TRITERPENE GLYCOSIDES OF Dianthus deltoides

II. STRUCTURE OF DIANTHOSIDE C

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As we have reported previously [1], from a methanolic extract of Dianthus deltoides L. three main triterpene glycosides have been isolated – dianthosides A, B, and C. In the present paper we give information on the determination of the structure of dianthoside C.

The Smith degradation of dianthoside C [2] and subsequent severe hydrolysis gave us the following results. After the first oxidation a glycoside was formed the carbohydrate chain of which consisted of fucose, glucuronic acid, and two molecules of xylose (densitometry) [3]. Subsequent oxidation of the glycoside obtained led to the destruction of all the monosaccharides mentioned except the glucuronic acid. Consequently, the fucose, glucuronic acid, and xylose are either centers of branching of carbohydrate chains or have 1-3 bonds.

The treatment of dianthoside C with methyl iodide and sodium hydride [4] in dimethylformamide yielded the full methyl ether, which was methanolysed and hydrolysed. The methylated monosaccharides so formed were separated chromatographically on silica gel and preparatively on paper and were identified by their constants, the relative retention times of the corresponding methyl glycosides, and by some chemical transformations and color reactions.

The following were isolated: 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose, 3,4-di-O-methyl-L-rhamnose, 2,4-di-O-methyl-D-xylose, 2-O-methyl-D-fucose, 4-O-methyl-D-xylose, and 2,4-di-O-methyl-D-glucuronic acid (in the form of 2,4-di-O-methyl-D-glucose).

Methylation showed that the centers of branching are the fucose and xylose, and the terminal monosaccharides are rhamnose, galactose, and arabinose.

Further information on the structure of the carbohydrate chains of dianthoside C was obtained by the reductive cleavage of its permethylate with lithium tetrahydroaluminate in dioxane. This formed the reduced permethylate of the acid glycoside (I) and the permethylate of the oligosaccharide (II). After the acid hydrolysis of (I), 2,4-di-O-methyl-D-glucose, 2,4-di-O-methyl-D-xylose, and 2,3,4,6-tetra-O-methyl-D-galactose were identified. This clearly shows the structure of the carbohydrate chain of the acid glycoside [1].

On being heated with acids, (II) decomposed into 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose, 3,4-di-O-methyl-L-rhamnose, 4-O-methyl-D-xylose, and 2-O-methyl-D-fucitol. The definitive structure of the carbohydrate chain attached to the carboxyl in dianthoside C was established by the enzymatic hydrolysis of the latter with diastase. In addition to a progenin consisting of gypsogenin, glucuronic acid, and xylose, we isolated a disaccharide and a trisaccharide.

On acid hydrolysis, the disaccharide split into rhamnose and xylose, and the trisaccharide into galactose, rhamnose, and xylose. The composition of the oligosaccharides shows that they were split from the carbohydrate chain attached to the carboxy group of the aglycone, and that the disaccharide is the product of the further decomposition of the trisaccharide. On acid hydrolysis, the full methyl ether of the disaccharide split into 2,3,4-tri-O-methyl-L-rhamnose and 3,4-di-O-methyl-D-xylose. It follows from

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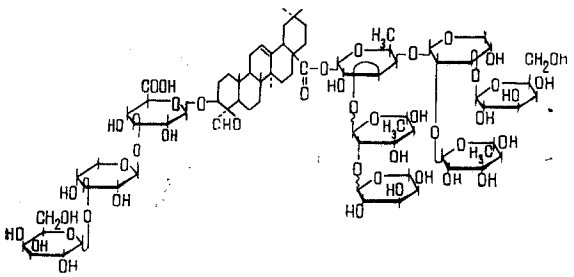
this, and also from an analysis of the composition of the methylated monosaccharides formed in the hydrolysis of (II) that the trisaccharide is branched and the galactose is attached to the xylose in position 3. The position of attachment of the trisaccharide to the fucose forming the branching center is the hydroxyl at C_4 of the latter, as follows from the results of the Smith degradation of dianthoside C. Consequently, the residue of the carbohydrate chain consisting of rhamnose and arabinose must be attached at C_3 of the rhamnose. The calculation of the configuration of some of the glycosidic bonds was performed by means of Klyne's rule.

