UDC 547

FLAVONOIDS OF ROBINIA PSEUDACACIA

N. P. Maksyutina

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Anthochlor pigments have been detected in various parts of the plant Robinia pseudacacia L. (black locust) and have been studied chemically [1-8]. The present paper gives the results of a study of the anthochlor pigments present in the unripe fruit of this plant.

Two glycosides have been isolated from valves of unripe fruit of Robinia pseudacacia. These substances are bioside derivatives of two aglycones—kaempferol and quercetin. The sugar component of the glycosides consists of rhamnose and galactose in the form of a biose.

The structural features of the biose residues of the glycosides were determined by differential IR spectroscopy and molecular rotation differences [9].

Table 1

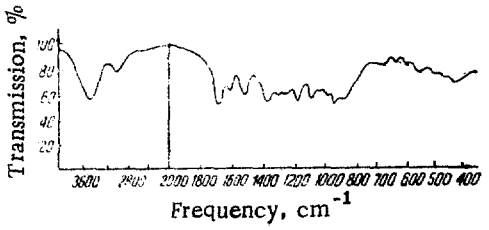
Spectral Characteristics of Bioquercetin and Its Aglycone

Solutions and reagents	Bioquercetin		Aglycone of bioquercetin	
	Bands, λ_{max} mμ			
	I	II	I	II
2×10^{-5} M absolute ethanol	365	257	371	255 and 270
" + sodium acetate	378	260	382	258
Δλ	13	3	11	3
" + sodium ethoxide	404	262	—	255
Δλ	39	5	—	0
" + zirconyl nitrate	415	260	458	265
Δλ	50	3	87	10
" + zirconyl nitrate + citric acid	360	258	430	260
Δλ	—5	1	59	5
" + boric acid + sodium acetate	387	267	396	265
Δλ	22	10	25	10

It has been established that one of the glycosides is kaempferol 3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside), which is identical with biorobin. We obtained the latter by the partial degradation of the glycoside robinin [10].

The second glycoside is apparently a new compound, and we have called it bioquercetin. On enzymatic and acid hydrolysis, bioquercetin splits off only the aglycone quercetin and the sugar in the form of monoses or a biose and does not form flavonoid monoglycosides.

The oxidative degradation of bioquercetin with hydrogen peroxide leads to the splitting out of the biose residue, which is obtained by chromatographic separation of the oxidation products on cellulose and is characterized by paper chromatography. In the butanol—acetic acid—water (4:1:5) system, the biose of bioquercetin has a R_f with respect to glucose of 0.80. The aniline phthalate derivative of the biose appears in the form of brown spots and with diphenylamine reagents it forms pink (with urea) or green (with p-anisidine) spots.



IR spectrum of bioquercetin.

The characteristic colorations with diphenylamine reagents and the splitting off of the biose residue by the stereospecific enzyme rhamnodiastase enables us to characterize the linkage of the sugars in the biose of bioquercetin as 1-6. The splitting of bioquercetin by snail enzyme, emulsin, and rhamnodiastase to the aglycone shows the presence of a β-linkage of the sugar with the aglycone and between the D-galactose and L-rhamnose.

The position of the biose residue at C_3 in bioquercetin

was demonstrated by the oxidation of the glycose with hydrogen peroxide and by spectral studies in the UV region (Table 1).

The molecular rotations were compared with the four possible phenyl galactosides (because of the absence of a monogalactoside from bioquercetin) and the results obtained were compared with the molecular rotation of the methyl rhamnosides. The figures given in Table 2 show that only in the case of a β-configuration and a furanose ring of D-galactose are comparable results obtained with respect to L-rhamnose in bioquercetin.

Table 2

Analysis of the Molecular Rotation of the Carbohydrate Moiety of Bioquercetin

Glycoside	M_D	K_{Ph}	$M_D \cdot K_{Ph}$	ΔC	K_M	$\Delta C \cdot K_M$	Configuration and size of the oxide ring of the sugars
Bioquercetin	—166.0	0.62	—103.0	—658.0	0.74	—487.0	
Phenyl α-D-galacto-pyranoside	+555.0	1.0	+555.0	—	—	—	
Phenyl β-D-galacto-pyranoside	—110.0	1.0	—110.0	—	—	—	
Proportion of the rotation of methyl rhamnoside for bioquercetin	—	—	—	+7.0	0.74	+5.2	
Phenyl β-D-galacto-furanoside	+403.0	1.0	+403.0	—	—	—	
Proportion of the rotation of methyl rhamnoside for bioquercetin	—	—	—	—506.0	0.74	—374.0	
Phenyl β-D-galacto-furanoside	—378.0	1.0	—378.0	—	—	—	
Proportion of the rotation of methyl rhamnoside for bioquercetin	—	—	—	+276.0	0.74	+204.0	β-D-galacto-furanose β-L-rhamno-pyranose
Methyl α-L-rhamno-pyranoside	—111.4	1.0	—111.4	—	—	—	
Methyl β-L-rhamno-pyranoside	+170.0	1.0	+170 0	—	—	—	
Methyl α-L-rhamno-furanoside	—158.0	1.0	—158.0	—	—	—	
Methyl β-L-rhamno-furanoside	—154.0	1.0	—154.0	—	—	—	

The analysis of the molecular rotation of bioquercetin permits the assumption of the presence in its biose of the furanose form of β-D-galactose and the pyranose form of β-L-rhamnose, and therefore bioquercetin must have the structure of quercetin 3-(β-D-galactofuranosyl-6-β-L-rhamnopyranoside). The IR spectrum of bioquercetin is given in the figure.

