THE FLAVONOIDS OF THE CRUCIFERAE

II. Neorobinin—a new glycoside from Cheiranthus allionii

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Glycosides of kaempferol are widely distributed in nature [1–3]. They are found in many plants in the form of mono-, di-, tri-, and polyglycosides with various sugars [4–9].

Table 1

Physicochemical Properties of Neorobinin and its Derivatives

Properties	Glycosides			
	Neorobinin	Biorobin	Rhamnorobin	Galactorobin
Form of the crystals	Thin needles	Thin needles	Plates	Thin needles
Mp, °C	195–197 (from water) 249–250 (from alcohol)	221–223 (from alcohol)	233–236 (from alcohol)	230–233 (from alcohol)
Solubility in water	Sparingly in the cold and readily in the hot	Readily in the hot	Insoluble	Insoluble
In alcohol	Very sparingly	Sparingly	Soluble	Soluble
$[\alpha]_D^{20}$, deg	−122.5 [c 1; pyridine–water (1:1)]	−75.0 (c 1; pyridine)	−200.0 (c 0.5; ethanol)	−120.0 (c 0.5; methanol)
Mol. wt.	740.0	594.0	432.0	448.0
Formula	$C_{33}H_{40}O_{19}$	$C_{27}H_{30}O_{15}$	$C_{21}H_{20}O_{10}$	$C_{21}H_{20}O_{11}$
R_f in 1) 15% acetic acid	0.77	0.69	0.16	0.16
2) butan-1-ol–acetic acid–water	0.45	0.54	0.74	0.74
Qualitative reactions				
with ferric chloride	Dark green	Green-brown	Green-brown	Green-brown
with zirconyl nitrate	Negative	Negative	Positive	Negative
Hydrolysis products				
aglycone	Kaempferol	Kaempferol	Kaempferol	Kaempferol
sugar	2 moles of rhamnose and 1 mole of galactose	1 mole of rhamnose and 1 mole of galactose	1 mole of rhamnose	1 mole of galactose

The present paper gives the results of a further study of the chemical structure of a flavonoid glycoside, isolated from Cheiranthus allionii Hort. (plains erysimum) and other plants, that we had previously identified as robinin [10]. As can be seen from Table 1, neorobinin (I) is a trioside derivative of kaempferol containing two molecules of L-rhamnose and one molecule of D-galactose.

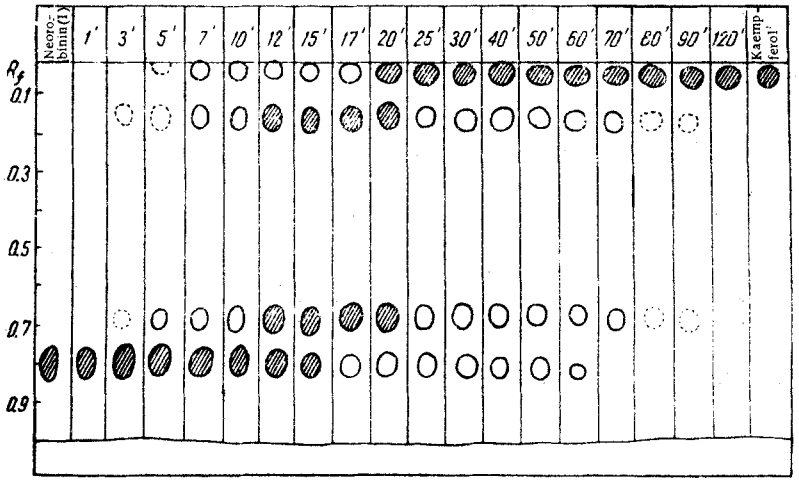
A spectroscopic investigation of this glycoside and its aglycone (Table 2) in the UV region using ionizing and complex-forming reagents [3, 11] shows that there are two free hydroxy groups in the glycoside in the 5 and 4' positions Consequently, the carbohydrate substituents in neorobinin are in positions 3 and 7.

