TRITERPENE GLYCOSIDES OF Gypsophilla trichotoma

IV. THE STRUCTURE OF TRICHOSIDE C

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As has been reported previously [1], in the plant Gypsophilla trichotoma (threefork gypsophila), family Caryophyllaceae, we have detected four triterpene glycosides – trichosides A, B, C, and D. The structures of the first two of them have not been established [2, 3].

The present paper gives the results of an experiment showing the structure of the third glycoside – trichoside C.

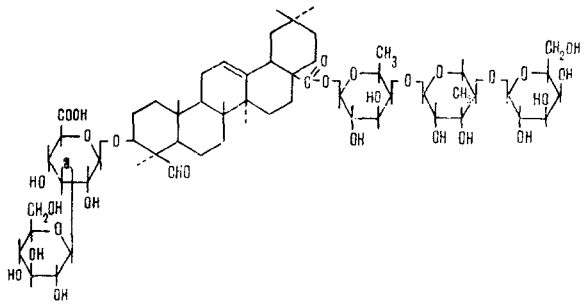
The acid hydrolysis of this glycoside led to the formation of the aglycone gypsogenin and its glucuronoside. Among the sugars, D-glucose, D-galactose, D-fucose, L-rhamnose, and D-glucuronic acid were found. Gas-liquid chromatography showed that the ratio of the sugars was 1:1:1:1:1.

The alkaline saponification of trichoside C gave a crystalline bioside of gypsogenin containing D-glucose and D-glucuronic acid. This bioside proved to be identical with the corresponding bioside obtained by the alkaline saponification of trichosides A and B. Consequently, the structure of the carbohydrate chain attached to the hydroxyl at C_3 of gypsogenin is the same for trichosides A, B, and C.

To determine the structure of trichoside C completely, it was necessary to investigate the structure of the oligosaccharide attached to the carboxy group of the gypsogenin. This oligosaccharide was split off in the alkaline hydrolysis of trichoside C. An acid hydrolysate of the oligosaccharide showed the presence of L-rhamnose and D-galactose. The absence of D-fucose can be explained by the assumption that it is directly attached to the carboxyl and is decomposed under the conditions of alkaline hydrolysis.

Among the residual sugars after the periodate oxidation of trichoside C we found only glucuronic acid, which shows the absence of 1→3 bonds in the acyloside carbohydrate chain.

The main information enabling the structure of the acyloside chain and, with this, the complete structure of glycoside C to be determined was obtained by exhaustive methylation and periodate oxidation of the trichoside. After the acid hydrolysis of the permethylate of trichoside C we found, in addition to 2,3,4,6-tetra-O-methyl-D-glucose and 2,4-di-O-methyl-D-glucuronic acid, which are derived from the components of the glycosidic carbon chain, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-D-fucose and 2,3-di-O-methyl-L-rhamnose. It follows from this that in the acyloside chain the rhamnose is attached to the hydroxyl of the fucose in position 4, and the terminal galactose is attached to the rhamnose



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(also at hydroxyl 4). Such a structure of trichoside C agrees completely with the results of its periodate oxidation, in which 6 moles of periodate were consumed and 2 moles of formic acid were formed. In this process, the glucuronic acid residue was not oxidized.

On the assumption that in plant glycosides the sugars of the D series normally have the β configuration and sugars of the L series the α configuration (Klyne's rule) [4], the structure above is proposed for trichoside C.

EXPERIMENTAL

Paper chromatography (PC) was carried out with type M ["slow"] paper of the "Goznak" Leningrad Mill, and column and thin-layer chromatography (TLC) with KSK and ShSK silica gels. The following solvent systems were used: 1) butan-1-ol–ethanol–25% ammonia (15 : 2 : 5); 2) the same (7 : 2 : 5); 3) chloroform–ethanol (25 : 1); 4) methyl ethyl ketone saturated with water; 5) pyridine–butan-1-ol–water (4 : 6 : 3), and 6) butan-1-ol–acetic acid–water (4 : 1 : 5). The sugars were revealed with o-toluidine salicylate, and the glycosides and aglycones with ethanolic phosphotungstic acid. The gas-liquid chromatography of the trimethylsilyl ethers of the sugars 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 176°C with hydrogen as the carrier gas at the rate of 55 ml/min.

Isolation of Trichoside C. The fraction obtained previously [1] containing trichosides B, C, and D was chromatographed on a column of KSK silica gel in system 1. The separation was monitored by TLC in the same system. The fractions containing trichoside C were combined and evaporated. Aqueous butanol deposited crystals in the form of white needles with mp 245-247°C $[\alpha]_D^{20}$ + 11° (c 1.29; aqueous methanol).

Acid Hydrolysis of Trichoside C. A mixture of 50 mg of the glycoside and 10 ml of 5% sulfuric acid was boiled for 8 h. The resulting precipitate was shown by TLC with markers in system 3 to contain gypsogenin β-D-glucuronoside. The acid hydrolysate was shown by PC in systems 5 and 6 and by TLC in system 2 to contain D-glucose, D-galactose, D-fucose, L-rhamnose, and D-glucuronic acid.

Alkaline Hydrolysis of Trichoside C. A mixture of 50 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 16 h, and was then neutralized with dilute sulfuric acid. The precipitate that deposited was recrystallized from aqueous methanol and then had mp 247-250°C (decomp.), $[\alpha]_D^{20}$ + 39° (c 1.02; aqueous methanol). According to TLC in systems 1, 2, and 6 and from its other properties, the substance was identical with the diglycoside obtained by the alkaline hydrolysis of trichosides A and B [2, 3]. Its acid hydrolysis gave D-glucose and D-glucuronic acid. The latter was not decomposed on periodate oxidation.

After the removal of the diglycoside, the neutralized aqueous solution was evaporated, and the residue was chromatographed on ShSK silica gel in system 1 and was then hydrolyzed with 50% sulfuric acid. The hydrolysate was found by PC in systems 5 and 6 to contain D-galactose and L-rhamnose.

Periodate Oxidation of Trichoside C. The glycoside (0.0843 g) was oxidized in the same way as trichoside B [3]. It consumed 6 moles of periodate per mole, giving two moles of formic acid. In a separate part of the reaction mixture, after acid hydrolysis, D-glucuronic acid was detected by PC in systems 5 and 6.

Methylation of Trichoside C. The glycoside (200 mg) was methylated by Hakomori's method [5]. The subsequent treatment was as for trichoside B [3]. PC and TLC in systems 2, 4, and 5 with markers permitted the identification of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-D-fucose, 2,3-di-O-methyl-L-rhamnose, and 2,4-di-O-methyl-D-glucuronic acid.

SUMMARY

The structure of trichoside C – a gypsogenin pentaoside from Gypsophilla trichotoma – has been established. The glycosidic carbohydrate chain forms the O-β-D-glucopyranosyl-(1→3)-O-β-D-glucuronopyranoside, and the acyloside chain forms the O-β-D-galactopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-fucopyranoside.

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