Thus, the formula of dianthoside C may be given as



EXPERIMENTAL

For chromatography we used type KSK silica gel and paper of type "M" ["slow"] of the Volodarskii Leningrad Mill and Schleicher und Schüll No. 2043 with the following systems of solvents: 1) butan-1-ol–acetic acid–water (4 : 1 : 5); 2) benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3); 3) benzene–ether (1 : 4); 4) butan-1-ol–ethanol–water (5 : 1 : 4); and 5) butan-1-ol saturated with water.

The R_g values of the methylated monosaccharides are given for system 4. The gas-liquid chromatography (GLC) of the methylated sugars was performed on a "Tswett-1" instrument [column 1 m long and 4 mm in diameter filled with Celite 545 with 15% of poly(butyleneglycol succinate)]. The retention times T_{rel} of the methyl glycosides of the monosaccharides were measured in relation to the retention time of methyl β-2,3,4,6-tetra-O-methyl-D-glucoside. Paper electrophoresis was performed on a type OE-202 instrument.

Smith Degradation of Dianthoside C. A solution of 0.25 g of dianthoside C in 100 ml of water was treated with 0.8 g of sodium paraperiodate and 1.2 ml of acetic acid, and the mixture was kept at room temperature for 8 days. The unchanged periodate was destroyed by the addition of ethylene glycol. The reaction mixture was evaporated, and the residue was dissolved in 25 ml of water and extracted with butan-1-ol (5 × 20 ml). The butanolic extracts were washed with water and evaporated, and the residue (0.2 g) was treated with 10 ml of water and 0.7 g of sodium tetrahydroborate, the mixture being heated at 70-80° C with stirring for 8 h. Then it was neutralized with KU-2 cation-exchange resin and evaporated with the addition of methanol (3 × 10 ml).

The product was dissolved in 20 ml of 1% sulfuric acid and the solution was heated at 60-70° C for 1 h and was then extracted with butan-1-ol (5 × 20 ml). The butanolic extracts were washed with water and evaporated. The resulting glycoside (0.15 g) was recrystallized from butan-1-ol. Mp 232-237° C, $[\alpha]_D^{20}$ + 43° (c 1.0; pyridine). A mixture of 20 ml of the glycoside and 3 ml of 5% hydrochloric acid was heated in the boiling water bath for 6 h. After neutralization with AV-17 anion-exchanger, paper chromatography in systems 1 and 2 showed the presence in the hydrolysate of fucose and xylose in a ratio of 1 : 1.87 (densitometry) [3], and glucuronic acid.

The product isolated (0.12 g) was reoxidized. The reaction was performed with 0.2 g of sodium paraperiodate and 0.6 ml of acetic acid in 50 ml of water. The experiment was performed in a similar manner to the preceding one. In the hydrolysate of the oxidized glycoside, glucuronic acid was found by paper chromatography in systems 1 and 2.

Full Methyl Ether of Dianthoside C. A solution of 3 g of dianthoside C in 30 ml of dimethylformamide was treated with 1 g of sodium hydride and 7 ml of methyl iodide and the mixture was stirred at room temperature for 3.5 h and then at the boiling point of the methyl iodide for 2.5 h. After this it was diluted with a saturated solution of sodium thiosulfate, the precipitate was filtered off, and the solution was extracted with chloroform (6 × 30 ml). The chloroform extracts were washed with water and evaporated.

The residue (2.8 g) was transferred to a column of silica gel (35 g). The permethylate of dianthoside C was eluted with 250 ml of chloroform–ethanol (50:1). Yield 2.4 g. Amorphous powder with the composition $C_{104}H_{176}O_{44}$, $[\alpha]_D^{20}$ + 13° (c 1.1; chloroform).

The permethylate of dianthoside C (1.4 g) was dissolved in 30 ml of 2% HCl in methanol and the solution was heated at 90° C for 6 h. Then it was diluted twofold with water and heated for another 2 h. The precipitate of gypsogenin that separated out was filtered off, and the filtrate was neutralized with AV-17 anion-exchange resin and evaporated. The residue (1 g) was transferred to a column (2.5 × 35 cm) of silica gel. Elution was performed with mixtures of chloroform and isopropanol (1 → 15%), 25-ml fractions being collected. The methyl ethers of the sugars were isolated.