Table 2

Physicochemical Characteristics of Neorobinin and its Derivatives

Solutions and reagents	Absorption bands	Neorobinin		Biorobin		Rhamnorobin		Galactorobin		Kaempferol	
		absorption maxima (λ, mμ) and shifts ($\Delta\lambda$)									
		λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$
2×10^{-5} M solution in anhydrous ethanol	I	352	—	355	—	365	—	355	—	367	—
	II	268	—	265	—	260	—	265	—	266	—
The same + sodium acetate	I	350	—2	375	20	365	0	365	10	380	13
	II	270	2	275	10	260	0	270	5	275	9
The same + sodium ethoxide	I	400	48	400	45	—	—	405	50		—
	II	270	2	275	10	260	0	272	7	270	4
The same + zirconyl nitrate	I	350 400	48	395	40	375 445	80	400	45	452	85
	II	270	2	265	0	255	—5	26.)	—5	265	—1
The same + zirconyl nitrate and citric acid	I	350	—2	345	—10	360 430	65	355	0	420	53
	II	260	—8	260	—5	270	10	265	0	270	4

To investigate the glycoside (I), in detail, we used partial acid and enzymatic hydrolysis. On acid hydrolysis of neorobinin with dilute hydrochloric acid it was found that, in addition to the aglycone, products of its partial hydrolysis were found. Complete acid hydrolysis with 0.16% hydrochloric acid took place in 1.5 hr. The hydrolysis products were analyzed by paper chromatography (cf. the chromatogram of the products of the stepwise acid hydrolysis of neorobinin).



The results of the stepwise hydrolysis (see figure) show that the maximum amount of intermediate products appears after 15–20 min. Consequently, using the results obtained, we carried out the stepwise hydrolysis of the glycoside (I) under the optimum conditions (14–15 min at 50°–60° C) and separated the products on columns of polyamide sorbent. The initial glycoside and four new substances (cf. Table 1) were isolated. A chemical investigation showed that three substances were glycosides and the fourth the aglycone, which was identified as kaempferol (IX) [10]. On acid hydrolysis of the most polar of the three glycosides, kaempferol and two monosaccharides, D-galactose (IV) and L-rhamnose (V), were obtained. Spectroscopic data (Table 2) showed that this glycoside contains free phenolic hydroxy groups in positions 5, 7, and 4'. Consequently, this compound is a 3-bioside of kaempferol, which we have called biorobin (II). By stepwise hydrolysis, the bioside was hydrolyzed to the 3-monoglycoside, in which the carbohydrate component was identified as D-galactose. Hence, it may be concluded that the aglycone in biorobin is directly linked to the D-galactose and the latter to the L-rhamnose. The biose of the glycoside (II) was split off by the enzymes of rhamnodiastase and compared with the known bioses (Table 3).

In respect to the coloration of the spots with various reagents and its chromatographic mobility, the biose (VIII) has much in common with robinobiose. The same biose (VIII) was obtained by the direct enzymatic hydrolysis of neorobinin. The second component of the enzymatic hydrolyzate of neorobinin was a monoglycoside of kaempferol which was also obtained by the acid hydrolysis of the glycoside (I). This glycoside contains free phenolic groups in positions 3, 5, and 4', and on acid hydrolysis it gives kaempferol and L-rhamnose. Consequently, it is kaempferol 7-rhamnoside (III), which we have called rhamnorobin.

Table 3

Paper Chromatographic Characteristics of the Biose Obtained by the Enzymatic Hydrolysis of Neorobinin and Biorobin

Sugar	R_f with respect to glucose	Revealing agent and coloration of the spots on the chromatogram		
		Aniline phthalate	DPA + p-anisidine	DPA + urea
Rutinose	0.85	Brown	Green	Pink
Robinobiose	0.78	Brown	Green	Pink
Biose of zhealin	1.11	Orange-brown	Yellow	No coloration
Biose of neorobinin and biorobin	0.80	Brown	Green	Pink
Rhamnose	2.00	Red-brown	Yellow	Pink

Note: Solvent system: butan-1-ol-acetic acid—water (4:1:5). DPA = diphenylamine.

The last of the glycosides formed in the acid hydrolysis of neorobinin is similar to the monoglycoside obtained by the stepwise hydrolysis of biorobin. This monoside contains three hydroxy groups in positions 5, 7, and 4', and the carbohydrate component is D-galactose. Thus, glycoside (IV), galactorobin, may be preliminarily characterized as kaempferol 3-galactoside.

The configuration of the glycosidic links and the sizes of the oxide rings in the monoglycosides were analyzed by comparing the molecular rotations with those of the corresponding phenyl glycosides [12] (Table 4). The results of the comparison of the molecular rotations shows the α-configuration of the glycosidic bond and the furanose form of L-rhamnose in rhamnorobin (III) and the β-glycosidic bond and the furanose form of the D-galactose in galactorobin (IV).

