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THE COMPOSITION OF ROBININ FROM ROBINIA VISCOSA

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From the flowers of Robinia viscosa Vent we have isolated a flavonoid glycoside in the form of bright yellow needles with mp 194–197° C. The glycoside is readily soluble in dimethylformamide and methanol and less readily in ethanol and hot water, and it is insoluble in ether. It is a kaempferol derivative with the empirical formula $C_{33}H_{42}O_{20}$ and contains 2 moles of rhamnose and 1 mole of galactose. The properties of the glycoside and its UV and IR spectra agree fully with the properties and spectra of robinin. [1–4].

To identify the glycoside obtained as robinin, we carried out a mild discontinuous stepwise acid hydrolysis with dilute mineral acid (0.16% HCl). In the first stage of hydrolysis (15 min), several intermediate products were formed which were characterized as kaempferol 7-α-L-rhamnofuranoside (α-rhamnorobin), kaempferol 7-β-rhamnofuranoside (β-rhamnorobin), kaempferol 3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside), identical with biorobin, kaempferol 3-β-D-galactofuranoside (galactorobin), and a certain amount of the initial glycoside.

The presence in the acid hydrolysate of the intermediate glycosides biorobin, galactorobin, and α- and β-rhamnorobins permits the assumption that they are formed from not one but two glycosides of the following structures: kaempferol 7-α-L-rhamnofuranosido-3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside) and kaempferol 7-β-L-rhamnofuranosido-3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside). We have called the first of them α-neorobinin and the second β-neorobinin. The residue of incompletely hydrolyzed glycoside after the first stage of hydrolysis, with properties similar to those of the initial trioside, was subjected to further stepwise hydrolysis under similar conditions (0.16% HCl, 30 min). As intermediate products were isolated a new monoglycoside of kaempferol, identified as kaempferol 7-α-L-rhamnopyranoside, together with galactorobin, biorobin, kaempferol, and a fraction of unhydrolyzed initial trioside.

Intermediate products of the second stage of acid hydrolysis could be formed from a trioside of the structure of kaempferol 7-α-L-rhamnofuranosido-3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside), which we have called α-isorobinin.

Products of the three stages of hydrolysis were separated on columns of polyamide sorbent. All the components were isolated in the individual state (Table 1). The IR spectra are given in the figure. The positions of the free phenolic hydroxy groups and of the carbohydrate substitutents in the glycosides investigated were determined by UV spectroscopy.

The configurations of the glycosidic links and the sizes of the oxide rings of the glycosides were established by comparing the molecular rotations of the compounds investigated and of the corresponding phenyl and methyl glycosides [5, 6]. The figures of Table 2 show that in α-rhamnorobin and α-rhamnoisorobin the L-rhamnose possesses the α-configuration of the linkage, and these glycosides differ only by the size of the oxide ring. In the first glycoside the rhamnose is present in the furanose form and in the second in the pyranose form while in β-rhamnorobin the rhamnose is present in the furanose form and is attached to the kaempferol by a β-glycosidic bond. In galactorobin and biorobin the carbohydrate substituents have the β-configuration of the glycosidic linkages and the furanose form of the oxide rings; in diorobin the rhamnose has the β-configuration of the link and a pyranose oxide ring and the galactose the β-configuration and a furanose ring.

When natural robinin was hydrolyzed enzymatically with rhamnodiastase, a biose was isolated consisting of 1 mole of rhamnose and 1 mole of galactose. The same biose was obtained from the intermediate product of the stepwise hydrolysis of robinin–biorobin. The stepwise splitting off of the rhamnose from biorobin with the formation of kaempferol D-galactoside shows the direct linkage of the D-galactose in robinin with the kaempferol. On comparing literature data on the structure of robinobiose [2], isolated previously from natural robinin, with the results of our investigation of robinin, substantial differences in the structure of the biose substituents can be seen. Thus, unlike robinobiose, the biose under consideration, which we have called neorobinobiose, has the furanose form of the sugars and the β-configuration of the glycosidic links both between the D-galactose and the L-rhamnose and between the D-galactose and the aglycone.

Consequently, natural robinin consists of a mixture of not less than four components. These compounds are kaempferol derivatives with a common biose substituent, neorobinobiose, in position 3 and differ by the configurations of the glycosidic linkages and the size of the oxide ring of the L-rhamnose in position 7. The structure of α- and β-neorobinins and α- and β-isorobinins, and also their transformation with the formation of intermediate products on stepwise acid hydrolysis are shown in the following scheme.