Fractions 1 and 2 (60 mg) yielded 2,3,4-tri-O-methyl-L-rhamnose with $[\alpha]_D^{20}$ + 28° (c 1.0; water) R_g 1.02. Literature data: $[\alpha]_D$ + 24.9 (water) [5], R_g 1.02 [6]. In GLC it coincided with a marker.

Fractions 3 and 4 (90 mg) consisted of 2,3,4,6-tetra-O-methyl-D-galactose with $[\alpha]_D^{20}$ + 110° (c 1.0; water), R_g 0.88. Literature data: $[\alpha]_D$ + 117.8° (water) [7], R_g 0.88 [6]. In GLC it coincided with an authentic sample.

Fractions 6 and 7 (80 mg) were separated by preparative rechromatography on Schleicher und Schüll paper (40 × 50 cm) in system 4. This yielded 25 mg of 3,4-di-O-methyl-L-rhamnose with $[\alpha]_D^{20}$ + 8° (c 1.1; water), R_g 0.84. Literature data: $[\alpha]_D \pm 0 \rightarrow +18.6°$ [8], R_g 0.84 [6]. It was oxidized by sodium periodate and reacted with triphenyltetrazolium chloride [9]. After demethylation with BCl_3 [10], L-rhamnose was identified by paper chromatography in systems 1 and 2. In addition, 35 mg of 2,3,4-tri-O-methyl-L-arabinose with $[\alpha]_D^{20}$ + 128° (c 1.2; water), R_g 0.81, was obtained. Literature data: $[\alpha]_D$ + 133.4° (water) [11], R_g 0.83 [12]. In GLC, it coincided with an authentic sample.

Fractions 9 and 10 (70 mg) consisted of 2,4-di-O-methyl-D-xylose with $[\alpha]_D^{20}$ – 32° (c 1.3; water), R_g 0.66. T_{rel} 1.5 and 2.0. Literature data: $[\alpha]_D$ – 30° (water) [13], R_g 0.66 [6], T_{rel} 1.50 and 1.97 [14]. It was not oxidized by potassium periodate. After demethylation, xylose was formed.

Fraction 13 (50 mg) consisted of 4-O-methyl-D-xylose $[\alpha]_D^{20}$ + 47° (c 1.1; water), R_g 0.39, T_{rel} 4.40. Literature data: R_g 0.39 [12], T_{rel} 4.35 [14]. It gave a positive reaction with triphenyltetrazolium chloride [9]. On demethylation with BCl_3, xylose was formed.

Fraction 14 (60 mg) consisted of 2-O-methyl-D-fucose, $[\alpha]_D^{20}$ + 67° (c 1.1; water), R_g 0.51. It had the same chromatographic behavior as an authentic sample. Literature data: $[\alpha]_D$ + 75° (water) [15], R_g 0.51 [6]. The reaction with triphenyltetrazolium chloride was negative [9].

Reductive Cleavage of the Permethylate of Dianthoside C with $LiAlH_4$. A solution of 0.5 g of the permethylate of dianthoside C in 10 ml of dry dioxane was treated with 0.2 g of $LiAlH_4$, and the reaction mixture was heated with stirring for 10 h. The excess of $LiAlH_4$ was destroyed by the addition of ethyl acetate and water. The solution was evaporated and the residue was dissolved in 15 ml of water and extracted with ether (7 × 10 ml). The ethereal extracts were washed with water and evaporated. The residue (0.3 g) was transferred to a column (1.5 × 15 cm) of silica gel and eluted with a mixture of chloroform and ethyl acetate (2:1) (50 ml), giving 0.2 g of (I) in the form of an amorphous powder with the composition $C_{61}H_{110}O_{21}$, $[\alpha]_D^{20}$ + 30° (c 2.4; chloroform).

