TRITERPENE GLYCOSIDES OF Gypsophila trichotoma

V. THE STRUCTURE OF TRICHOSIDE D

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In plants of Gypsophila trichotoma Wend (threefork gypsophila) we have found four triterpene glycosides – trichosides A, B, C, and D [1]. The structure of the first of these is already known [2]. The present paper gives experimental details for the establishment of the structure of trichoside D, the most complex glycoside.

In our first paper it was reported that trichoside D is composed of gypsogenin and D-galactose, D-glucose, D-glucuronic acid, D-fucose, D-xylose, L-arabinose, and L-rhamnose [1]. It has been determined by gas-liquid chromatography (GLC) of the silyl derivatives of the sugars that they are present in trichoside D in a ratio of 2:1:1:1:2:1:1, respectively; i.e., trichoside D is a gypsogenin nonaoside.

The subsequent investigation was directed to determining the structure of the carbohydrate chains of trichoside D.

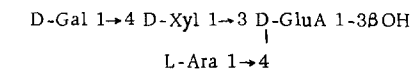
The progenin of trichoside D obtained by alkaline saponification – a gypsogenin tetraoside (without the acyloside chain) – on acid hydrolysis gave arabinose, xylose, and galactose, and also gypsogenin β-D-glucuronoside [3]. It is clear from this that the D-glucuronic acid is directly attached to the 3β hydroxyl of gypsogenin.

The gas-liquid chromatography of the sugars of the tetraoside showed that their ratio in it was 1:1:1:1. The products of the hydrolysis of the permethylated progenin of trichoside D were found to contain 2-O-methyl-D-glucuronic acid, 2,3-di-O-methyl-D-xylose, 2,3,4-tri-O-methyl-L-arabinose, and 2,3,4,6-tetra-O-methyl-D-galactose. Consequently, the terminal sugars are galactose and arabinose, one of them being attached to the glucuronic acid directly and the other through the xylose.

In order to determine the structure of the tetraoside, it was hydrolyzed with dilute acids. From the mixture of products we isolated a gypsogenin trioside containing D-glucuronic acid, D-galactose, and D-xylose.

After the exhaustive methylation of the trioside and the hydrolysis of the product, we found 2,4-di-O-methyl-D-glucuronic acid, 2,3-di-O-methyl-D-xylose, and 2,3,4,6-tetra-O-methyl-D-galactose. Thus, in the trioside, a chain consisting of galactose and xylose is attached to the glucuronic acid in position 3. This agrees well with the fact that on periodate oxidation of the trioside the glucuronic acid remains unchanged.

It follows from this that in the gypsogenin tetraoside (the progenin of trichoside D) the L-arabinose is attached to the glucuronic acid in position 4. Consequently, the glycosidic carbohydrate chain of trichoside D has the structure



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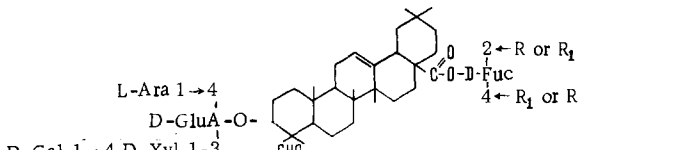
So far as concerns the acyloside chain, as has been shown previously [1], it contains D-glucose, D-galactose, D-xylose, D-fucose, and L-rhamnose, the D-fucose being attached directly to the carboxy group of the genin. It is obvious that the acyloside chain contains one molecule of each of the five sugars.

The exhaustive methylation of trichoside D gave a product in the hydrolysis of which we found, mentally excluding the sugars of the glycosidic chain, 3-O-methyl-D-fucose, 2,4-di-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-xylose, and 2,3,4,6-tetra-O-methyl-D-glucose. These results show that the acyloside chain like the glycosidic chain, is branched, the fucose being the center of branching and the glucose and galactose being the terminal sugars.

On the basis of the results obtained, the following partial structural formula may be proposed for trichoside D:



R = D-Glu 1-3L Rha or D-Glu 1-D-Gal

R_1 = D-Xyl 1-4 D-Gal or D-Xyl 1-L-Rha

EXPERIMENTAL

Paper chromatography (PC) was performed with type "M" ["slow"] paper of the "Gosznak" Leningrad factory, and thin-layer chromatography (TLC) with types KSK and ShSK silica gel. The following solvent systems were used: 1) butan-1-ol–ethanol–25% ammonia (15 : 2 : 5); 2) the same (7 : 2 : 5); 3) butan-1-ol–acetic acid–water (4 : 1 : 5); 4) butan-1-ol–pyridine–water (6 : 4 : 3); 5) methyl ethyl ketone saturated with water; 6) butan-1-ol–acetone–water (4 : 5 : 1); and 7) chloroform–ethanol (25 : 1). The sugars were revealed with o-toluidine salicylate, and the glycosides and aglycones with an ethanolic 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 g = 30 m silicone 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.

