SORBIFOLIN—A NEW FLAVONE GLYCOSIDE FROM SORBARIA SORBIFOLIA

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In a study of the polyphenolic compounds from the leaves of Sorbaria sorbifolia (L.) A. Br. (Ural falsespirea), we have isolated two compounds. One of them was identified as hyperoside, while the second proved to be a new compound which we have called sorbifolin.

Sorbifolin gives a positive cyanidin reaction [1], which shows its flavonoid nature. On acid hydrolysis it is cleaved into D-xylose, L-rhamnose, and an aglycone which, on acetylation, gives a tetraacetyl derivative.

Both the aglycone and the glycoside appear as dark spots on a paper chromatogram which shows that they lack a hydroxyl in position 3.

On the basis of the zirconium–citric acid test [2] and the cyanidin reaction with the addition of dry sodium biocarbonate, the presence of free OH groups at C-5 and C-4' may be assumed, and the positive Bargellini reaction (Table 1) shows the presence of adjacent hydroxyls. Since the aglycone belongs to the flavone group and has a OH group at C-4' in the side chain, the other three hydroxyls can be located only in positions 5, 6, and 7.

Table 1. Color Reactions of Sorbofolin and Its Monoglycoside and Aglycone

Qualitative reactions	Sorbifolin	Desxylosorbifolin	Scutellarein
Color reactions			
Cyanidin reaction	Red-orange		
Cyanidin reaction + + dry $NaHCO_3$	Yellow		—
Bryant's cyanidin reaction [7]	Red (in water)		Red (in octanol)
With 10% alkali (NaOH) [8], p. 474.	Yellow		Yellow-green
With zirconyl nitrate and citric acid		Light yellow	
With ferric chloride [8], p. 472.	Dark brown	Brownish green	Dark brown
Bargellini's reaction	Yellow (negative)	Yellow slowly changing to greenish (negative)	Bluish green (positive)
Reaction with diazotized sulfanilic acid	Yellow	Yellow	Red-orange
R_f values in systems*:			
1	0.47	0.65	0.82
2	0.37	0.29	0.18
3	—	—	0.33
4	—	—	0.56

*Systems: 1) ethyl acetate-formic acid-water (10 : 2 : 3), 2) 15% acetic acid; 3) chloroform-acetic acid-water (13 : 6 : 1); 4) benzene-ethyl acetate-acetic acid (23.5 : 74.5 : 2).

What has been said above is confirmed by the UV spectra (Table 2) with ionizing and complex-forming reagents [5, 6] and by the alkaline cleavage of the aglycone. The results obtained also show that the genin is 5,6,7,4'-tetrahydroxyflavone (scutellarein).

The position of attachment of the carbohydrate residues to the aglycone was established on the basis of the UV spectra of sorbifolin (see Table 2). The absence of a marked shift in the spectrum on the addition of sodium acetate (as compared with the aglycone) shows the substitution of position 7, which is confirmed by the negative Bargellini and diazotized sulfanilic acid reactions [9, 10].

Table 2. UV Spectra of the Bioside, the Monoside, and the Aglycone

Medium	Absorption bands	Bioside		Monoside		Aglycone	
		λ	Δλ	λ	Δλ	λ	Δλ
10^{-5} M solution in anhydrous ethanol	I	338	—	345	—	345	—
	II	288	—	292	—	292	—
Same + sodium acetate	I	338	0	344	−1	370	25
	II	290	2	292	0	285	−7
Same + sodium ethoxide	I	405	67	400	55	400	55
	II	283	−5	338	46	336	44
Same + aluminum chloride.	I	365	27	375	30	390	45
	II	290	2	310	18	304	12

The sequence of attachment of the sugars in the bioside was found by stepwise hydrolysis with 7% acetic acid. A scutellarein rhamnoside was obtained as an intermediate. Enzymatic hydrolysis with Aspergillus oryzae and rhamnodiastase led to the formation of the same rhamnoside and D-xylose. Consequently L-rhamnose is attached to the aglycone and D-xylose is the terminal sugar.

To establish the nature of the bond between the xylose and the rhamnose in the bioside we oxidized the aromatic part of the glycoside by Chandler's method [11] with subsequent paper chromatography of the bioside split off and the treatment of the chromatogram with specific reagents [12]. This showed the presence of a 1–4 bond between the xylose and the rhamnose.

The configuration of the glycosidic bonds and the sizes of the rings in sorbifolin were determined by comparing molecular rotations and by IR spectroscopy [13]. It was found that the L-rhamnose has an α- and the D-xylose a β-glycosidic linkage, and both sugars are present in the pyranose form. Thus, the structure of sorbifolin may be represented as scutellarein 7-O-α-L-rhamnopyranosyl-(4 → 1)-O-β-D-xylopyranoside.

EXPERIMENTAL

The substances for analysis were dried over P_2O_5 in vacuum at 110° C for 4 hr. The melting points were determined on a Kofler block. The ultraviolet spectra were obtained on an SF-4A spectrophotometer. The IR spectra were recorded by I. P. Kovalev on a UR-10 instrument.

The chromatography of the substances was carried out on "B" type paper of the Volodarskii Leningrad paper mill.

Isolation of sorbifolin. The combined flavonoids (7 g) were chromatographed on a column of polyamide and were eluted with butanol. The fractions containing the sorbifolin were evaporated in vacuum. The slightly yellowish acicular crystals that separated out were recrystallized from 40% ethanol, and then had a double mp: 230–234° C, 320–330° C; $[\alpha]_D^{23}$ − 133.2° (c 0.4; dimethylformamide).

