TRITERPENE GLYCOSIDES OF Gypsophila trichotoma

III. STRUCTURE OF TRICHOSIDE B

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As reported previously [1], in the plant Gypsophila trichotoma Wend (threefork gypsophila), family Caryophyllaceae we have found four triterpene glycosides – trichosides A, B, C, and D. In addition, information on the structure of trichoside A was obtained [2]. The present paper gives the experimental results enabling the structure of a second glycoside – trichoside B – to be established.

Acid hydrolysis of this glycoside, as in the case of trichoside A, yielded D-glucose, D-galactose, and D-glucuronic acid, and also gypsogenin and its glucuronoside. The gas-liquid chromatography of the silylated methyl glycosides [3] showed that the D-glucose, D-galactose, and D-glucuronic acid were present in a ratio of 2 : 1 : 1, i.e., trichoside B contains one molecule of glucose more than trichoside A.

When trichoside B was saponified with alkali, we obtained a crystalline diglycoside an acid hydrolysate of which contained D-glucose and D-glucuronic acid. On comparing the glycosides obtained in the alkaline cleavage of trichoside B and trichoside A, it was found that they were completely identical (mp, rotation, chromatography, periodate oxidation). The similarity of the structure of the two glycosides is also confirmed by the fact that in the initial stage of the acid hydrolysis of trichoside B traces of trichoside A and of a diglycoside common to both compounds were detected chromatographically.

The alkaline hydrolysate after the splitting out of the diglycoside and neutralization was heated with acid, and among the reaction products we found D-glucose and traces of D-galactose. Consequently, the second molecule of glucose is present in the acyloside moiety and is obviously terminal, while the galactose is attached to the aglycone.

Since the glycosidic carbohydrate chains of trichosides A and B are identical, for a final proof of the structure of trichoside B it was necessary to establish the structure of its acyloside chain. This was done by means of exhaustive methylation and periodate oxidation.

Hydrolysis of the product of methylation of trichoside B gave 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-galactose, and 2,4-di-O-methyl-D-glucuronic acid. It follows from this that the acylosidic carbohydrate chain of trichoside B consists of D-glucose and D-galactose, the latter being attached to the carboxy group of the gypsogenin and the glucose being attached to the galactose by a 1 → 4 bond.

These facts are in complete harmony with the results of periodate oxidation, in which 4.7 moles of periodate were consumed and 2 moles of formic acid were produced, the glucuronic acid remaining unaffected.

Thus, we propose for trichoside B the formula

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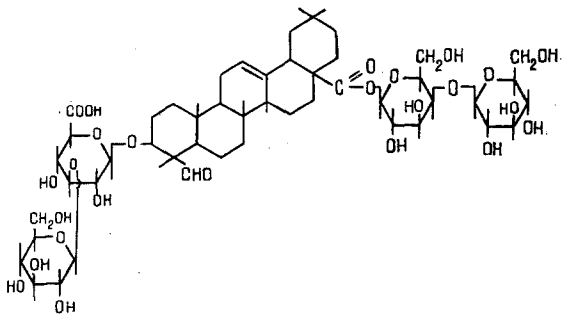

The configurations of the glycosidic bonds are given provisionally in accordance with Klyne's rule [4].

EXPERIMENTAL

Chromatography was performed on paper of type "M" ["slow"] of the "Goznak" Leningrad Mill and with silica gel of types KSK and ShSK and the following solvent systems: 1) butan-1-ol–ethanol–25% ammonia (15 : 2 : 5); 2) butan-1-ol–ethanol–25% ammonia (7 : 2 : 5); 3) butan-1-ol–acetic acid–water (4 : 1 : 5); 4) butan-1-ol–pyridine–water (6 : 4 : 3); 5) chloroform–ethanol (25 : 1); 6) benzene–acetone (2 : 1); and 7) methyl ethyl ketone saturated with water. The sugars were revealed with o-toluidine salicylate and the glycosides and aglycones with an alcoholic solution of phosphotungstic acid. The gas-liquid chromatography of the silylated methyl glycosides was performed on a UKh-1 chromatograph using a copper column (1 m × 4 mm) containing 5% of the silicone phase g = 30 M on Diaforit (0.2-0.315 mm) at a column temperature of 170° C with hydrogen as the carrier gas at a rate of flow of 55 ml/min.

