TRITERPENE GLYCOSIDES OF Dianthus deltoides. I

V. G. Bukharov, S. P. Shcherbak,
and A. P. Beshchekova

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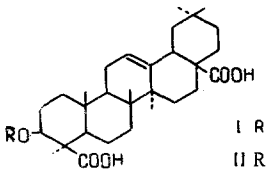
Dianthus deltoides L. (maiden pink) is a plant of the family Caryophyllaceae which has not previously been studied chemically. Other species of Dianthus contain saponins, but their individual glycosides have not been isolated [1]. We have studied Dianthus collected in the Tatar ASSR. An investigation of a methanolic extract of the whole plant in thin-layer chromatograms showed that it contains three glycosides, which we have called dianthosides A, B, and C (in order of increasing polarity).

Dianthoside A was isolated by the chromatography of an ethyl acetate extract from a methanolic extract on ÉDÉ-10P ion-exchange resin. On being heated with mineral acids, it decomposed into the aglycone, which was identified as gypsogenic acid, and glucose. From its molecular weight and the results of elementary analysis, dianthoside A is a monoside of gypsogenic acid.

The full methyl ether/ester obtained by treating dianthoside A with methyl iodide and sodium hydride in dimethylformamide [2] was cleaved by hydrolysis with the formation of dimethyl gypsogenate and 2,3,4,6-tetra-O-methyl-D-glucopyranose. Consequently, the glucose was attached to the hydroxyl of the gypsogenic acid, as is shown by the fact dianthoside A is not saponified by alkalis, the glucose having the β configuration of the glycosidic bond, as is shown by a calculation according to Klyne. The complete structure of dianthoside A is represented by (I)



I R β-D-Gp

II R β-D-Glp-6 β-D-Gp

Dianthoside B was isolated from a butanolic extract of a methanolic extract by partition chromatography on silica gel. On acid hydrolysis, it decomposed into gypsogenic acid and two molecules of glucose.

Treatment with diazomethane gave the methyl ester of dianthoside B, which with mineral acids formed not free gypsogenic acid but its dimethyl ester. This fact shows that in dianthoside B the carbohydrate chain is attached to the hydroxyl of gypsogenic acid. In order to obtain further information on the structure of the sugar chain, the full methyl ether/ester of dianthoside B was obtained. On hydrolysis, the latter formed, in addition to dimethyl gypsogenate, 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,3,4-tri-O-methyl-D-glucopyranose. The methylated monosaccharides were isolated and identified by means of their constants and were compared with authentic samples by gas-liquid chromatography. Calculation of the configurations of the glycosidic centers by Klyne's method showed that both glucose residues are attached by β-glycosidic bonds. On the basis of the results obtained, the structural formula of dianthoside B can be represented as (II).

In an aqueous extract of the methanolic extract dianthoside C remained, together with reserve sugars and oligosaccharides. It was obtained in chromatographically homogeneous form by gel filtration on Sephadex G-25. On acid hydrolysis, dianthoside C splits into gypsogenin, galactose (2 moles), arabinose (1 mole), xylose (2 moles), fucose (1 mole), rhamnose (2 moles), and glucuronic acid. In addition, a small amount of

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a progenin was formed which, on more severe hydrolysis, decomposed into gypsogenin and glucuronic acid. Dianthoside C contains two carbohydrate chains, since on alkaline hydrolysis it split out an oligosaccharide and formed an acid glycoside – desacyldianthoside C. The latter, on being heated with an acid, gave gypsogenin, galactose, xylose, and glucuronic acid.

The oligosaccharide, in its turn, was cleaved by acids into xylose, galactose, arabinose, and rhamnose.

The disappearance of the fucose can apparently be explained by its decomposition during alkaline hydrolysis [3]. As a comparison showed, in its qualitative and quantitative monosaccharide composition dianthoside C does not coincide with any of the known glycosides of gypsogenin.

EXPERIMENTAL

Chromatography was performed with type KSK silica gel, alumina, Sephadex G-25, and type "M" ("slow") paper of the Volodarskii Leningrad Paper Mill with the following solvent systems: 1) butan-1-ol–acetic acid–water (4 : 1 : 5); 2) benzene–butan-1-ol–pyridine–water (10 : 50 : 30 : 30); 3) benzene–ether (3 : 1); 4) butan-1-ol–ethanol–water (5 : 1 : 4); 5) butan-1-ol saturated with water; and 6) chloroform–ethyl acetate (3 : 1).

