THE FLAVONOIDS OF THE CRUCIFERAE I; ROBININ AND ZHEALIN FROM THE FRUIT OF CHEIRANTHUS ALLIONII

N. P. Maksyutina

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The flavonoid compounds of the plants of the family of Cruciferae have been very little investigated. In the literature there are only a few communications on the detection in plants of this family of derivatives of quercetin and isorhamnetin [1-3].

The fruit of Cheiranthus allionii (plains Erysimum), which we have previously investigated for their content of cardiac glycosides [4], contain, in addition to cardiac glycosides, a considerable amount of flavonoid compounds. It has been established that the flavonoids in the fruit are mainly glycosides of kaempferol, quercetin, and isorhamnetin, Using the method of chromatography on a polyamide, we have isolated from the fruit of the wallflower two flavonoid glycosides – a trioside of kaempferol identified as robinin (kaempferol-7-rhamno-3-galactorhamnoside in [5] and a new diglycoside which we have named zhealin (Table 1).

The chemical investigation of zhealin has shown that it is a derivative of quercetin with an original sugar component – a biose consisting of rhamnose and arabinose. We have found no information on the occurrence in natural compounds of flavonoids of this type of biose, consisting of a pentose and a methylpentose, in the literature available to us.

Zhealin, in contrast to many known biosides, is readily soluble in water which, in all probability, is due to the peculiarity of the structure of the sugar component. It hydrolyzes with 1% HCl with the formation of quercetin, rhamnose, and arabinose. On hydrolysis with 1% formic acid, in addition to quercetin, rhamnose, and arabinose, a certain amount of a monoglycoside identified as the arabinoside of quercetin is formed.

The oxidation of zhealin with hydrogen peroxide leads to the separation of the biose. Consequently, arabinose and rhamnose in the form of the biose can be present only at C-3, with the arabinose directly connected to the quercetin and the rhamnose being terminal. Spectroscopic investigations in UV light confirmed this. The data given in Table 2 show that the hydroxyl at C-3 is substituted by a sugar. The hydroxyls at C-5, C-7, C-3', and C-4' in zhealin are free. In the aglycone, four hydroxyls are free.

The biose isolated after the oxidation of zhealin with hydrogen peroxide was compared chromatographically with the bioses and monoses most frequently found in the flavonoids. The nature of the linkage of the sugars was determined from the coloration with specific reagents [6] and with respect to the action of various enzymes. It was established that neither zhealin nor the biose is decomposed by enzyme preparations from the fungus Aspergillus oryzae or the snail or by rhamnodiastase. This indicates, to some extent, the absence of a β-linkage in the samples of the sugars investigated.

The data of Table 3 show that the biose of zhealin is more mobile in the system studied than glucose, rutinose, and robinobiose. From the coloration of the biose of zhealin with reagents, a 1-2 linkage between the sugars may be assumed.

Taking into account the velocity of hydrolysis of zhealin and desrhamnozhealin and the data obtained in the chemical investigation, the new flavonoglycoside zhealin can be characterized as quercetin 3-α-1-arabopyranosido-2-α-1-rhamnofuranoside.

Experimental

Isolation and investigation of robinin: 5 kilograms of the seeds of Ch. allionii were extracted three times with 15-liter portions of a mixture of chloroform and alcohol (1:1). The extracts were evaporated and the residue was treated 8-11 times with 1-liter portions of ether and 3-4 times with 1-liter portions of chloroform to eliminate the fatty materials. The residue was treated with 0.8 liter of water and the solution was chromatographed on a column of 2.0 kg of Kapron. Elution was performed with water, 0.5-liter fractions being collected. The eluates with a positive reaction for flavonoids were combined and analyzed chromatographically in 15% acetic acid. The fractions containing a flavonoid with R_f = 0.82 were combined and evaporated in vacuum to small volume. After the evaporated eluate had cooled, needle-like crystals of the flavonoid deposited, and these were separated off and recrystallized from hot water. The yield of flavonoid after recrystallization was 5.2 g. Mp 195-197° (from water) or 248-250° (from alcohol).

Found %: C 60.47; H 6.23. $C_{33}H_{40}O_{19}$. Calculated %: C 60.99; H 6.06.

Hydrolysis of 0.50 g of the flavonoid with 1% HCl for 30 min in the water bath gave 0.21 g of aglycone with mp 277-278°, found to be identical with an authentic sample of kaempferol (Table 1). After neutralization, the acid hydrolyzate was analyzed by paper chromatography qualitatively and quantitatively for its content of sugars in the butanol-acetic acid-water (4:1:5) system. It was found to contain two moles of rhamnose and one mole of galactose.

Table 1

Physicochemical properties of the flavonoids of Ch. allionii and the products of their hydrolysis.

