TRITERPENE GLYCOSIDES OF Gypsophila patrinii

II. THE STRUCTURE OF PHILOSIDE A

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As reported previously [1], from the roots of Gypsophila patrinii we have isolated a high-molecular-weight glycoside of gypsogenin. In its chromatographic mobility on paper and thin-layer chromatograms it coincides with the known gypsoside [2], although its constants differed from those of the latter. On chromatograms two closely adjacent spots were clearly shown, which, by analogy with gypsoside, were ascribed to two forms of one and the same glycoside.

In the present paper we report the fact that by using other solvent systems for chromatography we have succeeding in separating these assumed forms into individual components which proved to be two different glycosides – philosides A and B. A proof of the structure of the first of them is given below.

On being heated with mineral acids, philoside A forms, in addition to gypsogenin, 1 mole each of D-glucose, D-fucose, and glucuronic acid, and also 2 moles each of D-galactose, D-xylose, and L-rhamnose. Consequently, philoside A is a nonaoside of gypsogenin. Its alkaline saponification yielded a pentaoside which, in its turn, on acid hydrolysis gave the aglycone, glucuronic acid, and 2 moles each of D-galactose and D-xylose. On comparing the carbohydrate composition of the initial philoside A and that of the pentaoside it can be seen that the O-acyl glycosidic carbohydrate chain of the former contains one residue each of D-fucose and D-glucose and two L-rhamnose residues.

The Smith degradation of philoside A and of the pentaoside formed the same product – gypsogenin glucuronoside (vaccaroside). This shows, in the first place, that the glucuronic acid is attached to the hydroxyl of the aglycone and, in the second place, the absence of (1→3) bonds between the monosaccharide units in the carbohydrate chains. We obtained further information on the structure of the glycoside from the results of the methylation of philoside A and the pentaoside.

The permethylates were synthesized by treating the glycosides with methyl iodide and sodium hydride in dimethylformamide solution [3], and they were then hydrolyzed. The methyl ethers of the sugars formed were separated by preparative paper chromatography and were identified by their constants, by some chemical reactions, and also by the relative retention times of the corresponding methyl glycosides on analysis by gas–liquid chromatography (GLC). The permethylate of philoside A was cleaved into 2,3-di-O-methyl-D-glucose, 3,4-di-O-methyl-D-fucose, 2,3,4-tri-O-methyl-L-rhamnose, 3,4,6-tri-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-xylose, and free glucuronic acid. The last four sugars were also formed from the permethylate of the pentaoside.

The results of methylation showed that in the carbohydrate chains of philoside A the glucose and glucuronic acid residues are the centers of branching, all their hydroxyls being bound with carbohydrate residues.

In the O-acyl glycosidic chain, the D-fucose residue is directly attached to the carboxyl of the aglycone. This conclusion was based on the alkaline saponification of philoside A, as a consequence of which the fucose residue was destroyed. The D-glucose residue is present in the middle of the chain, and the two rhamnose residues terminate it.

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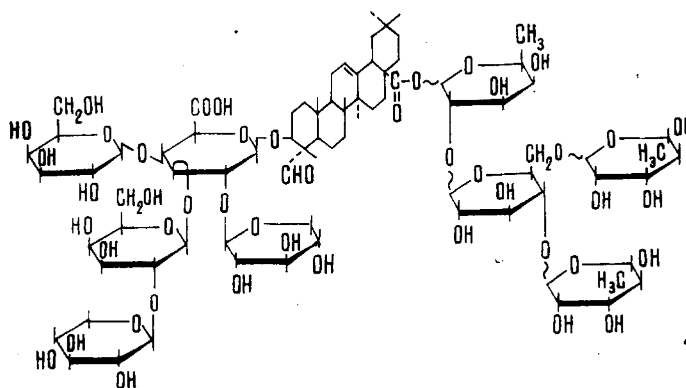
In the carbohydrate chain attached to the hydroxyl of the aglycone, one D-galactose and two D-xylose residues occupy terminal positions. However, their mutual arrangement remained obscure. Consequently, partial hydrolysis of the pentaoside was performed by heating it with dilute sulfuric acid. This gave a series of progenins, one of which contained glucuronic acid and two galactose residues. From this the complete methyl ether was prepared, and this was reduced with lithium tetrahydroaluminate and hydrolyzed. Since, in addition to tetramethylgalactose, 2-O-methyl-D-glucose, formed by the reduction of a monomethylglucuronic acid residue, was found in the hydrolyzate, the galactose residues are attached to its hydroxyls in positions 3 and 4. Accordingly, one of the xylose residues in philoside A has a (1→2) bond with the glucuronic acid.