The analytical results for all the compounds corresponded to the calculated figures.

The dry raw material (1400 g) was first defatted with chloroform and was then extracted exhaustively with methanol in a Soxhlet apparatus. The yield of methanolic extract was 170 g. It was dissolved in water (1.2 liter) and extracted with ethyl acetate (8 × 150 ml) and with butan-1-ol (9 × 200 ml). The weight of the ethyl acetate extract was 4 g, and that of the butanolic extract 37 g.

Dianthoside A. 3.5 g of the ethyl acetate extract was transferred to a column (5 × 15 cm) of ÉDÉ-10P ion-exchange resin. The neutral fraction was eluted with 10 liters of 80% ethanol (0.5 g) and the acid fraction with four liters of 10% acetic acid in aqueous ethanol (2.5 g). The latter was crystallized from isopropanol, and then had the composition $C_{36}H_{56}O_{10} \cdot 2H_2O$, mp 220-225°C, $[\alpha]_D^{20}$ +37° (c 1.1; pyridine), mol. wt. 716 (by titration).

The acetate, $C_{44}H_{64}O_{14}$, had mp 180-182°C (from aqueous ethanol), $[\alpha]_D^{20}$ +12° (c 2.4; chloroform).

Acid Hydrolysis of Dianthoside A. A mixture of 0.8 g of dianthoside A and 10 ml of 5% hydrochloric acid was heated for 6 h. The precipitate that deposited was filtered off and dried (0.3 g). The filtrate was neutralized with AV-17 anion-exchange resin and was chromatographed on paper in systems 1 and 2. Glucose was identified. The residue was transferred to a column of silica gel (2 × 15 cm). The genin was eluted with 80 ml of ether, mp 358-360°C (from isopropanol). It gave no depression of the melting point with gypsogenic acid, $[\alpha]_D^{20}$ +93° (c 1.07; pyridine). The melting point of the acetate of the genin was 340-343°C (from ethanol), $[\alpha]_D^{20}$ +72° (c 1.0; pyridine). The methyl ester of the genin (by treatment with diazomethane) had mp 241-243°C, $[\alpha]_D^{20}$ +76° (c 1.2; chloroform). The analytical results for the genin and its derivatives corresponded to the calculated figures.

An Attempt at the Alkaline Hydrolysis of Dianthoside A. A solution of 50 mg of dianthoside A in 5 ml of 5% KOH was heated at 90°C for 5 h, after which it was neutralized with KU-2 cation-exchange resin and evaporated in vacuum. Unchanged dianthoside A was recovered.

Full Methyl Ether/Ester of Dianthoside A. A solution of 100 mg of dianthoside A in 10 ml of dimethylformamide was treated with 1 ml of methyl iodide and 50 mg of sodium hydride and boiled with stirring for 3 h. Then it was poured into a saturated solution of sodium thiosulfate. The precipitate that deposited was filtered off, and the filtrate was extracted with chloroform (5 × 20 ml). The chloroform extracts were washed with water, evaporated, and transferred to a column of silica gel (15 × 1.5 cm). The full methyl ether/ester of dianthoside A was eluted with 100 ml of chloroform (60 mg). This gave $C_{40}H_{64}O_{10}$ with $[\alpha]_D^{20}$ +16° (c 1.7; chloroform).

The resulting product (20 mg) was dissolved in 5 ml of 2% hydrochloric acid in methanol and the solution was heated in the water bath for 6 h. Then it was diluted twofold with water and heated for another 1.5 h. The precipitate (7 mg) was filtered off. Dimethyl gypsogenate was identified by thin-layer chromatography in system 3.

The filtrate was neutralized with AV-17 anion-exchange resin, evaporated, and transferred to a column of silica gel (10 × 1.5 cm). Elution with 50 ml of chloroform gave 7 mg of 2,3,4,6-tetra-O-methyl-D-glucose $[\alpha]_D^{20}$ +83° (1.0; acetone), R_g 1.00 in system 4. According to the literature, $[\alpha]_D^{20}$ +83.9° (acetone) [4].

