ALLIOSIDIN FROM THE SEEDS OF ERYSIMUM ALLIONII

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Continuing an investigation of the seeds of the garden hybrid Erysimum allionii we have obtained yet another flavonoid O-glycoside, which we have called "alliosidin."

Alliosidin, $C_{27}H_{30}O_{15}$, $[\alpha]_D^{20}$ −74.0° (c 1.0; water) is a derivative of isorhamnetin. The sugar component of the substance isolated is a biose containing L-rhamnose and L-arabinose.

The position of the sugar component in alliosidin was determined by a spectral study in the UV region of the spectrum. With sodium acetate the glycoside produces a shift in the absorption maximum of the long-wave band by 20 mμ and with sodium ethoxide and zirconyl nitrate one of 49 mμ, which shows the presence of a free hydroxyl group at C-7 and also of hydroxyl groups at C-5 and C-4'. Very similar shifts in the maxima of the long-wave bands (11 and 51 mμ) were found for the monoglycoside of alliosidin (in desrhamnoalliosidin). However, with zirconyl nitrate in a neutral medium, the aglycone, unlike alliosidin and desrhamnoalliosidin, produces a shift in the maximum of the long-wave band by 83 mμ, and with citric acid it gives one of 65 mμ. This shows that the sugar component in alliosidin and desrhamnoalliosidin substitutes the hydroxyl group at C-3 of isorhamnetin.

The oxidation of alliosidin with hydrogen peroxide gave a biose consisting of L-rhamnose and L-arabinose, which also confirms the attachment of the sugar component in the form of a biose at C-13.

The order of attachment of the sugars in alliosidin was determined by partial hydrolysis of the bioside to the monoglycoside desrhamnoalliosidin, the sugar component of which was L-arabinose. In alliosidin, the arabinose is attached directly to the aglycone at C-3 and the rhamnose is attached to the arabinose.

The structural features of the biose substituent of the glycoside were analyzed by comparing the molecular rotations of the monoglycoside and the bioside (table). The molecular rotations taking into account the correction (K_{ph}) for desrhamnoalliosidin show the α-configuration and the pyranose form of the L-arabinose in the monoglycoside of alliosidin, since only this form of phenyl arabinoside has the same $[M]_D \cdot K_{ph}$ as the glycoside under investigation. The terminal L-rhamnose in alliosidin was considered with respect to the molecular rotation of the monoglycoside of alliosidin (see table). The results of the comparison of the contribution of the rotation of the terminal rhamnose in alliosidin and those of known methyl glycosides showed the α-configuration and the pyranose form of this monose of the glycoside.

Comparison of the Molecular Rotations of Alliosidin, Desrhamnoalliosidin, and Known Phenyl and Methyl Glycosides

Glycoside	$[M]_D$	K_{ph}	$[M]_D K_{ph}$
Desrhamnoalliosidin	+ 26.9	0.55	+ 14.7
Phenyl α-L-arabopyranoside	+ 13.5	1.0	+ 13.5
Phenyl β-L-arabopyranoside	+540.0	1.0	+540.0
Phenyl α-L-arabofuranoside	−359.0	1.0	−359.0
Phenyl β-L-arabofuranoside	− 59.0	1.0	− 59.0
Alliosidin	−439.6	0.59	−259.4
Desrhamnoalliosidin	+ 26.9	0.55	+ 14.7
Methyl α-L-rhamnofuranoside	−158.0	1.0	—

Note: For alliosidin, Δc 244.7, K_m 0.74. Δc · K_m 181.0; for methyl α-L-rhamnofuranoside Δc · K_m 158.0.

On chromatograms, the biose of alliosidin gives yellow spots with diphenylamine and p-anisidine and no coloration with diphenylamine and urea. The aniline phthalate reagent shows the biose in the form of pink-orange spots.

A comparison of molecular rotations with those of known methyl and phenyl glycosides, the characteristic colorations with the diphenylamine reagents, and the negative results of enzymatic hydrolysis with rhamnodiastase have shown the composition and configuration of the monoses in the biose of alliosidin, and their 1–2 linkage.

All that has been said gives grounds for assuming that alliosidin is a new glycoside with the structure isorhamnetin 3-[O-α-L-arabopyranosyl-(2 → 1)-α-L-rhamnofuranoside].

EXPERIMENTAL

Isolation of alliosidin. Five kilograms of seeds of Erysimum allioni was extracted with a mixture of chloroform and ethanol (1 : 1). The extract was evaporated and the residue was treated 11 times with ether and four times with chloroform, and the purified mass was dissolved in water. The aqueous solution was chromatographed on a column of Kapron (2 kg). Elution was carried out first with water and then with ethanol of increasing concentration. The eluates were analyzed by the cyanidin reaction and by paper chromatography in 15% acetic acid.

