STRUCTURE OF A TRITERPENE GLYCOSIDE FROM Gypsophilla acutifolia

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Gypsophilla acutifolia Fisch. (big gypsophilla), family Caryophyllaceae is used in some countries as a source for the industrial production of triterpene glycosides, although their chemical nature has not been studied. The results of a preliminary investigation have shown that the plant contains a saponin, which we have called acutifolioside [1].

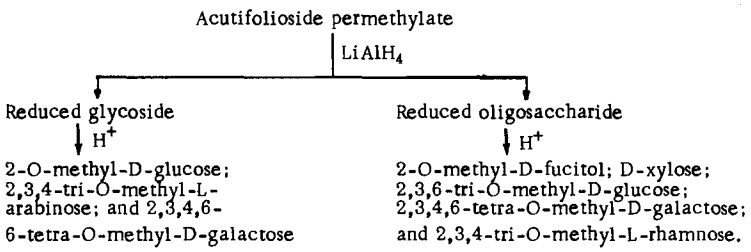
The gas–liquid chromatography of the sugars in the form of trifluoroacetates of the corresponding polyols [2] showed that acutifolioside includes four galactose residues and one residue each of glucuronic acid, glucose, arabinose, fucose, xylose, and rhamnose.

The structure of acutifolioside is largely similar to that of saponaside D [3]. In particular, the methods and approaches to the determination of its structure have been similar. In view of this, we did not consider it desirable to discuss in detail the whole course of the determination of the structure of acutifolioside but decided to direct attention only to the nodal points of the experiment.

As alkaline saponification showed, the acyloside part of the molecule of the glycoside includes galactose, glucose, xylose, fucose, and rhamnose, and the O-glycosidic moiety includes galactose and arabinose.

An analysis of the saponin methylated by Hakomori's method [4] showed the presence of completely-methylated rhamnose, arabinose, and galactose. Among the partially-methylated compounds by direct comparison we identified 2-O-methyl-D-fucose and 2,3,6-tri-O-methyl-D-glucose. The structure of the 2,3,6-tri-O-methyl-D-glucose was additionally confirmed by mass spectrometry [5]. The glucuronic acid was identified in the form of 2-O-methyl-D-glucose after preliminary cleavage of the permethylate of the saponin with lithium tetrahydroaluminate. In addition to the compounds mentioned, we found xylose in the form of the trimethylsilyl derivative in a hydrolyzate of methylated acutifolioside by paper and gas–liquid chromatography.

On making use of the possibility of selected cleavage in the saponin of the O-acylglycosidic bond under the action of lithium tetrahydroaluminate, we showed that the methylated monosaccharides are distributed over the functional groups of gypsogenin:



Among the products of the partial hydrolysis of the glycoside by oxalic acid we detected a bioside containing glucuronic acid and arabinose. When this was oxidized with sodium periodate, gypsogenin glucuronoside was formed. This shows a 1→3 bond between the pentose and the uronic acid. This type of bond is confirmed by the results of methylation, and the lithium tetrahydroaluminate reduction and hydrolysis of the methylated bioside. As the result of these processes we identified 2,3,4-tri-O-methyl-L-arabinose

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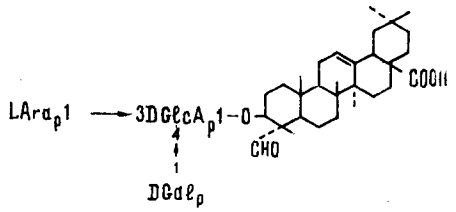
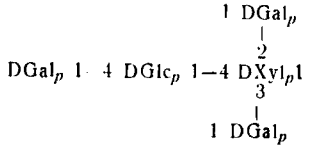
and 2,4-di-O-methyl-D-glucose. The remethylation of the glycoside obtained by the tetrahydroaluminate reduction of the permethylated bioside led to the isolation of 2,4,6-tri-O-methyl-D-glucose* in place of 2,4-di-O-methyl-D-glucose. In view of the fact that 2-O-methyl-D-glucose was obtained from the methylated acutifolioside, it may be assumed that the branching at the glucuronic acid is due to the presence of a galactose residue at the C_4 atom. Hence, a partial structure of acutifolioside has the form

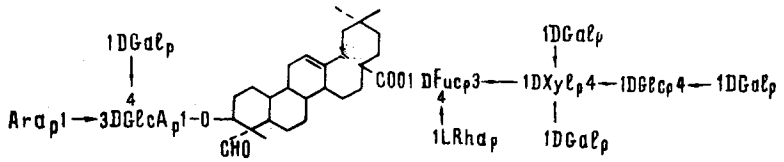


When acutifolioside was degraded with sodium periodate by Smith's method, a hederagenin trioside was isolated which included residues of glucuronic acid, fucose, and xylose. A second application of this procedure to the compound obtained enabled hederagenin fucoside to be obtained. Consequently, there is a 1 → 3 bond in the O-acyl-glycosidic part of the saponin molecule between the fucose and the xylose.

