A CHEMICAL INVESTIGATION OF PASTERNOSIDE

N. P. Maksyutina and V. I. Litvinenko

Khimiya Prirodnykh Soedinenii, Vol. 2, No. 1, pp. 20-26, 1966

The seeds of Pastinaca sativa L. (garden parsnip) have yielded three flavonoid glycosides, two of which have been identified as hyperin and rutin, while the third has proved to be new [1] and has been called pasternoside. From its physicochemical properties, the latter can be characterized as a flavonoid glycoside (Table 1).

When pasternoside was investigated spectroscopically (Table 2), its aglycone part was found to contain free hydroxy groups: in position 5 by a 48-mμ bathochromic shift with zirconyl nitrate and in position 7 on the basis of the 7-mμ bathochromic displacement of the absorption maximum of the long-wave band on ionization with anhydrous sodium acetate [6].

The acid hydrolysis of pasternoside gave an aglycone with free 3-, 5-, 7- and 4'-hydroxy groups and two monosaccharides – glucose and rhamnose. From the aglycone we prepared the tetraacetyl derivative; alkaline degradation (cf. Table 1) gave phloroglucinol and vanillic acid.

Table 1

Physicochemical Properties of Pasternoside and Its Derivatives

Properties	Pasternoside	Deglucopasternoside	Aglycone
Mp, °C	235–237	220–224	319–321
$[\alpha]_D^{20}$, degrees	–120.0 (c 1.3; pyridine)	–85.0 (c 2; pyridine)	—
Mol. wt.	624.0	462.0	316.0
Formula	$C_{28}H_{32}O_{16}$	$C_{22}H_{22}O_{11}$	$C_{16}H_{12}O_7$
Rf in systems			
1*	0.68	0.17	0.04
2**	0.00	0.16	0.70
	Qualitative reactions		
Cyanidin reaction	positive	positive	positive
Reaction with zirconyl nitrate and citric acid	negative	positive	positive
Coloration with ferric chloride	green	green-brown	green-brown
	hydrolysis products		
Hydrolysis with 2% HCl	isorhamnetin + glucose + rhamnose	isorhamnetin + rhamnose	—
with snail enzyme	deglucopasternoside and glucose	not hydrolyzed	—
with rhamnodiastase	deglucopasternoside and glucose	not hydrolyzed	—
Oxidation with H_2O_2 and saponification with NH_3	glucose	not saponified	—
Alkaline degradation	—	—	phloroglucinol and vanillic acid

*15% acetic acid

** Benzene-ethyl acetate-acetic acid (24.5:73.5:2). Paper impregnated with formamide.

The physicochemical properties of the aglycone of pasternoside showed that it is isorhamnetin (3, 5, 7, 4'-tetrahydroxy-3'-methoxyflavone) [8]. The appearance of two more hydroxy groups (in positions 3 and 4') in pasternoside after acid hydrolysis gives grounds for assuming that pasternoside is a diglycoside. The diglycosidic nature of pasternoside is also confirmed by the results of quantitative acidic hydrolysis, which gave 47% of aglycone (calculated: 46%).

Table 2

Spectral Characteristics of Pasternoside and Its Derivatives

Solutions and reagents	Bands	Pasternoside		Deglucopasternoside		Aglycone	
		Absorption maxima (λ, mμ) and displacements					
		λ_{max}	Δλ	λ_{max}	Δλ	λ_{max}	Δλ
2×10^{-5} M in abs. alcohol	I	357	—	367	—	372	—
	II	256 267	—	255 267	—	255 267	—
The same + sodium acetate	I	365	8	380	13	385	13
	II	256 268	1	255 —	—	268 274	7
The same + boric acid and sodium acetate	I	357	0	367	0	372	0
	II	255 —	—	255 —	—	255 —	—
The same + sodium ethoxide	I	425	68	350 415	58	345	—
	II	— 270	3	252 —	—	275	8
The same + zirconyl nitrate	I	405	48	385 455	88	375 455	83
	II	— —	—	270	3	265	—2
The same + zirconyl nitrate and citric acid	I	355	—2	367	0	375 435	63
	II	255 —	—	—	—	265	—2