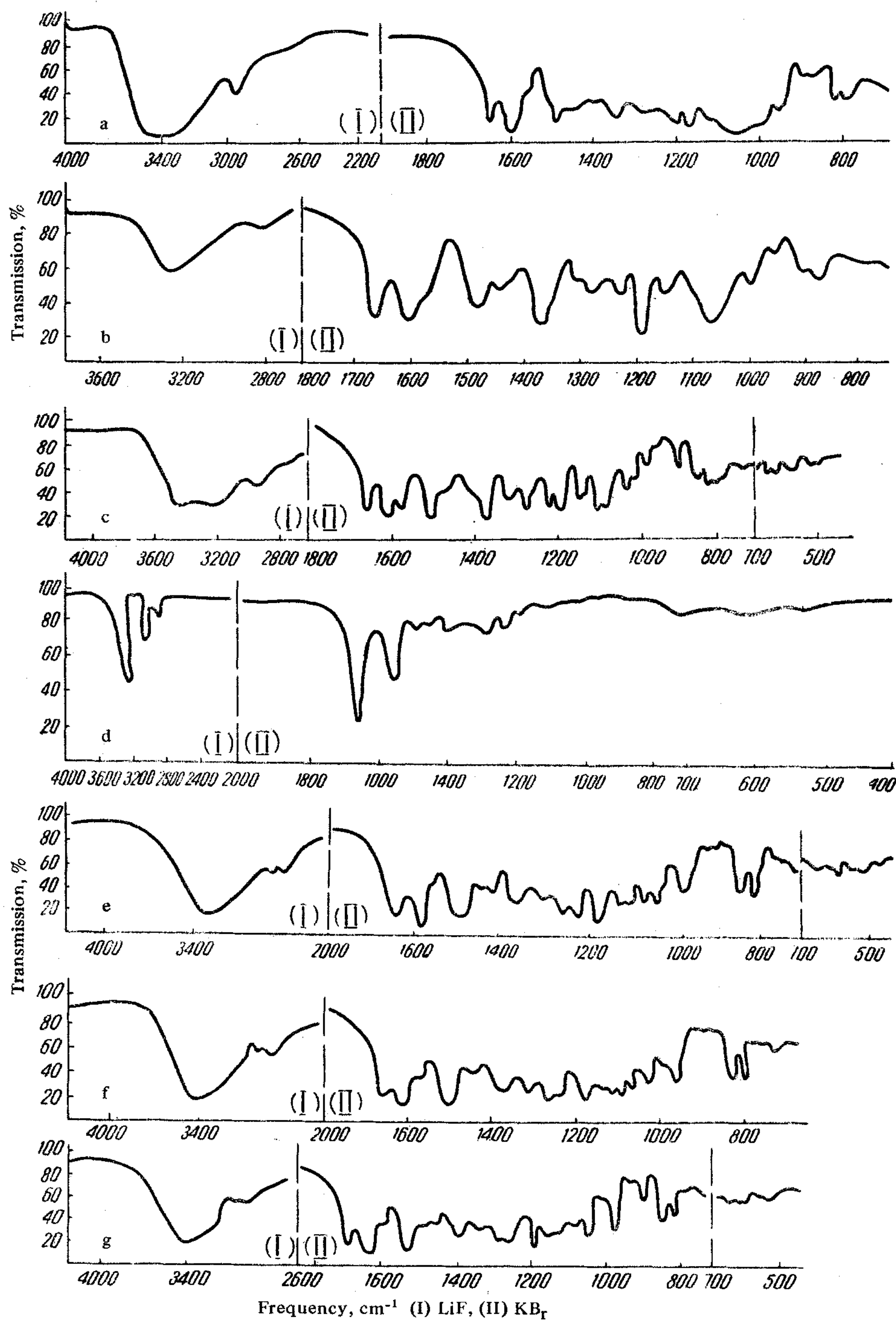
Table 1

Physicochemical Properites of Robinin and Its Derivatives

Compound	Mp, ° C	$[\alpha]_D^{20}$, deg	Formula	Mol wt	$R_f \times 100$ in the systems*		Structural features of the carbohydrate substituents			Results of IR spectroscopy, frequency cm^{-1} **			
										anomers		ring and C–O vibrations	
					1	2	position	configuration	form and nature of the sugar	α	β	pyranose	furanose
Robinin	194—197	—122.4 (c 1.0; pyridine)	$C_{33}H_{40}O_{19}$	740.0	77	20	3 7	β α and β	Robinobiose Rhamnopyranose Rhamnofuranose	— —	— —	— —	— —
α-Rhamnorobin	233—235	—200.0 (c 0.5; methanol)	$C_{21}H_{20}O_{10}$	432.0	16	64	7	α	Rhamnofuranose	840	—	—	1064, 1032
β-Rhamnorobin	232—234	—38.2 (c 0.5; methanol)	$C_{21}H_{20}O_{10}$	432.0	16	64	7	β	Rhamnofuranose	—	895	—	1064, 1032
α-Rhamnoisorobin	170—173	—126.0 (c 0.05; methanol)	$C_{21}H_{20}O_{10}$	432.0	16	64	7	α	Rhamnopyranose	840	—	1080, 1058 1036	—