A mixture of two flavonoids with R_f 0.60 and 0.62 was eluted with 20% ethanol. After the solvent had been eliminated under vacuum, the residue was rechromatographed on a column of 0.4 kg of Kapron powder. The alliosidin was eluted from the column first, and it crystallized from the evaporated eluates in the form of light yellow needles with mp 198–201° C. The substance is readily soluble in water, methanol, and dimethylformamide, and insoluble in ether. With metallic magnesium and HCl it gives a red coloration, with lead acetate a light yellow coloration, and with ferric chloride a green coloration. The flavonoid does not reduce an ammoniacal solution of silver nitrate.

Found, %: C 54.78, 54.65; H 5.12, 5.22. Calculated for $C_{27}H_{30}O_{15}$, %: C 54.56; H 5.07.

Hydrolysis of alliosidin. Alliosidin (0.45 g) was hydrolyzed with 1% HCl with heating in the water bath for 8 min. After cooling, crystals of the aglycone deposited, and they were identified by their melting point, a mixed-melting point, and chromatographic behavior as isorhamnetin. The acid hydrolysate after neutralization with an ion-exchange resin, was chromatographed on a column containing 45 g of cellulose powder. The column was filled and was eluted with the organic phase of a mixture of butan-1-ol, acetic acid, and water (4 : 1 : 5), 15-ml fractions being collected and analyzed by paper chromatography in the butan-1-ol–acetic acid–water system. Fractions 6–9 deposited as crystals of L-rhamnose and fractions 14–15 crystals of L-arabinose.

Preparation of the monoglycoside. Alliosidin (0.4 g) was hydrolyzed with 10% formic acid solution at 65–70° C for 1 hr. Immediately after cooling, the hydrolysate was deposited on several sheets of chromatographic cardboard (Whatman 33) and separated chromatographically in 15% acetic acid. The chromatographic sheets were observed in UV light and the spots of the monoglycoside with R_f 0.21–0.22 were cut out and the glycoside was extracted with hot 50% ethanol.

The eluates were evaporated and the monoglycoside was crystallized from water. It deposited in the form of yellow needles with the composition $C_{21}H_{20}O_{11}$, mp 232–234° C, $[\alpha]_D^{20}$ +6.0° (c 0.05; methanol).

Hydrolysis of the monoglycoside. The monoglycoside (0.05 g) was hydrolyzed with 1% HCl in the boiling water bath for 15 min. Crystals of the aglycone formed in the hydrolysate, and were identified by their IR spectrum and melting point as isorhamnetin. The acid hydrolysate was shown by paper chromatography in the butan-1-ol–acetic acid–water system to contain arabinose.

Oxidation of alliosidin. A solution of 0.25 g of alliosidin in 40 ml of water was mixed with 2 ml of 0.1 N ammonia solution. Then 4 ml of 30% hydrogen peroxide was added and the mixture was left at room temperature for 4 hr. The excess of hydrogen peroxide was eliminated by the addition of lead sulfide, and the mixture was filtered. The filtrate was made alkaline with ammonia (5 ml), evaporated to a volume of 0.3–0.4 ml, and purified chromatographically on 10 g of alumina. An amorphous powder of a sugar $C_{11}H_{20}O_9$ with $[\alpha]_D^{22}$ −12.2° (c 0.25; water) was obtained.

The biose was analyzed by paper chromatography with a set of reference samples, and the spots were revealed with aniline phthalate and diphenylamine reagents. With aniline phthalate the biose appeared in the form of pink-orange spots and with diphenylamine and p-anisidine in the form of yellow spots, while it gave no coloration with diphenylamine and urea. The R_f value of the spots of the biose in the butan-1-ol–acetic acid–water (4 : 1 : 5) system was 1.14 (referred to glucose).

Hydrolysis of the biose of alliosidin. The biose (0.002 g) of alliosidin was hydrolyzed with 1% HCl in the water bath for 10 min. The hydrolysate was analyzed chromatographically on paper in the BAW system. The hydrolysate showed two spots, which were identical with those of arabinose and rhamnose. In the properties and composition of the monoses, the biose of alliosidin is similar to the biose of zhealin [1], which we have isolated previously from wallflower seeds.

CONCLUSIONS

From the seeds of Erysimum allionii, we have isolated a new flavonoid biose, alliosidin, which has the structure of isorhamnetin 3-[O-a-L-arabopyranosyl-(2 → 1)-α-L-rhamnopyranoside].

REFERENCE

1. N. P. Maksyutina, KhPS [Chemistry of Natural Compounds], 1, 62, 1965.

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