Acid Hydrolysis of the Progenin of Trichoside D. A mixture of 100 mg of the progenin obtained as described in [1] and 5 ml of 5% sulfuric acid was heated in the boiling water bath for 6 h. The precipitate was filtered off, and the presence of gypsogenin and of gypsogenin β-D-glucuronoside was shown by the TLC method (with markers) in systems 1, 2, and 7 [3].

The filtrate was found by the PC method in systems 3 and 4 to contain arabinose, xylose, galactose, and glucuronic acid. According to GLC, the sugars were present in a ratio of 1 : 1 : 1 : 1.

Methylation of the Progenin of Trichoside D. The glycoside (100 mg) was methylated by Hakomori's method [4]. The permethylate was heated in a mixture of 4 ml of methanol and 1 ml of hydrochloric acid for 5 h, and then 5 ml of water was added, and the mixture was heated for another 3 h. The precipitate was filtered off, and the filtrate was neutralized with anion-exchange resin and evaporated. TLC in systems 3, 4, and 6, and also PC in system 5 (with markers) showed the presence of 2-O-methyl-D-glucuronic acid, 2,3-di-O-methyl-D-xylose, 2,3,4-tri-O-methyl-L-arabinose, and 2,3,4,6-tetra-O-methyl-D-galactose.

Isolation of a Gypsogenin Trioside. A mixture of 150 mg of the progenin of the trichoside in 10 ml of 0.125% sulfuric acid was heated for 4 h. The precipitate that deposited was separated off and was then chromatographed on a column of KSK silica gel in system 2, 10-ml fractions being collected. The fractions were monitored by TLC in systems 1 and 2. The eighth fraction yielded a chromatographically homogeneous substance an acid hydrolyzate which was shown by PC in systems 3 and 4 to contain glucuronic acid, galactose, and xylose.

Methylation of the Gypsogenin Trioside. The trioside (15 mg) was methylated by Hakomori's method [4]. The permethylate was treated in the same way as the permethylate of the progenin of trichose D. By TLC in systems 3, 4, and 6 and PC in system 5 with markers, 2,4-di-O-methyl-D-glucuronic acid, 2,3-di-O-methyl-D-xylose, and 2,3,4,6-tetra-O-methyl-D-galactose were detected.

Periodate Oxidation of the Gypsogenin Trioside. In the cold, 10 mg of the trioside was oxidized with 2 ml of 1% sodium periodate solution for two days. After the decomposition of the excess of periodate with

ethylene glycol, the solution was evaporated and the residue was hydrolyzed with 5% sulfuric acid. Glucuronic acid was found in the neutralized hydrolyzate by TLC in systems 3 and 4.

Methylation of Trichoside D. The methylation of 300 mg of the glycoside was performed by Hakomori's method [4]. The permethylate was heated (100°C, 5 h) in a 7% solution of perchloric acid in methanol. The mixture was diluted with water, and the aglycone was separated off, and then heating was continued for another 2 h. The reaction mixture was neutralized with Dowex-1 anion-exchange resin, and then 2-O-methyl-D-glucuronic acid, 2,3-di-O-methyl-D-xylose, 2,3,4-tri-O-methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-D-galactose, 3-O-methyl-D-fucose, 2,4-di-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-xylose, and 2,3,4,6-tetra-O-methyl-D-glucose were identified by TLC in systems 3 and 4 and PC in systems 5 and 6.

SUMMARY

The structure of the glycosidic carbohydrate chain and the partial structure of the acyloside chain of trichoside D – a new triterpene glycoside from Gypsophila trichotoma – have been established.

LITERATURE CITED

1. V. N. Luchanskaya, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 6, 434 (1970).
2. V. N. Luchanskaya, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 7, 151, 431, 608 (1971).
3. N. K. Abubakirov and K. Amanmuradov, Zh. Obshch. Khim., 34, 1661 (1964).
4. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).