The positions of the carbohydrate substituents in pasternoside were determined from the products of enzymatic hydrolysis with rhamnodiastase [2], snail pancreatic juice enzymes [3], the enzymes of the fungus Aspergillus oryzae [4], and from the products of oxidative degradation with hydrogen peroxide with subsequent saponification by ammonia [5]. It was established that the enzymes split off only glucose, and a monoglycoside was isolated which we have called deglucopasternoside, which exhibits a free hydroxy group at C_3. The presence of glucose in this position is also confirmed by its isolation on oxidative degradation with hydrogen peroxide, which is capable of oxidizing only the double bond between the C_2 and C_3 atoms with the formation of an ester bond with a carbohydrate present in position 3. A sugar ester, in contrast to other glycosidic bonds, is readily saponified with ammonia [5]. For the spectroscopic detection of the 3-hydroxy group and its distinction from a 5-hydroxy group, we carried out the complex-forming reaction with zirconyl nitrate, which permitted the two hydroxy groups in deglucopasternoside to be detected separately (see Table 2) [6].

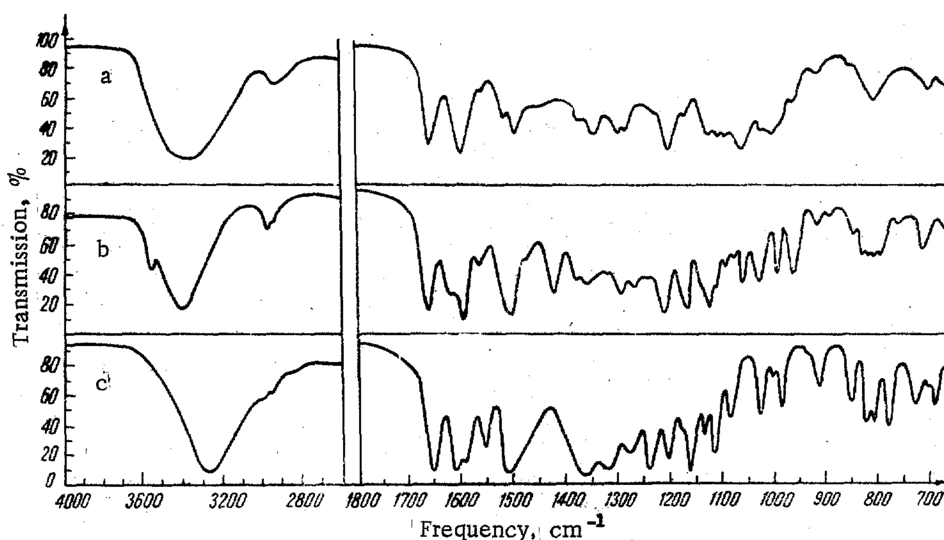
Table 3

Analysis of the Molecular Rotation of the Carbohydrate Part of Pasternoside and Its Derivatives

Glycoside	$[M]_D$	K_f	$[M]_D K_f$	ΔC	I	II
Pasternoside	—749.0	0.66	—494.0	—	—	—
Deglucopasternoside	—393.0	0.52	—204.0	—	α	Pyranoside
Derhamnopasternoside	—	—		—392.0	β	Furanoside
Phenyl rhamnoside	—254.0	1.00	—254.0	—	α	Pyranoside
Phenyl glucoside	—364.0	1.00	—364.0	—	β	Furanoside

Note: ΔC of the diglycoside = $[M]_D \cdot K_f$ of the diglycoside minus 1/2 $[M]_D \cdot K_f$ of the monoglycoside. I – configuration of the glycosidic bond, II – size of the oxide ring.

When deglucopasternoside was subjected to acid hydrolysis, we obtained isorhamnetin and rhamnose. The production of 67% of aglycone shows that this glycoside is a monorhamnoside. According to the spectroscopic investigation (cf. Table 2), the rhamnose is present in the monoglycoside in position 4'.



IR absorption spectra of pasternoside (a), deglucopasternoside (b), and isorhamnetin (c) in a potassium bromide tablet.