Dianthoside B. 37 g of a butanolic extract from the methanolic extract was dissolved in 50 ml of system 6 and the solution was filtered twice through a layer of alumina (10 × 5 cm). The filtrate was evaporated (30 g) and transferred to a column of silica gel (55 × 5 cm). Elution was performed with system 5, 200-ml fractions being collected. Fractions 4-9 contained chromatographically homogeneous dianthoside B (5 g), $C_{42}H_{66}O_{15} \cdot 2H_2O$ with mp 229-234°C (from butanol), $[\alpha]_D^{20}$ −18° (c 1.9; pyridine), mol. wt. 846 (by titration).

The acetate, $C_{56}H_{80}O_{22}$, had mp 159-162°C (from aqueous ethanol), $[\alpha]_D^{20}$ −23° (c 1.4; chloroform).

Dimethyl Ester of Dianthoside B. A solution of 250 mg of dianthoside B in 10 ml of dioxane was treated with 5 ml of an ethereal solution of diazomethane, and the mixture was kept at room temperature for 24 h. Then it was evaporated and the residue was crystallized from butan-1-ol, giving $C_{44}H_{70}O_{15} \cdot 2H_2O$ with mp 214-218°C, $[\alpha]_D^{20}$ −36° (c 1.1; pyridine).

The product obtained (50 mg) was dissolved in 5 ml of 5% hydrochloric acid and the solution was heated in the water bath for 6 h. The precipitate that deposited was filtered off and crystallized from ethanol, mp 243°C giving no depression of the melting point with dimethyl gypsogenate. Glucose was found in the filtrate after neutralization with AV-17 resin by paper chromatography.

Acid Hydrolysis of Dianthoside B. Dianthoside B (50 mg) was dissolved in 3 ml of 5% hydrochloric acid and hydrolyzed at 90°C for 6 h. The precipitate that deposited was filtered off and recrystallized from ethanol. The constants and chromatographic behavior of the product agreed with those of gypsogenic acid. Glucose was found in the filtrate by paper chromatography in systems 1 and 2.

Attempt at the Alkaline Hydrolysis of Dianthoside B. A mixture of 50 mg of dianthoside B and 10 ml of 5% KOH was heated at 90°C for 6 h. Then it was neutralized with KU-2 cation-exchange resin. Unchanged dianthoside B was recovered.

Full Methyl Ether/Ester of Dianthoside B. A solution of 300 mg of dianthoside B in 10 ml of dimethylformamide was treated with 1.5 ml of methyl iodide and 150 mg of sodium hydride. The reaction was performed in a similar manner to that described above, giving 350 mg of a product which was transferred to a column of silica gel (15 × 1.5 cm), after which elution with system 6 (150 ml) yielded the full methyl ether/ester of dianthoside B (240 mg). The IR spectrum of the product had no absorption bands in the hydroxyl region: $C_{49}H_{80}O_{15}$, mp 75-78°C, $[\alpha]_D^{20}$ − 27° (c 2.2; acetone).

The product obtained (100 mg) was heated in 10 ml of 2% hydrochloric acid in methanol as described previously. Twenty mg of the hydrolysate was separated on Schleicher and Schull No. 2043 paper preparatively in system 5. This gave 8 mg of 2,3,4,6-tetra-O-methyl-D-glucopyranose, $[\alpha]_D^{20}$ +82° (c.10; water), R_g 1 in system 4, and 7 mg of 2,3,4-tri-O-methyl-D-glucopyranose, $[\alpha]_D^{20}$ +71° (c 2.1; water), R_g 0.85 in system 4. According to the literature, $[\alpha]_D^{20}$ +66.8° (water) [5].

The methylated monosaccharides were identified with authentic samples by gas-liquid chromatography, as well. The analysis was performed on a Tswett-1 chromatograph using a column (1 m long and 0.4 cm in diameter) filled with Cellite-545 impregnated with 15% of poly(1,4-butylene succinate) with nitrogen as the carrier gas at a temperature of 175°C. The retention times were measured with respect to methyl 2,3,4,6-tetra-O-methyl-β-D-glucoside, the retention time of which was taken as unity. The chromatogram had peaks with retention times of 1 and 1.42 (methyl 2,3,4,6-tetra-O-methyl-β- and -α-D-glucosides) and 2.55 and 3.59 (methyl 2,3,4-tri-O-methyl-β- and -α-D-glucosides).

