UDC 547

FLAVONOIDS OF THE CRUCIFERAE

III. Glycosides of the Flowers of Syrenia Siliculosa

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We have studied the flowers of Syrenia siliculosa Andr. Z. which contain a considerable amount of flavonoid compounds as well as cardiac glycosides. The qualitative flavonoid composition of the flowers of this plant was established by two-dimensional paper chromatography. No less than eight flavonoid substances were detected.

A comparison of the intensities of the colorations after the spots had been revealed showed that Syrenia flowers contain the substances denoted on the plan of the two-dimensional chromatogram by the numbers 5 and 6 in considerably larger amounts than the other six flavonoids.

UV Spectra of Flassilin and its Derivatives

Solutions and reagents	Flassilin		Flassilin monoglycosides		Flassilin aglycone	
	Bands λ_{max}, mμ					
	I	II	I	II	I	II
$2 \cdot 10^{-5}$ M in anhydrous ethanol	360	265	365	266	371	255
The same + sodium acetate	375	265	376	265	382	258
Δλ	15	0	11	−1	11	−2
The same + sodium ethoxide	405	268	408	267	—	255
Δλ	45	3	43	1	—	0
The same + zirconyl nitrate	415	267	410	268	458	265
Δλ	55	2	45	2	87	10
The same + zirconyl nitrate + citric acid	358	266	364	266	430	260
Δλ	−2	1	−1	0	59	5
The same + boric acid	382	272	383	274	396	265
Δλ	22	7	18	8	25	10

By chromatography on Kapron, three flavonoid compounds were isolated from Syrenia flowers. Stepwise acid and enzymatic hydrolysis, the alkaline degradation of the aglycones, and the oxidation of the glycosides with hydrogen peroxide enabled two of them to be identified with glycosides isolated previously, pasternoside and desglucopasternoside [1]. The third glycoside, which we have called flassilin, is a new compound, in all probability. The aglycone of flassilin was identified as quercetin, and the sugar component was found to contain one molecule each of glucose and rhamnose. When flassilin was treated with dilute acid, stepwise hydrolysis took place with the formation first of quercetin monoglucoside and then of the aglycone.

The position of the sugar component was established by the oxidation of flassilin and of desglucoflassilin with hydrogen peroxide [2], by the reaction of the two glycosides with zirconyl nitrate and citric acid [3], and by their UV absorption spectra. The details of the UV spectra, which are given in the table, showed that the sugar component is present at C-3 in the form of a glucosidorhamnose.

The linkage of the sugars in the glycoside was established by treating the glucoside with a specific snail enzyme preparation [4]. In the first minutes of hydrolysis quercetin monorhamnoside and glucose were found, and after 5-6 min quercetin and rhamnose appeared in the hydrolyzate. This indicates a β-glycosidic linkage of the two monoses and that the rhamnose is directly attached to quercetin.

The high rate of acid hydrolysis with dilute acid indicates the furanose form of the monoses present in flassilin, which is also confirmed by the IR spectra (Fig. 2) and by a comparison of molecular rotations.

	$[M]_D$	K_{Ph}	$[M_D] \cdot K_{Ph}$	ΔC	K_M	$\Delta C \cdot K_M$
Flassilin	−528.0	0.60	−317.0	−219.0	0.74	−162.0
Desglucoflassilin	−180.0	0.54	−98.0	—	—	—
Methyl β-D-glucofuranoside	−148.0	1.0	−148.0	—	—	−14

$$K_{Ph} = \frac{\text{Mol. rot. of phenyl glucoside}}{\text{Mol. rot. of flavone glucoside}}; \quad K_M = \frac{\text{Mol. rot. of methyl glucoside}}{\text{Mol. rot. of phenyl glucoside}}.$$

Thus, the results obtained enable flassilin to be characterized provisionally as quercetin 3-(β-L-rhamnofuranosyl-β-D-glucofuranoside).