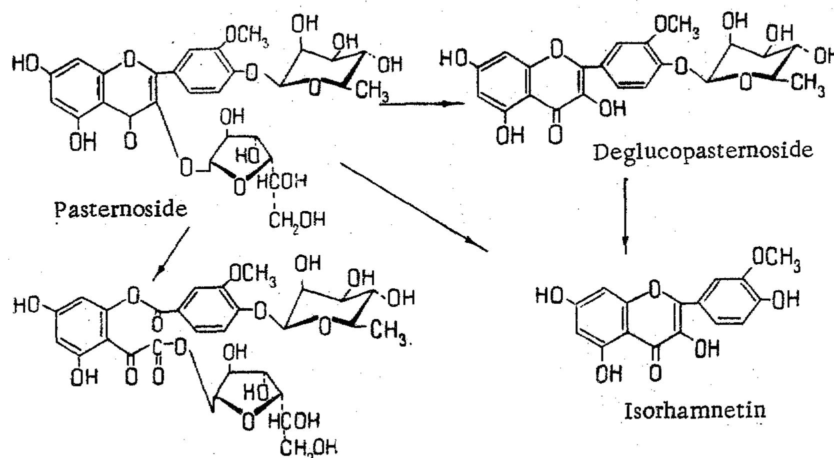
Thus, pasternoside may be characterized provisionally as isorhamnetin 3-D-glucoside 4'-L-rhamnoside.

In studying the configuration of the glycosidic bonds and the sizes of the oxide rings in the carbohydrate part of the molecule, we used the results of enzymatic hydrolysis and a comparison of molecular rotations (Table 3).

This showed that the D-glucose is attached to the isorhamnetin in position 3 by means of a β-glycosidic bond, and the L-rhamnose is attached to the aglycone in position 4' by an α-glycosidic bond. Analysis of molecular rotations (cf. Table 3) permits the assumption that in pasternoside D-glucose is present in the furanose form and L-rhamnose in the pyranose form [7].

Additional confirmation of the sizes of the oxide rings and the configuration of the glycosidic bonds of pasternoside is given by the results of a spectral investigation of the carbohydrate part of the molecule in the IR region [7]. Thus, the IR spectrum of deglucopasternoside exhibits the following bands: 1110 s., 1065 s., 1015 s., (ring vibrations of a pyranose and C—O groups), 993 m. (—O—C—O of a glycoside), 972 m. (CH_3 group), 846 m. (α-anomer) cm^{-1}; and derhamnopasternoside exhibits bands at 1080 s., 1036 s. (ring vibration of a furanose and C—O groups), and 1004 s. (—O—C—O of a glycoside) cm^{-1} (figure).

Thus, it has been established that pasternoside is isorhamnetin 3-β-D-glucopyranoside 4'-α-L-rhamnopyranoside.



Experimental

Pasternoside. This flavonoid glycoside was isolated from the fruit of garden parsnip.

Found, %: C 53.98; H 5.06. Calculated for $C_{28}H_{32}O_{16}$, %: C 53.86; H 5.12.

Acetylation of pasternoside. 0.3 g of pasternoside was acetylated with acetic anhydride (5 ml) at 18-20°C for 16 hr. On dilution with water, pasternoside acetate was deposited as an amorphous powder. The polyacetate has nine acetyl groups, determined by the Kuhn-Roth method.

Found, %: C 55.14; H 5.06. Calculated for $C_{46}H_{50}O_{25}$, %: C 55.08; H 4.99.

Oxidation of pasternoside with hydrogen peroxide. A suspension of 0.1 g of pasternoside in 20 ml of water was made alkaline with 1 ml of 0.1 N ammonia solution, 4 ml of 30% hydrogen peroxide was added, and the mixture was left at 18-20°C for 4 hr. Then freshly-prepared lead sulfide (a slight excess) was added and the mixture was left for 18 hr for the decomposition of the excess of hydrogen peroxide. The precipitate was filtered off, and the filtrate was treated with 5 ml of 25% ammonia solution and heated on a water bath (5 min). The resulting solution was concentrated to small bulk and the qualitative composition of the carbohydrates in the solution was investigated by paper chromatography. Only one sugar was found, which was identified as glucose.