Dianthoside C. Fifty grams of an aqueous extract of the methanolic extract was dissolved in 50 ml of water and transferred to a column of Sephadex G-25 (600 g). Fractions of 200 ml were collected. Fractions 1-4 contained dianthoside C (7.5 g) with the composition $C_{81}H_{130}O_{44} \cdot 3H_2O$, mp 270-275°C, $[\alpha]_D^{20}$ +28° (c 1.2; pyridine), mol. wt. 1885 (by titration).

The acetate, $C_{125}H_{174}O_{66}$, had mp 164-167°C; $[\alpha]_D^{20}$ − 14° (c 1.8; chloroform); and the methyl ether (by treatment with diazomethane), $C_{82}H_{132}O_{44} \cdot 3H_2O$ had mp 235-240°C, $[\alpha]_D^{20}$ +21° (c 1.5; pyridine).

Acid Hydrolysis of Dianthoside C. A solution of 2 g of dianthoside C in 50 ml of 5% hydrochloric acid was heated at the temperature of the boiling water bath for 7 h. The precipitate that deposited (0.55 g) was filtered off, dried, and transferred to a column of silica gel (15 × 1.5 cm). Elution with system 3 yielded 200 mg of the genin, mp 265-267°C, $[\alpha]_D^{20}$ +93° (c 1.0; ethanol). A mixture with gypsogenin gave no depression of the melting point. Then methanol eluted 70 mg of a product chromatographically different from gypsogenin. Crystallization from ethanol gave a substance with the composition $C_{36}H_{54}O_{10} \cdot 2H_2O$, mp 200-203°C, $[\alpha]_D^{20}$ +33° (c 1.1; pyridine).

The product (10 mg) was hydrolyzed with 2 ml of 15% hydrochloric acid for 7 h. The hydrolysate was shown by chromatography in systems 1 and 2 to contain glucuronic acid and its lactone. The filtrate after the hydrolysis of the dianthoside C was neutralized with AV-17 anion-exchange resin and was then found by chromatography on paper in systems 1 and 2 to contain galactose, arabinose, xylose, fucose, rhamnose, and glucuronic acid. A ratio of 1.85 : 1 : 1.90 : 0.80 : 1.70 was found by the aniline phthalate photocolorimetric method [6].

Alkaline Hydrolysis of Dianthoside C. Dianthoside C (4 g) was dissolved in 100 ml of 5% KOH solution in a tube filled with nitrogen and this was heated in the boiling water bath for 6 h. Then it was neutralized with KU-2 cation-exchange resin and evaporated, and the desacyldianthoside C was extracted from the solution with butan-1-ol (7 × 50 ml). The butanol extract was washed with water, evaporated, and transferred to a column of silica gel (30 × 2.5 cm). Elution was performed with system 5, 100-ml fractions being collected. Fractions 2-4 contained the desacyldianthoside C (1.8 g), with the composition $C_{53}H_{92}O_{22} \cdot 3H_2O$, mp 280-285°C (from butan-1-ol), $[\alpha]_D^{20}$ +23° (c 1.0; pyridine).

The product (50 mg) was hydrolyzed in 2 ml of 5% hydrochloric acid. After neutralization of the hydrolysate, it was found by paper chromatography in systems 1 and 2 to contain galactose, xylose, and glucuronic acid. An aqueous extract of the hydrolysate (1.7 ml) was transferred to a column of silica gel (50 × 2.5 cm). Elution was performed successively in systems 6 and 1, 80-ml fractions being collected. Fractions 15-19 contained an oligosaccharide (0.6 g). It was precipitated with acetone from aqueous methanol, mp 155-158°C, $[\alpha]_D^{20}$ +30° (c 1.2; water). A solution of 20 mg of the oligosaccharide in 2 ml of 5% hydrochloric acid was heated at 90°C for 6 h and was then neutralized with AV-17 anion-exchange resin. Galactose, arabinose, xylose, and rhamnose were identified by paper chromatography in systems 1 and 2.

SUMMARY

From Dianthus deltoides L. we have isolated three new triterpene glycosides: dianthosides A, B, and C. It has been shown that dianthoside A is the 3-O-β-glucopyranoside and dianthoside B the 3-O-[O-β-D-glucopyranosyl-(1 → 6)-β-glucopyranoside] of gypsogenic acid. Some information on the structure of dianthoside C – a new glycoside of gypsogenin – has been obtained.

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