the excess of formic acid, and then the aglycone and the monoside followed. The fraction containing the monoside was evaporated to dryness and the residue was crystallized from 60% alcohol. This gave bright yellow crystals of hyperin, mp 233°-234° C, $[\alpha]_D$ −59° C (c 0.1; dimethylformamide).

Found, %: C 54.07, 53.88; H 4.18, 4.32. Calculated for $C_{21}H_{20}O_{12}$, %: C 54.32; H 3.34.

When the intermediate compound was hydrolyzed with 1% hydrochloric acid, 62.8% of the aglycone was formed, which shows the presence of one molecule of galactose in it. The hyperin was identified by paper chromatography.

Carbohydrate component. The acid solution remaining after the hydrolysis of the rumarin was neutralized with barium carbonate and evaporated to 1-2 ml. Chromatographic investigation on "Leningrad rapid" paper was carried out in the presence of samples of sugars in three solvent systems: butan-1-ol–benzene–pyridine–water (5:1:3:2); picoline–ammonia–water (70:2:28); and ethyl acetate–pyridine–water (2:1:2). After the chromatogram had been developed, two spots were obtained with aniline phthalate, – a red spot and a brown spot. The R_f figures were, respectively, 0.28 and 0.37; 0.78 and 0.82; 0.40 and 0.48.

Oxidative degradation. A suspension of 0.1 g of rumarin in 20 ml of water was mixed with 2 ml of 0.1 N ammonia solution. The resulting solution was treated with 4 ml of 30% hydrogen peroxide and left for 4 hr at room temperature. The excess of hydrogen peroxide was eliminated by the addition of lead sulfide, and the mixture was filtered. The filtrate was evaporated to a volume of 0.2-0.3 ml and was analyzed by chromatography. After detection of the

chromatogram with aniline phthalate, a brown spot was found.

Repeat chromatograms were detected with diphenylamine reagents. Where the chromatogram was detected with a mixture of diphenylamine and p-anisidine the spot of the sugar under investigation was blue-green changing to deep blue; on detection with diphenylamine and urea it was red-purple.

Enzymatic degradation. A solution of 10 mg of the glycoside in 5 ml of alcohol was diluted with 20 ml of water, a solution of 10 mg of the enzyme rhamnodiastase was added, and the mixture was left at 30° C for a day. The mixture was boiled, treated with 10 ml of alcohol, and centrifuged. After evaporation under vacuum, the solution was chromatographed on paper in the butan-1-ol–acetic acid–water (4:1:5) system. The R_f values with respect to galactose were 0.43 for the biose and 1.24 for the arabinose; in the ethyl acetate–formic acid–acetic acid–water (18:1:3:4) system they were 0.20 for the biose and 1.4 for the arabinose.

A solution of the biose obtained by enzymatic degradation was hydrolyzed with 5% sulfuric acid for 1 hr. The products were chromatographed on paper, and D-galactose and L-arabinose were detected.

Summary

A new bioside of quercetin, rumarin, with the composition $C_{26}H_{28}O_{16}$, characterized as quercetin 3-(β-D-galactopyranosyl-6-α-L-arabinopyranoside), has been isolated from the fruit of Rumex maritimus L.

REFERENCES

1. O. K. Bagrii and P. E. Krivenchuk, Farm. zh., no. 5, 64, 1964.
2. E. F. Bryant, J. Am. Pharm. Ass. Sci. Ed., 39, 8, 480, 1950.
3. L. Hörhammer and R. Hansel, Arch. Pharm., 286, 425, 1953.
4. L. Hörhammer and K. H. Müller, Arch. Pharm., 287, 310, 1954.
5. S. M. Partridge, Nature (Lond.), 164, 443, 1949.
6. T. A. Geissman, The Chemistry of Flavonoid Compounds, N.Y., 1962.
7. D. W. Fox, W. L. Savage, and S. H. Wender, J. Am. Chem. Soc., 75, 2504, 1953.
8. B. V. Chendler and K. A. Harper, Austr. J. Chem., 14, 586, 1961.
9. R. W. Bailey, J. Chromatography, 8, 57, 1962.
10. H. Friedrich, Arch. Pharm., 295, 464, 1962.
11. H. Imaseki and T. Jamamoto, Arch. Bioch. Biophys., 92, 467–474, 1961.
12. J. Son-Bredenberg and K. P. Hietala, Acta Chem., Scand., 15, 936, 1961.
13. I. P. Kovalev and V. I. Litvinenko, KhPS, 233–240, 1965.
14. J. Staněk, M. Černý, I. Kocourek, and J. Pacak, Monosaccharidy, Prague, 184, 1960.

26 June 1965

Zaporozh'e Pharmaceutical Institute

Kharkov Chemical and Pharmaceutical Scientific Research Institute