Galactorobin	230—233	—120.0 (c 0.5; methanol)	$C_{21}H_{20}O_{11}$	448.0	16	62	3	β	Galactofuranose	—	895	—	1064, 1032
Diorobin	248—250	—80.0 (c 0.9; pyridine)	$C_{27}H_{30}O_{15}$	594.0	47	58	3 7	β β	Galactofuranose Rhamnopyranose	— —	895 895	— 1088, 1055 1016	1064, 1032 —
Biorobin	221—223	—75.0 (c 1.0; pyridine)	$C_{27}H_{30}O_{15}$	594.0	69	38	3 3	β β	Neorobinobiose	—	895	—	1083, 1052
β-Isorobin	192—195	—88.0 (c 0.05; methanol)	$C_{33}H_{40}O_{19}$	740.0	77	20	7	β	Neorobinobiose Rhamnopyranose	— —	895 898	1088, 1055 1016	1088, 1052 —

*1) 15% acetic acid; 2) butanol-acetic acid-water (4 : 1 : 2).

**The L-rhamnose in biorobin and diorobin was investigated with the elimination of the 3-galactofuranoside.



IR spectra of flavonoid glycosides: a) robinin, b) biorobin; c) diorobin; d) galactorobin; e) α-rhamnorobin; f) α-rhamnoisorobin; g) β-rhamnorobin.

Table 2

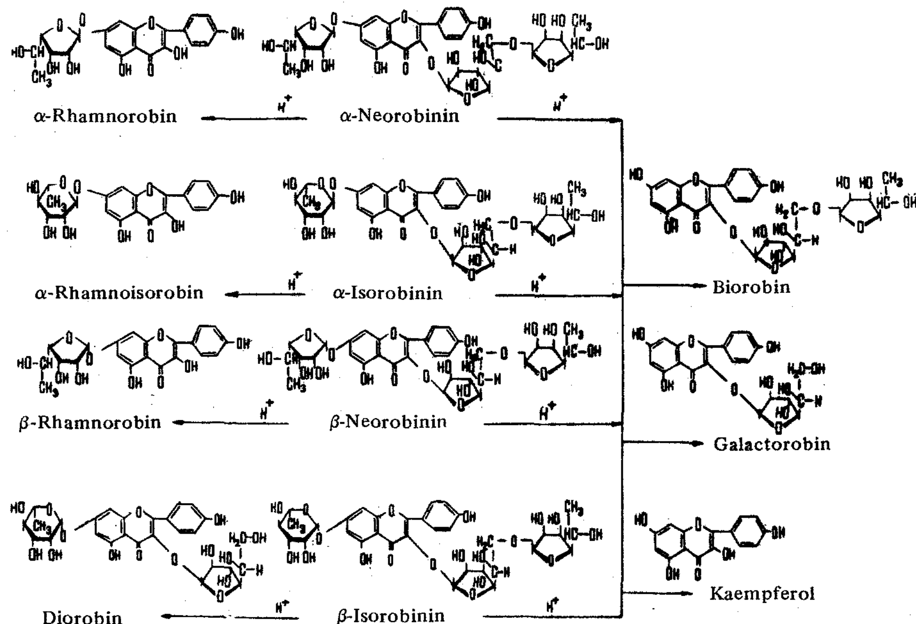
Analysis of the Molecular Rotations of the Carbohydrate Moieties of the Glycosides Obtained from Robinin Triosides