The configuration of the glycosidic bond and the form of the second mole of L-rhamnose in neorobinin and biorobin, together with the structure of the carbohydrate moiety in rhamnorobin and galactorobin, were investigated by IR spectroscopy [12] (Table 5).

The results of IR spectroscopy confirm the conclusions made on the basis of the molecular rotations, that the glycoside (III) is kaempferol 7-α-L-rhamnofuranoside, and the glycoside (IV) is kaempferol 3-β-D-galactofuranoside. The spectral characteristics of the rhamnose in biorobin likewise show the β-configuration of the glycosidic bond and its furanose form.

Table 4

Analysis of the Molecular Rotations of the Carbohydrate Moieties of the Glycosides Rhamnorobin (III) and Galactorobin (IV)

Glycoside	$[M]_D$	K_{pH}	$[M]_D \cdot K_{pH}$	A	B
Kaempferol 7-L-rhamnoside (III)	−864.0	0.55	−475.0	α	Furanoside
Phenyl L-rhamnoside	−410.0	1.00	−410.0	α	
Kaempferol 3-D-galactoside (IV)	−538.0	0.57	−307.0	β	
Phenyl D-galactoside	−379.0	1.00	−379.0	β	

Note: A) configuration of the glycosidic bond; b) size of the oxide ring.

Thus, from the results obtained biorobin can be characterized as kaempferol 3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside), and neorobinin as kaempferol 7-α-L-rhamnofuranosido-3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside). Moreover, these results show that the biose of neorobinin is not identical with robinobiose [1, 14] and the glycoside that we have obtained may therefore be considered as an isomeric form of robinin [13, 14].

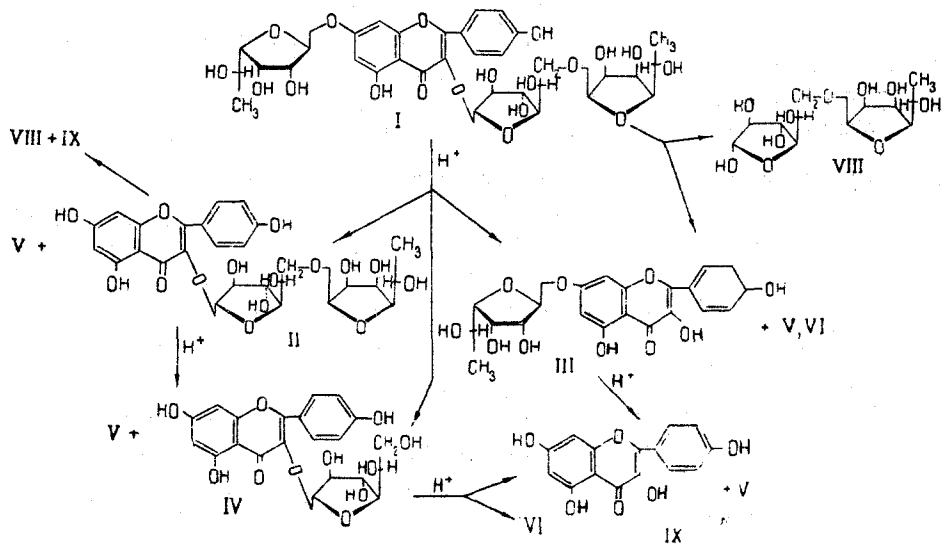


Table 5

Absorption Bands in the IR Spectrum of the Carbohydrate Part of the Kaempferol Glycosides Studied (λ_{max}, cm^{-1})

Glycoside	Ring vibrations of the furanoses	Configuration of the bonds	
		α	β
Kaempferol 7-L-rhamnoside (III)	1064 and 1032	840	–
Kaempferol 3-D-galactoside (IV)	1064 and 1032	–	895
The rhamnosidic moiety of biorobin (III)*	1083 and 1052	–	895

*The rhamnose in biorobin was investigated with the exclusion of the contribution of the 3-galactoside [12].

Experimental

The following systems were used for paper chromatography. 1) Butan-1-ol–acetic acid–water (4:1:5); 15% acetic acid.

