TRITERPENE GLYCOSIDES OF *Gypsophila patrinii*

III. THE STRUCTURE OF PHILOSIDE B

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In the preceding paper [1] we reported the isolation from the Gypsophila patrinii of two glycosides and a proof of the structure of one of them – philoside A. The present paper gives the results of a study of the structure of the second glycoside – philoside B.

The acid hydrolysis of philoside B led to the formation of gypsogenin and the following set of monosaccharides: D-galactose, D-xylose, D-fucose, L-arabinose, L-rhamnose, and glucuronic acid in a ratio of 2 : 2 : 1 : 2 : 1 : 1. Consequently, in the composition of the carbohydrate chains of philoside B, as compared with philoside A, the D-glucose has disappeared and L-arabinose has appeared.

The alkaline hydrolysis of philoside B formed a hexaoside which, on subsequent heating with acids, was cleaved into D-galactose, D-xylose (2 moles each), and L-arabinose. This shows that the hexaoside obtained differs from the pentaoside formed in the alkaline hydrolysis of philoside A by one L-arabinose residue. The carbohydrate chain attached by the O-acylglycosidic bond contains one residue each of D-fucose, L-arabinose, and L-rhamnose. At the same time, as in philoside A, the D-fucose residue is attached directly to the carboxyl of the aglycone, since it is destroyed on alkaline hydrolysis.

The Smith degradation of philoside B, like that of philoside A, gave a single product – vaccaroside – which shows the similarity of the structure of these glycosides.

We obtained further information on the structure of the carbohydrate chains from the results of the methylation of philoside B and of the intermediate glycosides.

The permethylate was hydrolyzed with dilute mineral acids. The following monosaccharide methyl ethers were isolated: 3,4-di-O-methyl-D-fucose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-D-xylose, 2,3,4-tri-O-methyl-L-rhamnose, 3,4,6-tri-O-methyl-D-galactose, 3,4-di-O-methyl-D-xylose, 2,3-di-O-methyl-L-arabinose, and free glucuronic acid. They were shown to be identical with authentic samples by paper chromatography and by their relative retention times in gas–liquid chromatography.

It follows from the facts given that in the trisaccharide chain attached to the carboxyl of the genin, a L-arabinose residue is attached to the D-fucose residue and, in its turn, a L-rhamnose residue is attached to the arabinose residue.

In the hexasaccharide chain (at the hydroxyl of the aglycone), the center of branching is, as also in philoside A, the glucuronic acid. As the results of methylation show, D-galactose, D-xylose, and L-arabinose (1 residue each) occupy the terminal positions in the carbohydrate chain, and in the middle of the chain there is another D-galactose residue and a D-xylose residue. Under the action of enzymes, philoside B splits off a molecule of D-galactose, having a (1→4) bond with the glucuronic acid residue, as follows from the results of methylation of the glycoside obtained.

If we compare the compositions of the methylated sugars of the acid glycosides obtained by the alkaline saponification of philosides A and B, it can be seen that they are completely identical with the ex-

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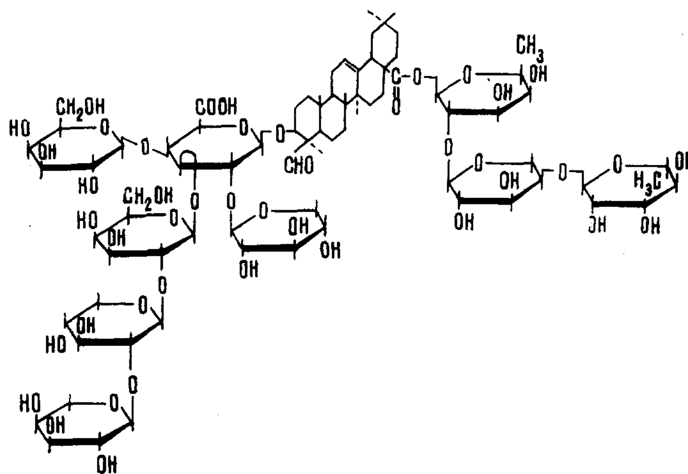
ception of 2,3,4-tri-O-methyl-L-arabinose and one 3,4-di-O-methyl-D-xylose residue. The formation of the latter can be explained by the assumption that in philoside B the residue of the terminal L-arabinose is attached to one of the D-xylose residues.

In order to determine to which of the two D-xylose residues the L-arabinose is attached, the enzymatic hydrolysis of philoside B was performed. This gave a trisaccharide consisting of D-galactose, D-xylose, and L-arabinose.

On acid hydrolysis, a permethylate of the trisaccharide was split into 3,4,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose, and 3,4-di-O-methyl-L-xylose. Consequently, the L-arabinose residue is attached to a disaccharide residue consisting of D-xylose and D-galactose which, by analogy with philoside A, probably has a (1→3) bond with the glucuronic acid residue.