Glycoside	$[M]_D$	K_{pH}	$[M]_D \cdot K_{pH}$	ΔC	$\Delta C \cdot K_{pH}$	Configuration and size of the oxide ring
α-Rhamnorobin	—864.0	0.55	—475.0	—	—	α-Furanoside
Phenyl L-rhamnoside	—410.0	1.00	—410.0	—	—	α-Furanoside
β-Rhamnorobin	—165.0	0.55	— 90.7	—	—	β-Furanoside
Phenyl L-rhamnoside	— 90.0	1.00	— 90.0	—	—	β-Furanoside
α-Rhamnoisorobin	—545.0	0.55	—899.7	—	—	α-Pyranoside
Phenyl L-rhamnopyranoside	—254.0	1.00	—254.0	—	—	α-Pyranoside
Biorobin	—368.0	0.65	—239.0	+140.0	+104.1	β-Furanoside
Galactorobin	—538.0	0.57	—307.0			
Methyl L-rhamnoside	+154.0	1.00	+154.0	—	—	β-Furanoside
Diorobin	—475.2	0.65	—268.9	+110.1	+159.6	β-Pyranoside
Phenyl L-rhamnoside	+210.0	1.00	+210.0	—	—	β-Pyranoside

Table 3

UV Spectra of Robinin and the Products of Its Stepwise Hydrolysis

Glycoside	Bands	2×10^{-5} M solution of the glycoside in absolute ethanol								
		ethanolic solution	ethanolic solution + sodium acetate		ethanolic solution + sodium ethoxide		ethanolic solution + zirconyl chloride		ethanolic solution + zirconyl chloride + citric acid	
		absorption maxima and their shifts, mμ								
		λ	λ	Δλ	λ	Δλ	λ	Δλ	λ	Δλ
Robinin	I	352	350	—2	400	48	400	48	350	—2
	II	268	270	2	270	2	269	1	265	—3
Biorobin	I	355	370	15	405	50	395	40	350	—5
	II	265	270	5	275	10	265	0	265	0
Diorobin	I	352	352	0	405	53	400	48	452	0
	II	265	265	0	270	5	270	5	260	—5
α-Rhamnorobin	I	365	365	0	—	—	445	80	425	60
	II	260	260	0	260	0	265	5	260	5
β-Rhamnorobin	I	365	365	0	—	—	445	80	425	60
	II	260	260	0	260	0	265	5	260	0
α-Rhamnoisorobin	I	365	365	0	—	—	448	83	415	50
	II	260	260	0	261	1	260	0	265	5
Galactorobin	I	355	367	12	405	50	405	50	355	0
	II	262	265	3	270	8	262	0	260	—2
Kaempferol	I	367	380	13	—	—	452	85	430	53
	II	266	275	9	270	4	266	0	265	—1



Experimental

Discontinuous stepwise acid hydrolysis of robinin. 20.0 g of robinin isolated from the flowers of Robinia viscosa was hydrolyzed with 0.16% HCl (3 *l*) at 50–60° C for 15 min. The hydrolysate was cooled and was rapidly transferred to a chromatographic column containing 0.8 kg of the polyamide Kapron. Elution was carried out with water and then with ethanol of various concentrations. The eluates were colled in 2-*l* fractions and were analyzed for their content of flavonoids in 15% acetic acid.

Fractions 4–8, on evaporation, gave a crystalline product $C_{33}H_{40}O_{19}$, mp 195–197° C, $[\alpha]_D^{20}$ −120.0° (c 1.0; pyridine), R_f in 15% acetic acid 0.77–0.80. The glycoside gave no depression of the melting point with the initial sample of robinin and it was called residue 1.

Fractions 9–10, eluted with water, contained practically no flavonoid compounds and therefore the subsequent elution of the column was carried out with ethanol of various concentrations.

Fractions 11–12, eluted with 10% ethanol, contained a flavonoid with R_f 0.69–0.70. It was impossible to achieve the crystallization of the flavonoid from the evaporated eluates of these fractions. Consequently, the substance of these fractions was rechromatographed on a column containing 0.1 kg of Kapron. A flavonoid with R_f 0.69–0.70 was obtained in the crystalline state. The flavonoid crystallized from concentrated solutions in the form of very thin matted crystals, $C_{27}H_{30}O_{15}$, mp 227–228° C. The glycoside has been named biorobin (see Tables 1 and 2).

Fractions 17–19 gave a crystalline flavonoid $C_{21}H_{20}O_{10}$, mp 232–234° C, $[\alpha]_D^{20}$ −38.2° (c 0.5; methanol). This flavonoid was characterized as β-rhamnorobin (see Tables 1 and 2, IR spectrum in the figure).

Fractions 23–24, eluted with 35% ethanol, gave a crystalline flavonoid $C_{21}H_{20}O_{11}$, mp 230–233° C, $[\alpha]_D^{20}$ −120.0° (c 0.5; methanol). This glycoside was characteriazed as galactorobin (see tables and figure).