Isolation of neorobinin. Four kilograms of the defatted seeds of Cheiranthus allionii was extracted with alcohol (3 × 12 *l*). The alcoholic extract was evaporated, the residue was dissolved in 2 *l* of water, and the solution was purified on polyamide (1.5 kg). The sorbent was washed with water until the reaction for cardiac glycosides was negative. Then the flavonoids were eluted with 20% ethanol (15 *l*). The eluates were concentrated to small bulk and rechromatographed on a column containing 1.0 kg of kapron powder. The flavonoids were eluted with water. The course of the separation was followed by means of qualitative reactions and paper chromatography in system 2. The first eluates, which contained a glycoside with R_f 0.77, were collected and evaporated in vacuum to small bulk. When the concentrated solution was allowed to stand, almost colorless, acicular crystals of neorobinin separated out, and after recrystallization from water these had mp 195°–197° C, $[\alpha]_D$ −122.5° C [c 1; pyridine–water (1:1)].

Found, %: C 53.78; H 5.28. Calculated for $C_{33}H_{40}O_{19}$, %: C 53.51; H 5.40.

Acid hydrolysis of neorobinin (I) to kaempferol (IX). A solution of 1.0 g of neorobinin in 200 ml of hot water was mixed with 3 ml of concentrated hydrochloric acid and hydrolysis was carried out for 30 min in a water bath. The

aglycone which separated on cooling (0.37 g) was recrystallized from dilute alcohol. Mp 277°–278° C, composition $C_{15}H_{10}O_6$. The aglycone was found to be identical in properties and with respect to degradation products with an authentic sample of kaempferol.

The acidic aqueous hydrolysate was neutralized with a 5% solution of alkali and evaporated in vacuum. The residue was extracted with ethanol (5 × 5 ml), the solution was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in 1 ml of ethanol, and the solution was analyzed chromatographically on paper in system 1. D-Galactose and L-rhamnose were detected in the hydrolysate. By quantitative chromatography of the sugars, it was established that neorobinin contained 2 moles of L-rhamnose and 1 mole of D-galactose.

Enzymatic hydrolysis of neorobinin (I) to rhamnorobin (III). A solution of 0.2 g of neorobinin in 200 ml of hot water was cooled to 35°–40° C, and 0.1 g of a preparation of rhamnodiastase was added. The mixture was left in a thermostat for fermentation for 4 hr. The hydrolysis products were analyzed chromatographically in systems 1 and 2. In both systems a new substance, with R_f 0.74 in system 1 and R_f 0.16 in system 2, was found in addition to the initial glycoside. Analysis of the carbohydrates showed the presence of a biose similar in chromatographic behaviour and qualitative reactions [diphenylamine (DPA) + urea, and diphenylamine + p-anisidine] to the robinobiose from robinin.

The fermentation mixture was heated to boiling and, after cooling and the separation of the enzyme precipitate, it was chromatographed on a column containing 0.2 kg of kapron. The flavonoids were eluted with water and the unchanged residue of neorobinin was separated. Subsequent elution with 50% ethanol gave a fraction containing a flavonoid with R_f 0.16 in system 2. The eluates were evaporated in vacuum to small bulk. The precipitate which separated out on cooling was recrystallized from dilute alcohol. This gave a substance with mp 233°–236° C, $[\alpha]_D$ −200° C (c 0.5; methanol).

Found, %: C 57.90; H 4.53. Calculated for $C_{21}H_{20}O_{10}$, %: C 58.33; H 4.63.

The flavonoid gave a positive reaction with zirconyl nitrate and citric acid, and a greenish brown coloration with ferric chloride, and it reduced an ammoniacal solution of silver nitrate. The acid hydrolysis of this substance with 0.5% hydrochloric acid solution gave kaempferol and L-rhamnose. For the results of spectroscopic investigations, see Table 2.

Partial hydrolysis of neorobinin (I) to biorobin (II), rhamnorobin (III), and galactorobin (IV). (Data on the determination of the optimum conditions for the stepwise hydrolysis are given in the figure.) The preparative isolation of the partial hydrolysis products was carried out as follows: 20.0 g of neorobinin was dissolved in 5 *l* of hot water, and 24 ml of concentrated hydrochloric acid was added to the solution; hydrolysis was carried out at 50°–60° C for 14–15 min. The hydrolysate was rapidly cooled to 20° C and chromatographed on a column containing 1.2 kg of kapron. The flavonoid compounds were eluted with water and alcohol of various concentrations. The first 9 *l* of eluate, which did not contain the substances under investigation, was discarded, and the subsequent eluates were collected in 2-*l* fractions and analyzed by paper chromatography in system 2. Fractions containing individual flavonoid components were combined, evaporated, and crystallized from water or dilute alcohol. Fractions 1–6 gave 2.3 g of the initial glycoside, fractions 8–10 1.1 g of biorobin, fractions 18–20 2.6 g of rhamnorobin, and fractions 28–30 0.3 g of galactorobin. The remaining fractions contained mixtures of glycosides and were used for reseparation.