Isolation of Trichoside. The fraction containing trichosides B, C, and D obtained previously [1] was chromatographed on a column of KSK silica gel in system 1. The separation of the glycoside was monitored by chromatography in a thin layer (TLC) of silica gel in the same system. The fractions containing trichoside B were combined and evaporated. Aqueous butanol deposited acicular crystals with mp 228-230° C (decomp.), $[\alpha]_D^{20}$ + 12.3° (c 0.7; aqueous methanol).

Acid Hydrolysis of Trichoside B. A mixture of 100 mg of the trichoside and 2.5 ml of 5% sulfuric acid was heated in the boiling water bath. The hydrolysis process was monitored by TLC in systems 2 and 4. In the first 20 min of hydrolysis, trichoside A, a diglycoside, and gypsogenin β-D-glucuronoside [5] were detected in the reaction mixture. After the end of hydrolysis (6 h) the precipitate was filtered off, and TLC in system 5 showed the presence in it of gypsogenin and its glucuronoside.

The acid hydrolysate, after neutralization with barium carbonate, was found by TLC in system 2 and by paper chromatography (PC) in systems 3 and 4 to contain D-glucose, D-galactose, and D-glucuronic acid.

Alkaline Hydrolysis of Trichoside B. A mixture of 30 mg of the glycoside and 5 ml of a 10% solution of caustic potash in 70% ethanol was heated in the boiling water bath for 8 h and was then neutralized with dilute sulfuric acid. After recrystallization from aqueous ethanol, the precipitate that had deposited had mp 247-250°C (decomp.); $[\alpha]_D^{20}$ + 38° (c 0.9; aqueous methanol).

Acid hydrolysis of the diglycoside isolated showed that it contained D-glucose and D-glucuronic acid. On periodate oxidation of the diglycoside, the glucuronic acid was not affected. TLC in systems 1, 2, and 3 also showed the identity of the diglycosides obtained by the alkaline hydrolysis of trichoside A and of trichoside B.

The neutralized aqueous solution after the splitting out of the diglycoside was evaporated, and the residue was purified by chromatography on ShSK silica gel in system 1, after which it was hydrolyzed with 5% sulfuric acid. After neutralization with barium bicarbonate, the hydrolysate was shown by PC in systems 3 and 4 to contain D-glucose and traces of D-galactose.

Periodate Oxidation of Trichoside B. The glycoside (0.1704 g) was oxidized with a 1% solution of sodium metaperiodate. A blank experiment was performed in parallel. The consumption of periodate was determined by titration with 0.1 N sodium thiosulfate solution, and the formic acid liberated was titrated with 0.01 N caustic soda. The consumption of periodate per mole of glycoside was 4.7 moles, and two molecules of formic acid were produced.

The residual sugars were determined in a separate part of the reaction mixture after the addition of ethylene glycol and acid hydrolysis. Glucuronic acid was detected by PC and TLC in systems 2, 3, and 4.

Methylation of Trichoside B. The glycoside (300 mg) was methylated by Hakomori's method [6]. Of the permethylate, 100 mg was heated in a 7% solution of perchloric acid in methanol (100°C, 5 h). The mixture was diluted with water, the aglycone was separated off, and heating was carried out for another 2 h. Then the reaction mixture was neutralized with Dowex-1 anion-exchange resin, and PC in system 7 and TLC in system 6 with markers showed the presence of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-galactose, and 2,4-di-O-methyl-D-glucuronic acid.

SUMMARY

The structure of trichoside B – a gypsogenin tetraoside from Gypsophila trichotoma Wend. – has been established. The glycosidic carbohydrate chain is O-β-D-glucopyranosyl-(1 → 3)-O-β-D-glucuronopyranosyl and the acyloside chain is O-β-D-glucopyranosyl-(1 → 4)-O-β-D-galactopyranosyl.

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