When enzymatic hydrolysis was carried out under somewhat different conditions, it was possible to isolate a glycoside without terminal galactose. After the methylation of this glycoside, reduction with lithium tetrahydroaluminate, and acid hydrolysis, we identified 4-mono-O-methyl-D-glucose, in addition to other methyl ethers of sugars, which permits the unambiguous identification of the site of attachment of the terminal galactose in philoside B. On the basis of the facts presented, the following structure is proposed for philoside B:



EXPERIMENTAL

Chromatography was performed with type KSK silica gel, Schleicher and Schüll No. 2043 paper, and "Silufol" metal plates with a thin layer of SiO_2, Kavalier (Czechoslovakia), using the following solvent systems: 1) butan-1-ol–acetic acid–water (4 : 1 : 5); 2) benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3); 3) and 4) ethyl acetate–methanol–water (10 : 1 : 1) and (10 : 2 : 3), respectively; 5) and 6) ethyl acetate–methanol–water–acetic acid (10 : 3 : 2 : 2) and (10 : 3 : 1 : 1), respectively; and 7) and 8) butan-1-ol–methanol–water (10 : 1 : 1) and (10 : 2 : 3), respectively. The R_g values of the methylated sugars are given for the butan-1-ol–ethanol–water (5 : 1 : 4) system.

The gas–liquid chromatography of the methylated sugars was performed on a Tsvet-6A instrument at 175°C in a column 2 m long and 3 mm in diameter filled with Celite-545 with 15% of poly(butane-1,4-diol succinate). To determine the densities of the spots on the chromatograms we used an ERJ-65 automatic densitometer.

Acid Hydrolysis of Philoside B. A mixture of 200 mg of the glycoside and 20 ml of 5% sulfuric acid was heated in the boiling-water bath for 10 h. The precipitate that deposited was separated, and it was identified as vaccaroside by TLC in system 1. The filtrate was neutralized with AV-17 anion-exchange resin, and the following sugars were identified by paper chromatography in systems 1 and 2: D-galactose, D-xylose, L-arabinose, D-fucose, and L-rhamnose in a ratio of 2 : 2 : 2 : 1 : 1.

Alkaline Hydrolysis of Philoside B. A solution of 0.3 g of the glycoside in 25 ml of 5% KOH was heated at 80°C in a nitrogen-filled tube for 10 h. The reaction mixture was neutralized with KU-2 cation-exchange resin, evaporated to small volume, and extracted with butan-1-ol (3 × 15 ml). The wash-water from the butanol extract was evaporated and the residue was chromatographed on a column of silica gel (2 × 15 cm), with elution by ethyl acetate (200 ml), system 3 (250 ml), and system 4. The last eluate con-

tained a hexaoside (0.15 g) with mp 240-245°C (from butan-1-ol), $[\alpha]_D$ +7.2° (+9.5) (c 4.5; pyridine). The product obtained (25 mg) was hydrolyzed by being heated with 10 ml of 5% sulfuric acid. After the separation of the precipitate that had deposited and neutralization with AV-17 anion-exchange resin, the filtrate was shown by paper chromatography in systems 1 and 2 to contain D-galactose, D-xylose, and L-arabinose (2 : 2 : 1).

The aqueous extract was evaporated and the residue was chromatographed on a column of silica gel (1.5 × 15 cm), with elution by means of system 5. The first fractions contained an oligosaccharide with R_f 0.8 (system 6) – 35 mg. The oligosaccharide was hydrolyzed with 5% sulfuric acid. Arabinose, rhamnose, and fucose (traces) were identified by paper chromatography in systems 1 and 2.

Smith Degradation of Philoside B. A solution of 0.17 g of philoside B, 0.47 g of sodium paraperiodate, and 2 ml of acetic acid in 30 ml of water was kept in the dark at room temperature for 9 days. The excess of sodium periodate was decomposed by the addition of 0.5 ml of propylene glycol, the reaction mixture was evaporated, the dry residue was dissolved in 10 ml of water, and the solution was extracted with butan-1-ol (5 × 10 ml). After being washed with water, the butanolic extracts were evaporated, the dry residue was dissolved in 15 ml of methanol, the solution was diluted with 10 ml of water, 0.2 g of sodium tetrahydroborate was added, and the mixture was stirred at room temperature for 6 h. Then it was neutralized with KU-2 cation-exchange resin and evaporated, the dry residue (0.15 g) was dissolved in a mixture containing 20 ml of ethanol, 10 ml of water, and 5 ml of 5% sulfuric acid, and the solution was heated at 60-70°C for 1 h. No free monosaccharides were detected in the hydrolyzate by paper chromatography. After neutralization with AV-17 anion-exchange resin, the hydrolyzate was evaporated, and the residue was deposited on a column of silica gel (2.5 × 25 cm) and eluted successively with 100 ml of ethyl acetate, 100 ml of system 3, and 200 ml of system 4. Evaporation of the last eluate yielded 50 mg of vaccaroside with mp 218-220°C (aqueous ethanol) [2].