Properties	Zhealin	Desrhamnoside of zhealin	Aglycone of zhealin	Robinin	Aglycone of robinin
Mp, °C	197-199	234-236	308-311	195-197	177-278
Solubility					
In water	Good	Poor	Very poor	Poor	Very poor
In alcohol	Good	Soluble	Soluble	Very poor	Soluble
In ether	Insoluble	Poor	Good	Insoluble	Good
Formula	$C_{26}H_{28}O_{15}$	$C_{20}H_{18}O_{11}$	$C_{15}H_{10}O_7$	$C_{33}H_{40}O_{19}$	$C_{15}H_{10}O_6$
Mol. wt.	580.6	434.2	302.0	660.3	286.4
Specific optical activity	-85° (in water)	-	-	-120.4° (pyridine-ethanol 1:1)	-
Reaction with zirconyl	-	-	+	-	+
Reaction with $FeCl_3$	Dark green	Dark Green	Brown-green	Brown	Green
Reaction with lead acetate	Yellow precipitate	Yellow precipitate	Orange precipitate	Yellow solution	Yellow solution
R_f in 15% acetic acid	0.60	0.20	0.03	0.82	0.05
R_f in benzene-ethyl acetate-acetic acid-formamide (23.5:74.5:2:1) system	0.00	0.26	0.64	0.00	0.72
Hydrolysis with 1% HCl	Quercetin +rhamnose	Quercetin + arabinose	-	Kaempferol + rhamnose +galactose	-
Hydrolysis with 1% HCOOH	Desrhamnozhealin +rhamnose + arabinose + quercetin	Quercetin + arabinose	-	The same	-
Oxidation with H_2O_2	Arabinosidorhamnose	Arabinose	-		Phloroglucinol + p-hydroxybenzoic acid
Hydrolysis with 1% HCOOH	Desrhamnozhealin +rhamnose + arabinose +quercetin	Quercertin +arabinose	-	The same	-
Oxidation with H_2O_2	Arabinosidorhamnose	Arabinose	-	Rabinobiose	-
Hydrolysis with 20% KOH	-	-	Phloroglucinol +pyrocatechuic acid		Phloroglucinol + p-hydroxybenzoic acid

0.3 g of the flavonoid was subjected to the enzymatic action of 0.2 g of rhamnodiastase at 38° for 24 hr. The products of enzymatic hydrolysis were analyzed for their contents of sugars in the butanol-acetic acid-water (4:1:5) system and for their content of phenols in the benzene-ethyl acetate-acetic acid-formamide (23.5:74.5:2:1) system.

The analysis showed the presence of robinobiose, rhamnose, and kaempferol.

Table 2

UV spectra of zhealin and its aglycone.

Medium (ethanolic solution +...)	Zhealin		Aglycone of zhealin	
	I	II	I	II
Ethanolic solution +				
$0.5 \cdot 10^{-5}$ mole λ_{max}, mμ	355	255 265	372	256
NaAc λ, mμ	375	265	380	275
Δλ, mμ	+ 20	0	+ 8	+ 19
H_3BO_3 + NaAc λ_{max}, mμ	375	261	390	256 261
Δλ, mμ	+ 20	— 4	+ 18	+ 5
Na ethoxide λ_{max}, mμ	405 335	273	320	245 266
Δλ, mμ	+ 50	+ 8	— 52	+ 10
$AlCl_3$ λ_{max}, mμ	305 410	275	365 430	270
Δλ, mμ	+ 55	+ 10	+ 58	+ 14
$AlCl_3$ + HCl λ_{max}, mμ	300 405	270	300 430	266
Δλ, mμ	+ 50	+ 5	+ 58	+ 10

The flavonoid (0.2 g) was oxidized with H_2O_2 by a published method [6]. After oxidation, 0.04 g of robinobiose was obtained in the form of an amorphous hygroscopic powder. It was shown to be identical with an authentic sample of robinobiose. The data obtained in the comparison agreed completely with those given in the literature. The identity was also confirmed directly by comparison with an authentic sample of robinin.

Table 3

Paper-chromatographic characteristics of the biose of zhealin and comparison sugars.

Sugar	R* glucose	Coloration in the staining agent		
		Aniline phthalate	Diphenylamine + p-ansidine	Diphenylamine + urea
Rutinose	0.85	Brown	Green	Pink
Robinobiose	0.78	Brown	Green	Pink
Biose of zhealin	1.11	Pink-orange	Yellow	No color
Glucose	1.00	Brown	Green	Pink
Rhamnose	2.02	Red-brown	Yellow	Pink
Arabinose	1.45	Red	Yellow-green	Red

* In the butanol-acetic acid-water (4:1:5) system.