The position of the other xylose residue was determined by the enzymatic hydrolysis of philoside A. When it was treated with diastase, one galactose residue was split off. To determine the position of its attachment, the product of enzymolysis was hydrolyzed with alkali, methylated, reduced with lithium tetrahydroaluminate, and hydrolyzed. 2,3,4-Tri-O-methyl-D-xylose, 3,4,6-tri-O-methyl-D-galactose, and 4-mono-O-methyl-D-glucose were identified in the hydrolyzate. Thus, it is clear that the galactose residue split off had a (1→4) bond and the disaccharide residue consisting of galactose and xylose, correspondingly, had a (1→3) bond with the glucuronic acid residue.

The structure of the main glycoside of Gypsophila patrinii – philoside A – follows from what has been said above. After calculation by Klyne's method of the configurations of some glycosidic bonds, the following structure is proposed for philoside A:



EXPERIMENTAL

Chromatography was performed with type KSK silica gel, Schleicher and Schüll No. 2043 paper, and metal plates with a thin layer of SiO_2 ["Silufol," Kavalier (Czechoslovakia)], and with the following solvent systems: 1) butan-1-ol–acetic acid–water (4 : 1 : 5); 2) acetic acid–methanol–water (1 : 4 : 4); 3) and 4) butan-1-ol–methanol–water (5 : 3 : 2) and (5 : 2 : 1), respectively; 5) benzene–butanol–pyridine–water (1 : 5 : 3 : 3); 6), 7), and 8) ethyl acetate–methanol–water (10 : 1 : 1), (10 : 2 : 3), and (20 : 3 : 6), respectively; and 9) butan-1–ethanol–water (5 : 1 : 4). The R_g values of the methylated monosaccharides are given for system 9.

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 a ERJ-65 automatic densitometer.

The dry comminuted roots of the plant (2.9 kg) were first extracted with chloroform and were then extracted exhaustively with 80% aqueous methanol at the boil. The extract obtained (450 g) was dissolved in 2 liters of water and extracted with butan-1-ol (3 × 1 liter). The process was monitored by thin-layer chromatography in system 1. The residual aqueous solution was evaporated to a volume of 1 liter, deposited on 1 kg of ÉDÉ-10P anion-exchange resin, and left for 15 h. Then the resin was washed with 3 liters of water, 1.5 liters of 30% acetic acid, and 6 liters of solvent system 2. After the evaporation of the two latter eluates, 40 g of a mixture of glycosides without reserve sugars was obtained which was separated on a column of silica gel (5 × 100 cm) in system 3. The separation was monitored by TLC in systems 3 and 4. After repeated separation, 6.4 g of philoside A was isolated with mp 258-262°C (from system 1), $[\alpha]_D$ −4.9° (c 1.1; pyridine). Acetate: $C_{128}H_{178}O_{68}$, mp 175-177°C (from aqueous ethanol), $[\alpha]_D$ −13° (c 2.3; pyridine).

In addition, 0.6 g of philoside B was obtained with mp 232-235°C (system 4), $[\alpha]_D$ 0° (c 2.8; aqueous pyridine). Acetate: $C_{137}H_{190}O_{74}$, mp 200-202°C (aqueous ethanol), $[\alpha]_D$ +5.3° (c 3.8; pyridine).

Acid Hydrolysis of Philoside A. A mixture of 0.25 g of philoside A and 25 ml of 5% sulfuric acid was heated in the boiling-water bath for 8 h. The precipitate that had deposited was separated off, and the filtrate was neutralized with AV-17 anion-exchange resin and chromatographed on paper in systems 1 and 5. Galactose, glucose, xylose, fucose, and rhamnose in a ratio of 2 : 1 : 2 : 1 : 2 and glucuronic acid were identified by comparison with authentic samples.

Alkaline Hydrolysis of Philoside A. A solution of 1 g of philoside A in 50 ml of 5% KOH was heated in a nitrogen-filled tube at 80°C for 12 h. Then the reaction mixture was neutralized with KU-2 cation-exchange resin, evaporated, and extracted with butan-1-ol (5 × 15 ml). The butanolic extract was washed with water, evaporated, deposited on a column of silica gel (2 × 15 cm), and extracted successively with ethyl acetate (200 ml), system 6 (250 ml), and system 7. The last eluate contained a pentaoside (0.48 g) with mp 255-257°C (from butan-1-ol), $[\alpha]_D$ 0° (c 2.7; pyridine). This product (30 mg) was hydrolyzed in 10 ml of 5% sulfuric acid. After neutralization, galactose and xylose (2 moles of each) and glucuronic acid were found in the hydrolyzate by paper chromatography by systems 1 and 5.