IR spectrum, cm^{-1}: 3375 (HO of carbohydrates), 2980 and 2530 (CH of carbohydrates); 1663 (C=O of a γ-pyrone); 1608, 1572, 1518, and 1490 (C=C of an aromatic system); and 972 (CH_3 of carbohydrates). The qualitative reactions, R_f values, and UV spectrum of the glycoside are given in Tables 1 and 2.

Found, %: C 53.57, 53.82; H 5.15, 5.22. Calculated for $C_{26}H_{28}O_{14} \cdot H_2O$, %: C 53.61; H 5.15.

Acid hydrolysis of sorbifolin. The glycoside (0.4 g) was hydrolyzed with 3% methanolic HCl at 100° C for 2 hr. Mp of the crystals that deposited: 345–348° C (from methanol). IR spectrum, cm^{-1}: 1668 (C=O of a γ-pyrone), 1590, 1565, and 1490 (C=C of an aromatic system), 3448, 3320, and 3100 (phenolic OH groups). The qualitative reactions, R_f values and features of the UV spectrum are given in Tables 1 and 2.

The acetate of the aglycone, obtained by the usual method, had mp 236–238° C (from ethanol). Four acetyl residues were found in it [14].

Alkaline cleavage of the aglycone. A solution of 10 mg of the aglycone in 20 ml of 20% caustic potash solution was heated at 100° C for 30 min, cooled, and neutralized with 20% H_2SO_4 to pH 5. The cleavage products were extracted with ether, the extract was evaporated, and the residue was dissolved in the minimum amount of ethanol and chromatographed in the butan-1-ol–benzene–acetic acid–water (2 : 10 : 2 : 1) system. The products of alkaline cleavage were found to contain p-hydroxybenzoic acid (R_f 0.80).

Sugar component of sorbifolin. The filtrate after the separation of the aglycone was neutralized with KU-2 ion-exchange resin in the OH form and evaporated to 5 ml; 2 g of activated carbon was added and the mixture was boiled in the water bath for 1 hr. The concentrated filtrate was analyzed by paper chromatography in the following systems: 1) phenol saturated with water; 2) butan-1-ol–methyl ethyl ketone–borate buffer (0.1 N solution of borax–0.1 N solution of boric acid) (1 : 1 : 2). D-Xylose and L-rhamnose were detected.

Phenylosazone of the total sugars. To 3 ml of the sugar syrup were added 1 ml of freshly-distilled phenylhydrazine and 1 ml of glacial acetic acid. The mixture was heated at 100° C for 2 hr. Then it was allowed to stand in the cold for 12 hr, whereupon crystals deposited. They were filtered off and were washed with 10% acetic acid, water, ethanol, and ether. An experiment was carried out in parallel with a mixture of 10 ml of xylene and 10 ml of rhamnose. When the osazones obtained were chromatographed in the chloroform–formamide system, the R_f values obtained coincided completely.

Splitting off of the biose. The splitting off of the biose in sorbifolin was carried out as described by Chandler and Harper [11]. When the chromatograms were treated with a mixture of diphenylamine and p-anisidine, the spot of the sugar under investigation was colored blue-green; when it was treated with diphenylamine and urea it was colored orange-brown.

Enzymatic cleavage of sorbifolin. An aqueous solution of rhamnodiastase (2.5 g in 100 ml of water) was added to a solution of 2 g of the glycoside in 500 ml of 10% ethanol and the mixture was left in a thermostatted place at 38° C for 4 days. The course of hydrolysis was monitored by paper chromatography. After the end of the period given, a new substance had appeared in the fermentation medium.

To obtain the hydrolysis products, the enzyme was precipitated with a tenfold amount of boiling ethanol. The precipitate was filtered off and the filtrate was evaporated to 100 ml and deposited on a column of polyamide. The solution of sugar eluted with water was concentrated to 5 ml. On the 4th day, crystals of D-xylose with mp 148–151° C, giving no depression of the melting point with an authentic sample of D-xylose, precipitated. The monoside was eluted with 60% ethanol. After the evaporation of the eluate, the residue was crystallized from 30% ethanol. The substance obtained had a double mp: 234–236° C and 334–340° C; $[\alpha]_D^{23}$ −98° (c 0.16; dimethylformamide).

IR spectrum, cm^{-1}: 3400 (HO of carbohydrates), 2980 and 2940 (CH of carbohydrates), 1670 (C=O of a γ-pyrone); 1610, 1578, and 1490 (C=C of an aromatic system); and 972 (CH_3 of carbohydrates). For the UV spectrum, see Table 2, and for the qualitative reactions and R_f values, see Table 1.

Found, %: C 58.51, 58.42; H 4.53, 4.61. Calculated for $C_{21}H_{20}O_{10}$, %: C 58.33; H 4.63.

The same products were obtained by the hydrolysis of the bioside with 7% acetic acid.

The acid hydrolysis of the substance isolated as a result of enzymatic cleavage led to L-rhamnose and scutellarein.

It is an interesting fact that when the bioside and the monoside were fermented with the pancreatic juice of the grape snail for 15 days, they were cleaved into the aglycone and sugars.

CONCLUSIONS

1. From the leaves of Sorbaria sorbifolia (L.) A. Br. a new glycoside sorbifolin, has been isolated, and its structure has been established as scutellarein 7-O-α-L-rhamnopyranosyl-(4 → 1)-O-β-D-xylopyranoside.

2. The enzymatic and stepwise acid hydrolyses of sorbifolin have yielded a new monoside, desxylosorbifolin (scutellarein 7-O-α-L-rhamnopyranoside).

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