Biorobin (II). The glycoside crystallized from the evaporated aqueous alcoholic eluate in the form of thin needles. After recrystallization from hot water, a substance was obtained with mp 221°–223° C, $[\alpha]_D$ −75.0° C (c 1; pyridine) (other properties are shown in Table 1).

Found, %: C 54.62; H 5.60. Calculated for $C_2H_{30}O_{15}$, %: C 54.54; 5.55.

The acid hydrolysis of 0.5 g of biorobin gave 0.23 g of the aglycone with mp 277°–278° C. The acid hydrolysate was treated exactly as described for neorobinin, and the sugars were analyzed. D-Galactose and L-rhamnose were found in a ratio of 1:1. The stepwise hydrolysis of biorobin with 0.16% hydrochloric acid gave a small amount of a monoglycoside and a considerable amount of the aglycone. Enzymatic hydrolysis with rhamnodiastase was carried out as for neorobinin. The aglycone, kaempferol, and a biose similar to robinobiose were obtained (Table 5).

Rhamnorobin (III). The glycoside was isolated in the form of an amorphous powder when the eluates were evaporated to small bulk. After crystallization from dilute alcohol, the glycoside had mp 233°–236° C, $[\alpha]_D$ −200° C (c 0.5; methanol) (other properties are shown in Table 1).

Found, %: C 57.98; H 4.53. Calculated for $C_{21}H_{20}O_{10}$, %: C 58.33; H 4.63.

The acid hydrolysis of 0.2 g of rhamnorobin gave 0.11 g of kaempferol and L-rhamnose.

Galactorobin (IV). The glycoside, which was obtained in the amorphous state by the evaporation of the eluates, was crystallized from water–alcohol, mp 230°–233° C, $[\alpha]_D$ −120.0° C (c 0.5; methanol).

Found, %: C 56.31; H 4.48. Calculated for $C_{21}H_{20}O_{11}$, %: C 56.25; H 4.46.

The properties of the glycoside are given in Tables 1, 2, and 5. Acid hydrolysis yielded kaempferol (0.06 g from 0.1 g of the glycoside) and D-galactose.

Summary

1. A new glycoside neorobinin has been isolated from the seeds of Cheiranthus allionii. On the basis of the results of chemical and spectroscopic investigations, the structure of kaempferol 7-α-L-rhamnofuranoside-3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside) has been proposed for it.

2. On partial hydrolysis, neorobinin is split into a series of intermediate substances, the following of which have been isolated and characterized: kaempferol 7-α-L-rhamnofuranoside (rhamnorobin), kaempferol 3-β-D-galactofuranoside (galactorobin), and kaempferol 3-(β-D-galactofuranosyl-6-β-L-rhamnofuranoside) (biorobin).

REFERENCES

1. W. Karrer, Vorkommen und Konstitution der Organische Pflanzestoffe, Birkhäuser Verlag, 579, 1958.
2. T. A. Geissman, in: Biochemical Methods of Plant Analysis [Russian translation], Moscow 453, 1960.
3. T. A. Geissman, The Chemistry of Flavonoid Compounds, Pergamon Press, N. Y., 1962.
4. H. J. Gehrmann, L. Endress, R. Cobet, and U. Fiedler, Naturwiss., **42**, 181, 1955.
5. V. N. Spiridonov, A. P. Prokopenko, and D. G. Kolesnikov, ZhOKh, **34**, 4128, 1964.
6. H. Wagner and R. Luck, Naturwiss., **42**, 607, 1965.
7. M. Kanao, M. Shimokoriyama, Acta and Phytochim., **15**, 229, 1949.
8. N. Waliaschko, Arch. Pharm., **242**, 210, 1904; **247**, 447, 1909.
9. F. E. Mumford, D. H. Smith, and P. G. Heytler, Biochem. J., **91**, 517, 1964.
10. N. P. Maksyutina, KhPS, 63, 1961.
11. V. I. Litvinenko and N. P. Maksyutina, KhPS [Chemistry of Natural Compounds], 420, 1965.
12. I. P. Kovalev and V. I. Litvinenko, KhPS [Chemistry of Natural Compounds], 233, 1965.
13. G. Zemplen and R. Bognar, Ber., **74**, 1783, 1941.
14. J. B. Harborne, Biochemistry of Phenolic Compounds, Acad. Press, N. Y., 134, 1964.

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