The product obtained was dissolved in 10 ml of 2% HCl in methanol and hydrolyzed as described above. The hydrolysate was neutralized with AV-17 anion-exchange resin and evaporated. The residue (0.1 g) was chromatographed on a column of silica gel (1.5 × 15 cm) under the conditions given above. The substances isolated were 14 mg of 2,3,4,6-tetra-O-methyl-D-galactose, 17 mg of 2,4-di-O-methyl-D-xylose and 14 mg of 2,4-di-O-methyl-D-glucose. The latter had $[\alpha]_D^{20}$ + 40° (c 1.2; water), R_g 0.07 (paper electrophoresis). Literature data: $[\alpha]_D$ + 43.4° (water) [16], R 0.05 [17]. It was not oxidized by sodium periodate.

The aqueous extract after the reduction of the dianthoside C was neutralized with KU-2 cation-exchange resin and evaporated, and the residue was transferred to a column of silica gel (1.5 × 15 cm). Elution with 40 ml of a mixture of chloroform and ethyl acetate (3:1) gave 0.1 g of (II) in the form of an amorphous powder with $[\alpha]_D^{20}$ + 59° (c 2.1; chloroform). It was hydrolyzed in 5 ml of 2% HCl. After neutralization of the hydrolysate, paper chromatography with markers showed the presence in it of 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose, 3,4-di-O-methyl-L-rhamnose, and 4-O-methyl-D-xylose. In addition, 2-O-methyl-D-fucitol (R_g 0.51) was identified by treating the chromatogram with sodium periodate.

Enzymatic Hydrolysis of Dianthoside C with Diastase. A solution of 0.5 g of dianthoside C in 50 ml of phosphate buffer with pH 5.5 was treated with 50 mg of diastase, and the mixture was kept at 30° C for 3 days. The substrate was extracted with butan-1-ol (5 × 15 ml), and the butanolic extract was washed with water and evaporated. The residue (0.3 g) was transferred to a column (1.5 × 15 cm) of silica gel. System 5 (50 ml) eluted the progenin of dianthoside C with the composition $C_{41}H_{64}O_{14} \cdot 2H_2O$ (100 mg). Mp 227-233°C, $[\alpha]_D^{20}$ −22° (d 1.2; pyridine).

The progenin (20 mg) was hydrolyzed in 2 ml of 5% hydrochloric acid. By paper chromatography in systems 1 and 2 with authentic samples, xylose and glucuronic acid were identified. The treatment of 50 mg of the progenin in 5 ml of dimethylformamide with 50 mg of sodium hydride and 1 ml of methyl iodide gace the full methyl ether. Amorphous powder with $[\alpha]_D^{20}$ −27° (c 1.2; chloroform). On acid hydrolysis, the latter was split into 2,3,4-tri-O-methyl-D-xylose (R_g 0.95) and 2,4-di-O-methyl-D-glucuronic acid (R_g 0.08).

The aqueous extract after enzymatic hydrolysis was neutralized with KU-2 and AV-17 ion-exchange resins and evaporated, and the residue (70 mg) was deposited on Schleicher und Schüll paper and chromatographed in system 1 for 2 days. This yielded 20 mg of disaccharide with mp 160-165° C, $[\alpha]_D^{20}$ + 55° (c 1.1; water), R_f 0.13 in system 1. On being heated with dilute mineral acids, it decomposed into xylose and rhamnose. In order to obtain the full methyl ether, 20 mg of the disaccharide was dissolved in 5 ml of dimethylformamide, and 20 ml of sodium hydride and 1 ml of methyl iodide were added. After the usual working up, the full methyl ester was isolated. Sirup with $[\alpha]_D^{20}$ + 15° (c 1.2; chloroform). On being heated with 2% HCl in methanol, it split into 2,3,4-tri-O-methyl-L-rhamnose (R_g 1.02) and 3,4-di-O-methyl-D-xylose (R_g 0.76). Literature data for 3,4-di-O-methyl-D-xylose: Rg 0.75 [12]. In addition to the disaccharide, the aqueous extract yielded by preparative paper chromatography 15 mg of a trisaccharide with mp 170-175°C, $[\alpha]_D^{20}$ + 50° (c 1.2; water), R_f 0.07 in system 1.

The trisaccharide (10 mg) was hydrolyzed in 2 ml of hydrochloric acid as described above. The hydrolysate was shown by chromatography in systems 1 and 2 to contain xylose, rhamnose, and galactose.

SUMMARY

The structure of dianthoside C – a triterpene glycoside from Dianthus deltoides L. – has been established.

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