Preparation of deglucopasternoside. 2 g of pasternoside was dissolved in 1*l* of 10% ethanol with heating, the solution was cooled to 38°C, a solution of snail enzyme (0.2 g in 100 ml of water) was added, and the mixture was left for 3 hr in a thermostat at 38°C. The fermentation process was followed by means of paper chromatography in 15% acetic acid; it was found that after 3 hr the pasternoside had been almost completely broken down to the monoside. The enzyme was denatured by heating the solution to the boil, and the monoglycoside was extracted with ethyl acetate (three 0.8-*l* portions). The ethyl acetate extract was evaporated under vacuum, the residue was dissolved in the minimum amount of ethanol, and the solution was diluted with water and left to crystallize. The needle-like crystals were filtered off, recrystallized from 50% ethanol, and dried under vacuum (0.1 mm Hg) at 100°C. The flavonoid had mp 220-224°C, $[\alpha]_D^{20}$ −85.0° (c 2.0; pyridine).

Found, %: C 57.36; H 4.69. Calculated for $C_{22}H_{22}O_{11}$, %: C 57.14; H 4.76.

The qualitative reactions of the compound under investigation are shown in Table 1, the results of a spectroscopic investigation in the UV region in Table 2, and the IR spectrum in Fig. 1, b. The IR spectra of pasternoside and its derivatives were taken on a UR-10 spectrophotometer in a potassium bromide tablet by I. P. Kovalev.

Acid hydrolysis of deglucopasternoside. 0.5 g of the monoglycoside was hydrolyzed with 1% hydrochloric acid in 50% aqueous alcohol with heating in the water bath for 20 min. The aglycone, which separated out in the form of yellow needles, was filtered off and recrystallized from 30% ethanol. The yield of aglycone was 0.33 g, mp 319-321°C, composition $C_{16}H_{12}O_7$. The aglycone was sparingly soluble in water, somewhat more soluble in alcohol and ether, and readily soluble in propylene glycol, dimethylformamide, and pyridine.

Acetylation of the aglycone as described above gave a tetraacetate with the composition $C_{24}H_{20}O_{11}$, mp 205-208°C.

Alkaline degradation. A solution of 0.1 g of the aglycone in 10 ml of 20% caustic potash solution was heated for 2 hr in a water bath, cooled, diluted with water to 100 ml, neutralized with 5% hydrochloric acid, and extracted with ether (three 150-ml portions). The residue after the evaporation of the ether was dissolved in a small amount of alcohol, and the solution was analyzed by paper chromatography in the benzene-ethylacetate-acetic acid-formamide (24:73.5:2:1) system. Phenols (resorcinol and phloroglucinol) and aromatic acids (p-hydroxybenzoic, vanillic, and protocatechuic) were used for comparison. The chromatogram was detected with diazotized sulfanilic acid (0.1%) and a 3% aqueous alcoholic solution of caustic soda.

By their chromatographic behavior, the products of the alkaline degradation of the aglycone were identified as phloroglucinol and vanillic acid.

Analysis of the carbohydrates of deglucopasternoside. The acid hydrolyzate of the monoglycoside was neutralized with a solution of alkali and evaporated to dryness under vacuum. The residue was treated with absolute alcohol (3 × 15 ml) the precipitate was separated off, and the solution was concentrated. This operation was repeated several times in order to eliminate mineral salts. The syrup was dissolved in 40 ml of water and purified on a column containing 50 g of cellulose powder. The column was washed with distilled water and the fractions containing the carbohydrate were collected. The sugar was analyzed by paper chromatography in the butan-1-ol-acetic acid-water (4:1:5) system and was identified as L-rhamnose. The purified syrup was crystallized from a mixture of ethanol and acetone. The crystals of the sugar had mp 122-125°C, composition $C_6H_{12}O_5$, and $[\alpha]_D^{20}$ +8.8° (c 1.0; water). The identity of the sugar with L-rhamnose was also confirmed by the similarity of the properties of the phenylosazones.

Hydrolysis of deglucopasternoside with rhamnodiastase. With heating, 0.02 g of deglucopasternoside was dissolved in 20 ml of water, the solution was cooled to 38°C and mixed with a solution (0.01 g) of rhamnodiastase in 5 ml of water, and the mixture was left for fermentation in a thermostat at 38°C. The hydrolysis was followed by paper chromatography in the 15% acetic acid system. No hydrolysis was observed during two days.

Summary

1. The new flavonoid glycoside pasternoside has the structure of isorhamnetin 3-β-D-glucopyranoside 4'-α-L-rhamnopyranoside.

2. The enzymatic hydrolysis of pasternoside has given the monoglycoside deglucopasternoside, which has been shown to be isorhamnetin 4'-α-L-rhamnopyranoside.

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23 March 1965

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