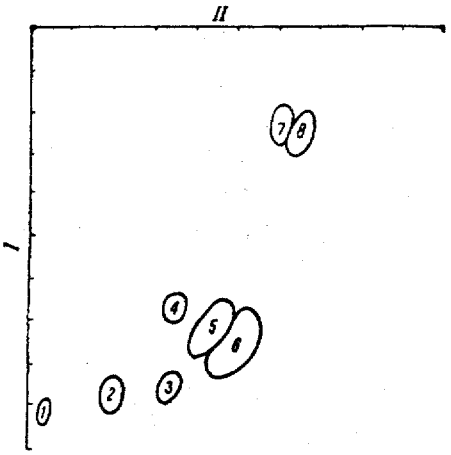

Fig. 1. Two-dimensional paper chromatogram of an extract of flowers of Syrenia siliculosa. I) 15% acetic acid; II) butanol–acetic acid–water (4:1:5). 5) pasternoside; 6) flassilin; 7) deglucopasternoside.

Experimental

Chromatographic analysis. The flowers of Syrenia siliculosa (20 g) were extracted three times with 70% ethanol (60-ml portions), the extract was evaporated in vacuum, the residue was dissolved in 10 ml of hot water, and the solution was chromatographed on Gosznak paper (30 × 30 cm). The chromatograms were revealed with 10% methanolic alkali (see Fig. 1).

Isolation and separation of the flavonoids flassilin, pasternoside, and desglucopasternoside. Two kilograms of Syrenia siliculosa flowers were extracted with two 10-*l* portions of ethanol. The ethanolic extracts were evaporated in vacuum and the residue was treated several times with 0.5-*l* portions of water. The aqueous extracts were filtered, purified with ether, and chromatographed on a column containing 0.8 kg of Kapron. The column was eluted with water and then with ethanol, 1-*l* fractions being collected from the moment when flavonoids appeared in the eluate.

Fractions 1–6 contained the flavonoid flassilin. After they had been evaporated to small bulk, bright yellow crystals with mp 182–185° C deposited. Fractions 9–15 contained pasternoside, which crystallized from the evaporated eluates in the form of light yellow needles with mp 235–237° C. Fractions 9–23, eluted from the column with 40% ethanol, contained desglucopasternoside, which crystallized in the form of short yellow needles with mp 222–224° C.

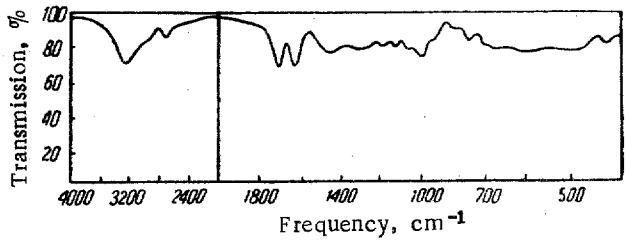

Fig. 2. IR spectrum of flassilin.

Flassilin. The glycoside is sparingly soluble in water and alcohol and insoluble in ether, gives a dark green coloration with ferric chloride, a bright yellow coloration with alkali, reduces ammoniacal silver nitrate solution, and does not give a positive reaction with zirconyl chloride and citric acid. $[\alpha]_D^{20}$ −66.5° (c 1.0; dimethylformamide).

Found, %: C 56.12; H 4.58. Calculated for $C_{27}H_{26}O_{14}$, %: C 55.92; H 4.53.

Acid hydrolysis of flassilin. With heating, 0.5 g of the glycoside was dissolved in 200 ml of water, and then 3 ml of hydrochloric acid was added in the solution and it was heated for 15 min. The precipitate that deposited was filtered off, washed with water and dried. Yield of the aglycone 0.25 g (50% on the glycoside).