In order to avoid the spreading of the zone of the aglycone kaempferol and its contamination with the remaining zones of the glycosides sorbed on the polyamide (absorbing UV light), the column was separated mechanically into eight fractions. The top fractions, with a bright yellow color in visible light, were heated with 60% ethanol. Evaporation of the extracts from these fractions yielded kaempferol.

The lowest two fractions contained a small amount of galactorobin and the middle fractions (the 4th and 5th) a flavonoid glycoside which, on elution with 40% ethanol and consequent evaporation, separated out in the form of a crystalline product $C_{21}H_{20}O_{10}$ with 233–236° C, $[\alpha]_D^{20}$ −200.0° (c 0.5; methanol). This glycoside was characterized as α-robinin (see tables and figure).

Second stage of the hydrolysis. Residue 1 (6.8 g) was dissolved in 1 *l* of 0.16% HCl and was hydrolyzed at 50–60° C for 30 min. Immediately after being cooled, the hydrolysate was chromatographed on a column of 0.36 kg of polyamide. The hydrolysis products were separated in the same way as in the first stage of hydrolysis. This yielded biorobin, galactorobin, kaempferol, and 2.6 g of a triglycoside $C_{33}H_{40}O_{19}$ with mp 192–195° C, $[\alpha]_D^{20}$ −88.0° (c 0.05; methanol)

and a new monoglycoside with mp 170–173° C, $[\alpha]_D^{20}$ −126.0° (c 0.05; methanol) which we have called α-rhamnoisorobin.

Third stage of the hydrolysis. The 2.6 g of the triglycoside obtained after the second stage of hydrolysis was dissolved in 0.5 *l* of 0.16% HCl at 50–60° C and was hydrolyzed for 45 min. The hydrolysate was separated on a column of 140.0 g of polyamide under the conditions described above for the first stage of the hydrolysis. In this way the hydrolysate yielded biorobin, galactorobin, kaempferol, and a new glycoside $C_{27}H_{30}O_{15}$, mp 248–250° C, $[\alpha]_D^{20}$ −80.0° (c 0.05; methanol) which we have called diorobin (see Tables 1 and 2).

Determination of the position of the carbohydrate substituents in the glycosides. The positions of the free hydroxy groups in the intermediates obtained from natural robinin that we have investigated were determined spectroscopically in the UV region. The positions of the carbohydrate substituents were established simultaneously (Table 3).

Enzymatic hydrolysis of the glycosides. Robinin, β-isorobinin, biorobin, diorobin, galactorobin, α- and β-rhamnorobins, and α-rhamnoisorobin were fermented with rhamnodiastase, emulsin, an enzyme preparation of snail pancreatic juice, etc. As a result of the hydrolysis, robinin, β-isorobinin, and biorobin yielded a biose which we have called neorobinobiose. The properties of the biose have been described previously [5].

Oxidative degradation of biorobin and robinin. Biorobin and robinin (0.2 g) were oxidized with hydrogen peroxide in an ammoniacal medium at room temperature for 4 hr. The excess of hydrogen peroxide was eliminated with lead sulfide and the products obtained were separated chromatographically. A biose identical with the neorobinobiose obtained by the enzymatic leavage of biorobin and robinin was isolated.

Conclusions

1. Several isomeric forms of triglycosides have been found in the natural flavonoid product robinin.

2. The three-stage discontinuous hydrolysis of robinin has given individual fragments in the form of mono-, bi-, and diglycosides showing the structure of the initial isomeric robinin triosides. Six intermediate glycosides have been isolated preparatively: biorobin, diorobin, α-rhamnorobin, β-rhamnorobin, α-rhamnoisorobin, and galactorobin. The structures of the glycosides isolated have been established.

3. An investigation of the intermediate products of the hydrolysis of robinin has shown that robinin contains not less than four triosides—α-and β-neorobinins and α- and β-isorobinins.

4. All the forms of the robinin triosides contain a biose at C-3 differing from the robinobiose described in the literature by the size of the oxide rings of the L-rhamnose and D-galactose. This biose, which we have called neorobinobiose, contains the furanose forms of L-rhamnose and D-galactose and also a β-configuration of the links both between the monoses and between the galactose and the kaempferol.

5. The different forms of the robinins differ from one another by the configurations of the glycosidic links and by the sizes of the oxide rings of the L-rhamnose at C-7.

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