Full Methyl Ether of Philoside B. To 50 mg of philoside B in 30 ml of absolute dimethyl sulfoxide in a current of nitrogen was added 3 ml of the methylsulfinylmethyl anion prepared in the following way: in a current of nitrogen, 70 mg of sodium hydride was stirred with 3 ml of absolute dimethyl sulfoxide at 50°C for 1 h, forming a homogeneous green solution. The reaction mixture was stirred for 10 min, and then 8 ml of CH_3I was added, and it was left for 15 h. Then 20 ml of water was added and the mixture was extracted with chloroform (3 × 50 ml). The chloroform extracts were washed with sodium thiosulfate solution and with water and evaporated, and the residue was hydrolyzed. By paper chromatography in systems 1 and 2 and by GLC using the retention times in relation to methyl 2,3,4,6-tetra-O-methyl-β-D-glucoside we identified 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-xylose, 2,3,4-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-rhamnose, 3,4-di-O-methyl-D-fucose, 3,4,6-tri-O-methyl-D-galactose, 3,4-di-O-methyl-D-xylose, and 2,3-di-O-methyl-L-arabinose.

Enzymatic Decomposition of Philoside B. A) A solution of 0.15 g of the initial glycoside in 50 ml of phosphate buffer (pH 4.01) was treated with 20 mg of diastase and the mixture was kept at 39°C for 22 h. Then the precipitate was separated off. The substrate was neutralized, evaporated, and deposited on a column of silica gel (2.2 × 30 cm). Elution was performed successively with 200 ml of butan-1-ol, 200 ml of system 7, and 250 ml of system 8. The last two eluates were evaporated, the dry residue (70 mg) was treated with butan-1-ol (5 × 5 ml), and the butanolic extracts were evaporated. A product was obtained with mp 118-120°C (from butan-1-ol), R_{Rha} 0.32; after reduction with $NaBH_4$ – $[\alpha]_D$ 0° (c 1.7; pyridine).

The product (10 mg) was heated in 5 ml of 5% sulfuric acid for 4 h. After neutralization, the hydrolyzate was found by paper chromatography in systems 1 and 2 to contain D-galactose, D-xylose, and L-arabinose in a ratio of 1 : 1 : 1. Another sample of the product (10 mg) was dissolved in 5 ml of dimethylformamide and treated with 40 mg of sodium hydride, and then, with stirring, 4 ml of methyl iodide was added dropwise. The reaction mixture was stirred for 6 h at 40°C and was then worked up in the usual way. A hydrolyzate of the permethylate was shown by paper chromatography in systems 1 and 2 to contain 3,4,6-tri-O-methyl-D-galactose, 3,4-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-L-arabinose.

B) A mixture of 0.5 g of philoside B in acetate buffer (pH 4.1) and 50 mg of diastase was thermostated at 40°C for four days. Then the substrate was neutralized with KU-2, and the product was extracted with butan-1-ol. The butanolic extract was evaporated and the dry residue (0.2 g) was deposited on a column (2 × 25 cm) of silica gel and was eluted with system 8, 20-ml fractions being collected. Fractions 5-8 contained an individual glycoside (60 mg), 246-250°C, $[\alpha]_D$ –0.8° (c 4.8; pyridine). In its acid hydrolyzate, galactose, arabinose, xylose, fucose, and rhamnose (1 : 2 : 2 : 1 : 1) were identified by paper chromatography in systems 1 and 2.

In a current of nitrogen, 3 ml of the methylsulfinylmethyl anion prepared as described above was added dropwise to 40 mg of the glycoside in 3 ml of absolute dimethyl sulfoxide. Then 10 ml of CH_3I was added, and the mixture was left for 15 h. After this, it was worked up in the usual way.

The permethylate (30 mg) in 4 ml of a mixture of absolute benzene and absolute ether (1 : 1) was added dropwise to a suspension of $LiAlH_4$ in absolute ether, and the mixture was stirred with gentle heating for 5 h. The complex was decomposed with 5% acetic acid, and the product was extracted with ether. The acid hydrolyzate of the product obtained was shown to contain 2,3,4-tri-O-methyl-D-xylose, 2,3,4-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-rhamnose, 3,4,6-tri-O-methyl-D-galactose, 3,4-di-O-methyl-D-xylose, 4-mono-O-methyl-D-glucose, and 2,3-di-O-methyl-L-arabinose.

SUMMARY

The structure of philoside B – a gypsogenin nonaoside isolated from the roots of Gypsophila patrinii – has been established.

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