Isolation and investigation of zhealin. After the isolation of robinin, the flavonoids were eluted from the column with 20% ethanol, 0.5-liter fractions being collected. The eluates were analyzed by chromatography in 15% acetic acid. The fractions containing a flavonoid with R_f = 0.60 were combined and evaporated to small volume. Since crystallization of the flavonoid did not set in even after prolonged standing, the evaporated eluate was rechromatographed on a column containing 0.4 kg of Kapron. Elution was first carried out with water, and the fractions not containing the flavonoid with R_f = 0.60 were rejected. When zhealin appeared in the eluate, elution was continued with 20% ethanol.

The combined eluates were evaporated in vacuum to small volume (approximately 10 ml) and left for crystallization. On standing, very fine needles deposited, and these were filtered off, washed with a small amount of cold water, and dried in a high vacuum over P_2O_5 for 3 hr at 100°. After drying, the mp was 197-199°, the yield of crystalline flavonoid being 2.7 g. The glycoside is very readily soluble in water and alcohol, more sparingly soluble in ethyl acetate, and insoluble in ether and chloroform (Table 1).

Found %: C 53.52; H 4.93. $C_{26}H_{28}O_{15}$. Calculated %: C 53.79; H 4.82.

With a check on the hydrolysis products in 15% acetic acid every minute, 0.1 g of zhealin was hydrolyzed with 1% HCl for 5 min in the water bath. Crystals of the aglycone in the form of yellow needles with mp 308-311° deposited from the still hot hydrolyzate (Table 1).

After neutralization, the acid hydrolyzate was evaporated to dryness and treated with ethanol. The ethanolic

solution was analyzed for its content of sugars with a set of standard sugars. Rhamnose and arabinose were found in the zhealin hydrolyzate.

A solution of 20 mg of the aglycone of zhealin in 20 ml of 20% KOH solution was heated for 30 min in the boiling water bath, rapidly cooled, and neutralized with 10% HCl to pH 5, and then extracted several times with 10 ml portions of ether. The ethereal extracts were evaporated and the residue, after dissolution in the minimum amount of alcohol, was chromatographed in the benzene-ethyl acetate-acetic acid-formamide (23.5:74.5:2:1) system. Various phenols and phenolic acids were used as standards. The products of alkaline degradation were found to contain phloroglucinol and pyrocatechuic acid, which shows the presence in the aglycone of free hydroxyls at C-5, C-7, C-3', and C-4'. Spectroscopic investigations in UV light confirmed the presence of free hydroxyls in these positions of the aglycone of zhealin (Table 2). The aglycone of zhealin is identical in all its properties with quercetin and gives no depression of the melting point with an authentic sample.

Zhealin (0.54 g) was oxidized with H_2O_2 by a published method [6]. The oxidation products were purified on a column containing 20 g of neutral alumina. This gave 0.12 g of an amorphous powder of the biose. $[\alpha]_D^{22} = 0.00$, after 3 hr, -11.0 (water).

Found %: C 43.88; H 6.91. $C_{11}H_{20}O_9$. Calculated %: C 44.59; H 6.68.

The biose was analyzed chromatographically and compared with rutinose and robinobiose (Table 3). Hydrolysis of the biose with 1% HCl gave 1 mole of rhamnose and 1 mole of arabinose.

Acetylation of 0.05 g of the biose was carried out in a mixture of 1 ml of absolute pyridine in 1 ml of acetic anhydride. After 24 hr acetylation at room temperature, the mixture was poured into 100 ml of ice water. The precipitate which deposited was filtered off and washed with a small amount of ice water. This gave 0.028 g of an amorphous powder with $[\alpha]_D^{20}$ -25.4° (chloroform).

Found %: C 49.56; H 5.92. $C_{23}H_{32}O_{15}$. Calculated %: C 50.36; H 5.83.

A solution of 0.50 g of zhealin in 10 ml of water was treated with 0.1 ml of concentrated formic acid. The solution was heated for 1 hour at 65-70°. The hydrolyzate was deposited on a sheet of chromatographic paper (Whatman 33) and chromatographed in 15% acetic acid. The chromatogram was dried and the strip of the glycoside with $R_f = 0.22$ was cut out. The glycoside was extracted from the paper with 50% ethanol with heating and the extract was evaporated to small volume and left for crystallization. The yellow needles with mp 220-224° which deposited were recrystallized from alcohol. After recrystallization, the glycoside melted at 234-236°. Yield 0.038 g (Table 1).

Found %: C 55.48; H 4.20. $C_{20}H_{18}O_{11}$. Calculated %: C 55.30; H 4.15.

The glycoside obtained (10 mg) was hydrolyzed with 1% HCl with heating for 5 min. Crystals completely identical with quercetin deposited. After neutralization, the acid hydrolyzate was analyzed for its content of sugars. Only arabinose was found in the hydrolyzate.

SUMMARY

1. The triglycoside robinin and a new diglycoside, zhealin, have been isolated from the fruit of Cheiranthus allionii.

2. Zhealin has been identified as quercetin 3-α-arabopyranosyl-2-α-1-rhamnofuranoside.

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