The aqueous extract was treated with ÉDÉ-10P anion-exchange resin and evaporated, and the residue was dissolved in 30 ml of aqueous ethanol and hydrolyzed by heating with 2% hydrochloric acid. D-Glucose and L-rhamnose were found in the hydrolyzate by paper chromatography in systems 1 and 5.

Smith Degradation of Philoside A. A solution of 0.5 g of the glycoside in 120 ml of water was treated with 1.4 g of sodium periodate and 2 ml of acetic acid, and the mixture was kept at room temperature for 8 days. The unchanged periodate was decomposed by the addition of 0.5 ml of propylene glycol, and the reaction mixture was evaporated, diluted with 30 ml of water, and extracted with butan-1-ol (5 × 25 ml).

The butanolic extracts were washed with water and evaporated, the dry residue (0.46 g) was dissolved in 25 ml of methanol, to this solution were added 20 ml of water and 0.5 g of sodium tetrahydroborate, and the mixture was stirred without heating for 8 h. Then it was neutralized with KU-2 cation-exchange resin and evaporated, with the addition of methanol (3 × 10 ml). The dry product (0.4 g) was dissolved in 60 ml of 70% ethanol and 8 ml of 5% sulfuric acid and was heated at 60-70°C for 1 h. No free monosaccharides were found in the hydrolyzate by paper chromatography. The hydrolyzate was evaporated, the residue was deposited on a column of silica gel (2.5 × 30 cm), and elution was performed successively with 150 ml of ethyl acetate, 150 ml of system 6, and 250 ml of system 7. Evaporation of the last eluate yielded 0.27 g of vaccaroside [4] with mp 218-220°C (from aqueous ethanol), $[\alpha]_D$ +21.8° (c 6.1; pyridine).

Smith Degradation of the Pentaoside. In the same way as described above, 0.5 g of the pentaoside yielded 0.3 g of vaccaroside with mp 218-220°C (from 80% ethanol), $[\alpha]_D$ +20.4° (c 4.42; pyridine).

Full Methyl Ether of Philoside A. To a solution of 0.6 g of the initial glycoside in 30 ml of dimethylformamide were added 0.15 g of sodium hydride and, with stirring, over 1 h, 6 ml of methyl iodide. The mixture was heated to 75°C, after which it was stirred at this temperature for another 6 h, with the addition of 0.05 g of sodium hydride and 3 ml of methyl iodide. Then it was diluted with 50 ml of water and was extracted with chloroform (3 × 50 ml). The chloroform extracts were washed with sodium thiosulfate solution and with water and were evaporated. The residue was deposited on a column containing 15 g of silica gel and was eluted with chloroform with the addition of methanol (5 → 15%). This gave 0.18 g of the permethylate in the form of an amorphous powder with $[\alpha]_D$ +20° (c 1; chloroform).

The permethylate (0.1 g) was heated with 3 ml of absolute methanol containing 4% of HCl at 80°C for 4 h. The mixture of methyl glycosides of the fully methylated monosaccharides was extracted with chloroform and analyzed by GLC. Peaks were observed with retention times (in relation to that of methyl 2,3,4,6-tetra-O-methyl-β-D-glycoside) of 0.47, 0.59, and 1.9. Literature figures: 0.46 (methyl 2,3,4-tri-O-methyl-β-L-rhamnoside), 0.46 and 0.57 (methyl 2,3,4-tri-O-methyl-α- and -β-D-xylosides) and 1.80 (methyl 2,3,4,6-tetra-O-methyl-β-D-galactoside) [5].

The permethylate (0.1 g) was heated in aqueous methanol (1 : 1) containing 3% of HCl on the boiling-water bath for 3 h. The hydrolyzate was neutralized with AV-17 anion-exchange resin and evaporated to dryness, and the residue, in 50-mg portions, was separated preparatively on chromatographic paper (29 × 58 cm) in system 9. Several substances were isolated: 2,3-di-O-methyl-D-glucose (22.7 mg), $[\alpha]_D$ +6.8° (c 1; benzene–methanol), R_g 0.52 (literature figures: $[\alpha]_D$ +5.9 [5]), positive reaction with KIO_4, negative reaction with triphenyltetrazolium chloride [7]; on demethylation by heating a sample with 48% HBr, glu-

cose was identified; 3,4-di-O-methyl-D-fucose (22.1 mg), $[\alpha]_D$ +41.5° (c 1; benzene–methanol), R_g 0.68, reaction for an α-diol group and the reaction with triphenyltetrazolium chloride positive [7]; and 3,4,6-tri-O-methyl-D-galactose (19 mg), $[\alpha]_D$ +45° (c 1; ethanol), R_g 0.72, reactions with KIO_4 and triphenyltetrazolium chloride positive [7]; on demethylation galactose was identified.