Experimental

Isolation of biorobin and bioquercetin. One kilogram of the dry valves of unripe fruit (without seeds) was extracted twice with 10-*l* portions of water, and the extract was chromatographed on 0.8 kg of polyamide sorbent. The first eluates were rejected and the zone with absorption in the UV region was eluted with water for 18 days. The biorobin passed into the eluate first, being detected by paper chromatography in 15% acetic acid, R_f 0.68–0.69. The fractions containing only the spot of biorobin were collected separately, evaporated to small volume, and rechromatographed on 0.2 kg of polyamide. Biorobin was isolated from the concentrated aqueous eluates, mp 221–223° C.

The bioquercetin was eluted from the first column with hot water 18–19 days after the beginning of the chromatographic separation of the aqueous extract. The use of alcohol to accelerate the elution is undesirable, since anthocyanidin glycosides pass into the eluates. The aqueous eluates containing only bioquercetin (with R_f 0.61 in 15% acetic acid) were combined and evaporated to small bulk and were rechromatographed on polyamide (0.2 kg). This gave bioquercetin with mp 201–203° C.

Biorobin. The substance is readily soluble in water and sparingly soluble in methyl and ethyl alcohols and in

acetone, and is insoluble in ether. With magnesium and hydrochloric acid the bioside gives a red coloration, with Wilson's reagent a yellow coloration, and with ferric chloride in aqueous solution a dark brown and in alcoholic solution a dark green-brown coloration. The reaction of biorobin with zirconyl chloride and citric acid was negative, $[\alpha]_D^{20}$ −75.0° (c 1.0; pyridine).

Found, %: C 54.68, 54.47; H 4.95, 5.05. Calculated for $C_{27}H_{30}O_{15}$ (594.10), %: C 54.54; H 5.07.

The biorobin was hydrolyzed by being heated with 0.5% hydrochloric acid for 30 min. Crystals of the aglycone kaempferol with mp 275–276° C deposited from the hot hydrolyzate. The acid hydrolyzate after neutralization was chromatographed on a column of 0.24 kg of cellulose. The sugars were eluted with butanol–acetic acid–water (4:1:1). L-Rhamnose was isolated from fractions 7–12 and identified; D-galactose was obtained from fractions 14–18.

The hydrolysis of biorobin with 0.1% hydrochloric acid at 60° C for 20 min gave a monoglycoside which was isolated from the hydrolyzate by chromatography on polyamide. The properties of the glycoside were identical with those of the galactorobin isolated by the stepwise hydrolysis of robinin [10].

The enzymatic hydrolysis of biorobin by an enzyme preparation of snail pancreatic juice led to the formation of galactorobin, kaempferol, D-galactose, L-rhamnose, and a biose identical with the biose of robinin [10]. Rhamnodiastase split the biorobin isolated into kaempferol and the biose.

The oxidative degradation of biorobin with hydrogen peroxide also gave the biose identical with the biose of robinin [10].

The nature of the glycosidic linkages and the sizes of the oxide rings were determined by IR spectroscopy and by polarimetry. The results of these investigations are similar to those that we have described previously [10].

Bioquercetin. The glycoside is readily soluble in water, sparingly soluble in alcohol and acetone, and insoluble in ether; with magnesium and hydrochloric acid it gave a dark red coloration, with Wilson's reagent a yellow coloration, and with ferric chloride in aqueous solution a brown and in alcoholic solution a green-brown coloration. Bioquercetin reduced an ammoniacal solution of silver nitrate and formed a yellow-orange precipitate with basic lead acetate. R_f in 15% acetic acid 0.61 and in butanol–acetic acid–water (BAW) (4:1:5) 0.30; $[\alpha]_D^{20}$ −27.2° (c 0.10; dimethylformamide).

Found, %: C 53.32, 53.10; H 4.81, 4.88. Calculated for $C_{27}H_{30}O_{16}$ (610.0), %: C 53.10; H 4.92.

0.6 g of bioquercetin was hydrolyzed with 2% hydrochloric acid in the water bath for 15 min. The aglycone quercetin with mp 312–313° C was isolated in a yield of 0.28 g.

Chromatography on cellulose of an acid hydrolyzate gave D-galactose and L-rhamnose. The sugars were isolated and identified by the methods given for biorobin.

Bioquercetin was subjected to enzymatic hydrolysis with rhamnodiastase, snail enzyme preparation, and emulsin. When the hydroyzates were chromatographed in the butanol–acetic acid–water system, it was found that with all these enzymes hydroysis takes place as far as the aglycone without a stage of the formation of a monoglycoside. Analysis of the sugars in the enzymatic hydrolyzate showed that a biose with a R_f with respect to glucose of 0.78 was first split off predominantly, and then the biose was split by the enzymes to galactose and rhamnose with R_f values with respect to glucose of 1.08 and 2.02. Hydrolysis began directly after the addition of the enzymes and was complete in 14–18 hr. The largest amount of biose was found in the hydrolyzate from rhamnodiastase.

0.2 g of bioquercetin was hydrolyzed with rhamnodiastase for 18 hr, the enzyme was precipitated with ethanol at the boil, and the biose in the form of a sirup was isolated chromatographically on cellulose (50 g) in the BAW system.

By the method described previously [8], 0.05 g of bioquercetin was oxidized with hydrogen peroxide. A paper-chromatographic analysis of the oxidation products of bioquercetin showed that a biose with R_f with respect to glucose of 0.78 in the BAW system had been split off in oxidation. With diphenylamine reagents, the biose gave colorations analogous to those of the biose obtained by degradation with rhamnodiastase.

Summary

The valves of unripe fruit of Robinia pseudacacia L. have yielded two flavonoid biosides: the known biorobin and the new bioquercetin, which has the structure of quercetin 3-(β-D-galactofuranosyl-6-β-L-rhamnopyranoside).

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