The aglycone had mp 314–315° C and composition $C_{15}H_{10}O_7$; after recrystallization from aqueous alcohol it gave no depression of the melting point with an authentic sample of quercetin and had an identical R_f value in the benzene–ethyl acetate–acetic acid–formamide (23.5:74.5:2:1) systems. The products of alkaline degradation confirmed the identity of flassilin aglycone as quercetin.

The acid hydrolyzate was treated in the usual way [2] and was analyzed in the butanol – acetic acid – water (4:1:5) and pyridine – ethyl acetate – acetic acid – water (1:4:1:5) systems. The hydrolyzate gave two spots with R_f values corresponding to rhamnose and glucose. 0.5 g of flassilin was dissolved in 200 ml of hot water, treated with 1 ml of hydrochloric acid, and hydrolyzed at 45° C for 15 min. The hydrolyzate was rapidly neutralized and was analyzed by paper chromatography in the benzene – ethyl acetate – acetic acid – formamide (23.5:74.5:2:1) system. Three spots were seen, one with R_f 0.62 identical with quercetin, another at the start identical with the initial flassilin, and the third with R_f 0.22, which had appeared as a result of partial hydrolysis.

To isolate the desglucoflassilin formed, the hydrolyzate was chromatographed on 100 g of Kapron sorbent. The flavonoid with R_f 0.22 was eluted with 40% ethanol and, after evaporation of the eluates, was crystallized from aqueous ethanol. The glycoside isolated, with mp 186–187° C, was sparingly soluble in water, gave a green coloration with ferric chloride and a yellow precipitate with lead acetate, reduced ammoniacal filter solution, and gave a positive reaction with magnesium and hydrochloric acid and a negative one with zirconyl nitrate and citric acid. $[\alpha]_D^{20}$ −40.4° (c 0.05; methanol). Acid hydrolysis with 1% hydrochloric acid for 20 min led to the splitting off of quercetin and rhamnose.

Found, %: C 58.46; H 4.21. Calculated for $C_{21}H_{18}O_{10}$, %: C 58.60; H 4.18.

Oxidation with H_2O_2. Flassilin and desglucoflassilin (0.1 g each) were oxidized by Chaudler and Harper's method [2]. Glucosidorhamnose and rhamnose respectively, were obtained.

Enzymatic hydrolysis of flassilin and desglucoflassilin. A suspension of 0.01 g of snail enzyme preparation in 2 ml of water was added to a solution of 0.01 g of flassilin in 150 ml of water, and the mixture was left in a thermostated vessel at 37° C. Every 2 min for 1 hr, samples were taken for chromatographic analysis for carbohydrates and flavonoids. This showed that flassilin was hydrolyzed after only 2 min with the formation of desglucoflassilin and after 4–6 min the aglycone quercetin was found in the hydrolyzate.

By analyzing the carbohydrate components of the hydrolyzate, it could be seen that in the first few minutes of hydrolysis only glucose was formed and then with the appearance of quercetin in the hydrolyzate, rhamnose was found in addition to glucose. The complete hydrolysis to the aglycone took 30 min. Under similar conditions, 1.0 g of flassilin was hydrolyzed for 10 min. The hydrolyzate was separated chromatographically on Kapron, giving 0.3 g of quercetin monoglycoside identical with the desglucoflassilin obtained after acid hydrolysis.

Pasternoside. Composition $C_{28}H_{28}O_{14}$, mp 235–237° C, giving no depression of the melting point with an authentic sample of pasternoside from parsnip. The glycoside and its derivatives were identical in all their properties with the latter [6].

Desglucopasternoside. Composition $C_{22}H_{20}O_{10}$ with mp 222–224° C, giving no depression of the melting point with an authentic sample of desglucopasternoside from parsnip and identical with the latter in all its properties [6].

Summary

Three flavonoid glycosides have been isolated from the flowers of Syrenia siliculosa Andr. Z.: pasternoside, desglucopasternoside, and a new flavonoid flassilin having the structure of quercetin 3-β-L-rhamnofuranosyl-β-D-glucofuranoside.

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