TRITERPENE GLYCOSIDES OF Gypsophylla trichotoma

II. THE STRUCTURE OF TRICHOSIDE A

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In a preceding paper [1] we reported that various organs of the plant Gypsophylla trichotoma Wend. (threefork gypsophila), family Caryophyllaceae were found to contain four triterpene glycosides – trichosides A, B, C, and D.

The present paper gives a proof of the structure of trichoside A – the least polar glycoside, which is present in the roots, leaves, and flowers of the plant. The amount of trichoside A in methanolic extracts is extremely low, and only repeated chromatography of the extracts on silica gel and Sephadex enabled us to isolate it in the individual state.

The migration of trichoside A on thin-layer chromatograms (TLC) already showed that it has short carbohydrate chains of not more than three or four sugars. On acid hydrolysis, D-glucuronic acid, D-glucose, and D-galactose were detected.

The precipitate that deposited from the aqueous hydrolyzate contained a group of substances all of which, on hydrolysis, formed glycosides of gypsogenin [1, 2] and gypsogenin itself. Hydrolysis with more dilute acids gave gypsogenin β-D-glucuronoside [3].

The gas-liquid chromatography of the silylated methyl glycosides of the sugars obtained from trichoside A [4, 5] showed that the D-glucose, D-galactose, and D-glucuronic acid were present in a ratio 1 : 1 : 1. Consequently, trichoside A is a trioside of gypsogenin with D-glucuronic acid attached to the hydroxyl in position 3.

Alkaline hydrolysis showed the position and qualitative composition of the carbohydrate chains, giving a saponified glycoside (the progenin of trichoside A). The acid hydrolyzate of the latter was found to contain D-glucose and D-glucuronic acid, while the alkaline hydrolyzate of trichoside A contained a small amount of D-galactose.

This means that the D-galactose is attached to the carboxy group of the aglycone and the D-glucose is attached to the D-glucuronic acid.

On determining the residual sugars by the periodate oxidation of trichoside A, only the D-glucuronic acid remained unoxidized. It follows from this that the D-glucose is attached to the D-glucuronic acid in position 3.

These results were confirmed by a study of the products of the acid hydrolysis of the completely methylated trichoside A, as a result of which 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-galactose, and 2,4-di-O-methyl-D-glucuronic acid were found.

Thus, the following formula may be proposed for trichoside A:

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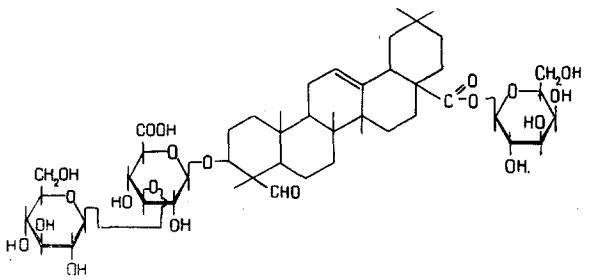

The β-glycosidic configurations for the D-glucose–D-glucuronic acid and the D-galactose–gypsogenin bonds are given provisionally.

EXPERIMENTAL

Chromatography was performed with type "M" ["slow"] paper of the "Goznak" Leningrad mill, type KSK silica gel, and Sephadex G-75, with the following solvent systems: 1) butan-1-ol–ethanol–25% ammonia (10 : 2 : 5), 2) butan-1-ol–acetic acid–water (4 : 1 : 5), 3) chloroform–ethanol (25 : 1), and 4) benzene–acetone (2 : 1). The sugars were detected by means of aniline phthalate and o-toluidine salicylate, and the glycosides and aglycones with an ethanolic solution of phosphotungstic acid.

The gas-liquid chromatography of the sugars was performed after the previous methanolysis of the glycosides and silylation [4] on a UKh-1 chromatograph with a copper column (1 m × 4 mm) filled with 5% of G-30-M silicone phase on Diaphorit (0.2-0.315 mm) at a column temperature of 170° C with hydrogen as the carrier gas (55 ml/min).

Isolation of Trichoside A. The fractions containing the glycosides A, B, and C obtained previously [1] were combined and dissolved in water, and the solution was passed through a column of Sephadex G-75. The first portions of eluate, containing glucosides A and B, were evaporated and chromatographed on a column of silica gel in system 1. Trichoside A was isolated with mp 310-312° C (from methanol), $[\alpha]_D^{20}$ + 1.8° (c 3.2; aqueous methanol).

Hydrolysis of Trichoside A. The glycoside (20 mg) was heated in 5% sulfuric acid (5 ml) in a boiling water bath for 5 h. The precipitate that deposited was filtered off, and the hydrolyzate was neutralized with barium carbonate, evaporated, and chromatographed on paper in system 2. D-Glucose, D-galactose, and D-glucuronic acid were detected. The precipitate was found by TLC on silica gel in system 3 to contain gypsogenin and gypsogenin β-D-glucuronoside [3].

Alkaline Hydrolysis of Trichoside A. The glycoside (50 mg) was heated in 5 ml of a 10% solution of caustic soda in methanol in the boiling water bath for 8 h. The reaction mixture was neutralized with dilute sulfuric acid, the ethanol was distilled off in vacuum, and the mixture was treated with 10 ml of water. The precipitate that deposited was filtered off, and the filtrate was chromatographed on paper in system 2. A faint spot of D-galactose was found. The precipitate (progenin of trichoside A) was hydrolyzed in 5 ml of 10% sulfuric acid. The hydrolyzate, neutralized with the barium carbonate, was found by paper chromatography in system 2 to contain D-glucose and D-glucuronic acid.

Determination of the Residual Sugars on the Periodate Oxidation of Trichoside A. The glycoside (50 mg) was oxidized with a 1% solution of sodium periodate (50 ml) at 5° C for a day. After the addition of ethylene glycol to decompose the excess of periodate, the reaction mixture was evaporated in vacuum and was then hydrolyzed by being heated with 5% sulfuric acid. D-glucuronic acid was found in the neutralized and purified hydrolyzate by paper chromatography in system 1.

Methylation of Trichoside A. The glycoside (100 mg) was methylated by the Hakomori method [6]. The completely methylated product (50 mg) was heated in a 7% solution of perchloric acid in absolute methanol at 100° C for 5 h. The mixture was diluted with water, the aglycone was separated off, and heating was continued for another 2 h.

The reaction mixture was neutralized with Dowex-1 anion-exchange resin, and then 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-galactose, and 2,4-di-O-methyl-D-glucuronic acid were identified by TLC on silica gel in system 4.

SUMMARY

The structure of trichoside A – a gypsogenin trioside from Gypsophylla trichotoma Wend. – has been established. The O-glycosidic moiety is the O-β-D-glucopyranosyl-(1 → 3)-O-β-D-glucopyranoside grouping and the O-acyl moiety is D-galactose.

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