Full Methyl Ether of the Pentaoside. The pentaoside (80 mg) was methylated as described above. This gave 55 mg of permethylate, which was heated in aqueous methanol (1 : 1) containing 3% of HCl on the boiling-water bath for 3 h. The hydrolyzate was shown to contain as described above 3,4,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-xylose, and 2,3,4,6-tetra-O-methyl-D-galactose.

To the pentaoside (40 mg) in a mixture of absolute ether and dioxane (1 : 1) was added 20 mg of lithium tetrahydroaluminate in 20 ml of absolute ether. The resulting mixture was stirred with heating for 5 h and was then diluted with 20 ml of 5% acetic acid and extracted with ether (3 × 40 ml). The extract was evaporated and the residue was heated with 10 ml of 6% HCl in aqueous methanol. After neutralization, glucose was identified in the hydrolyzate by paper chromatography with an authentic sample in systems 1 and 5.

Partial Hydrolysis of the Pentaoside. To a solution of 0.83 g of the pentaoside in 60 ml of propanol were added 30 ml of water and 3 ml of concentrated H_2SO_4. The mixture was heated at 85°C for 25 min, rapidly cooled, and neutralized with AV-17 anion-exchange resin, and the solution was evaporated. The dry residue (0.4 g) was deposited on a column (2.5 × 60 cm) of silica gel and was eluted first with 250 ml of ethyl acetate and then with system 8, 15-ml fractions being collected. Fractions 12-16 yielded 30 mg of a progenin with mp 270-275°C $[\alpha]_D$ + 13.7° (c 1.6; pyridine). Galactose and glucuronic acid were identified in the acid hydrolyzate by paper chromatography in systems 1 and 5.

The glycoside (30 mg) was methylated under the usual conditions and was then reduced with lithium tetrahydroaluminate as described above. The hydrolyzate was shown by paper chromatography in system 9 to contain 2-mono-O-methyl-D-glucose with R_g 0.17 and 2,3,4,6-tetra-O-methyl-D-galactose with R_g 0.9. Literature figures: R_g 0.2 and 0.88, respectively [8]. For the 2-O-methyl-D-glucose the reaction for an α-diol grouping was positive and the reaction with triphenyltetrazolium chloride, negative.

Enzymatic Hydrolysis of Philoside A. To a solution of 0.5 g of philoside A in 50 ml of phosphate buffer (pH 4.5) was added 50 mg of diastase, and the mixture was kept at 37°C for 3 days. The substrate was filtered, evaporated to a small volume, and extracted with butan-1-ol (5 × 15 ml). The butanolic extract was evaporated, and the dry residue (0.4 g) was deposited on a column (2.4 × 40 cm) of silica gel and eluted first with ethyl acetate (200 ml) and then with solvent system 7. Evaporation of the last eluate yielded 0.38 g of a glycoside with mp 250-253°C (from butan-1-ol), $[\alpha]_D$ −11.6° (c 2.24; pyridine). After heating with 5% H_2SO_4, the hydrolyzate was shown by paper chromatography in systems 1 and 5 to contain galactose, glucose, xylose, fucose, and rhamnose in a ratio of 1 : 1 : 2 : 1 : 1 and glucuronic acid.

The product isolated on enzymolysis (0.2 g) was subjected to alkaline saponification under the conditions given. This yielded 70 mg of a glycoside with mp 235-240°C (from butan-1-ol) $[\alpha]_D$ + 15.5° (c 3.4; pyridine), which, on acid hydrolysis, was cleaved into glucuronic acid, xylose, and galactose. The glycoside was methylated under the usual conditions and was then reduced with lithium tetrahydroaluminate. The product isolated was hydrolyzed by fractions.

The reduction product (10 mg) was heated in 2 ml of methanol containing 4% of HCl. The hydrolyzate was shown by paper chromatography in system 9 with the reagent for an α-diol grouping to contain methyl 4-mono-O-methyl-D-glucoside (R_g 0.64).

The product (10 mg) was hydrolyzed in 2 ml of 2% hydrochloric acid, and by paper chromatography in system 9 with authentic samples 4-mono-O-methyl-D-glucose (R_g 0.23), 3,4,6-tri-O-methyl-D-galactose (R_g 0.75), and 2,3,4-tri-O-methyl-D-xylose (R_g 0.9) were identified.

SUMMARY

From the roots of Gypsophila patrinii two high-molecular-weight triterpene glycosides have been isolated – philosides A and B. The structure of philoside A has been established; it is a gypsogenin nonaoside.

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