The problem of proving the position of the terminal monosaccharides proved to be very complicated, and it was solved by studying the products of the partial diastase hydrolysis of the oligosaccharide obtained by the saponification of the initial glycoside with 10% aqueous ethanolic alkali. By preparative chromatography on paper we isolated from it two new oligosaccharides. The first of them, according to the results of periodate oxidation and methylation, had the structure of D-glucopyranosyl-(1 → 4)-D-xylose, and the second consisted of glucose, galactose, and xylose. Its reducing end was also xylose. When a permethylate of this oligosaccharide was cleaved, completely-methylated galactose, 2,3,6-tri-O-methyl-D-glucose and free xylose were identified. Hence, the most probable structure for the second oligosaccharide is:



As the structure of the pentasaccharide shows, the rhamnose residue in the saponin is located on the C_4 atom of the fucose. Thus, on the basis of the material presented, it is possible to propose the following structure for acutifolioside from Gypsophilla acutifolia:



The saponin from Gypsophilla acutifolia Fisch. is the first example of a triterpene glycoside with such an unusually high degree of branching of the carbohydrate chains.

EXPERIMENTAL

The following solvent systems were used for chromatography: 1) butan-1-ol–ethanol–water (10 : 2 : 5); 2) butan-1-ol–acetic acid–water (4 : 1 : 5); 3) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); 4) benzene–acetone (2 : 1); and 5) chloroform–acetone (4 : 1). Gas–liquid chromatography was performed on a Hewlett-Packard model 5750 instrument with a flame ionization detector. For quantitative determinations, a Hewlett-Packard 3370A integrator was used. The stainless steel column, 3 m long and 3 mm in internal diameter, was filled with 1% of XE-60 on Gas Chrom Z 80/100 mesh, the carrier gas was nitrogen at the rate of 36 ml/min, and the temperature was 280°C.

Alkaline Saponification of Acutifolioside. A mixture of 15 g of the glycoside (I) and 500 ml of a 10% aqueous alcoholic solution of caustic potash was heated in a current of nitrogen at 100°C for 5 h. Then the reaction mixture was neutralized and extracted with isoamyl alcohol. The organic layer was concentrated and purified on silica gel in system 1. This gave 5 g of the glycoside (II) with mp 201-203°C, $[\alpha]_D^{20}$ +51°

*The samples of some of the methylated sugars were kindly given to us by P. Kovacs (Chemical Institute of the Slovak Academy of Sciences, Czechoslovakia).

(c 2.0; methanol). According to paper chromatography in system 3, substance (II) consisted of galactose, arabinose, glucuronic acid, and gypsogenin.

The oligosaccharide (III) from the aqueous layer was deposited on a column of silica gel and chromatographed in system 1. Then the oligosaccharide was eluted with pure methanol.

The oligosaccharide (III) (10 mg) was hydrolyzed with 2% H_2SO_4 at 100°C for 4 h. Galactose, glucose, xylose, and rhamnose were identified by paper chromatogarphy in systems 2 and 3.

Methylation and Lithium Tetrahydroaluminate Cleavage of Acutifolioside. By Hakomori's method, 5 g of the saponin (I) was converted into the permethylated compound and this was then subjected to methanolysis to the monosaccharides. The aqueous and chloroform fractions of the decomposition products were separated preparatively on silica gel in system 4. The organic layer was found to contain completely methylated arabinose, rhamnose, and galactose, while the aqueous layer contained 2-O-methyl-D-fucose, 2,3,6-tri-O-methyl-D-glucose, methyl 2-O-methyl-D-glucuronate, and free xylose.

After tetrahydroaluminate decomposition of the fully-methylated acutifolioside, in the reduced oligosaccharide 2-O-methyl-D-fucitol, free xylose (in the form of the trimethylsilyl derivative), 2,3,6-tri-O-methyl-D-glucose, and fully-methylated rhamnose and galactose were identified by thin-layer and gas–liquid chromatography, and the reduced glycoside was found to contain 2-O-methyl-D-glucose, 2,3,4-tri-O-methyl-L-arabinose, and 2,3,4,6-tetra-O-methyl-D-galactose.

Partial Hydrolysis of Acutifolioside. The glycoside (8 g) was hydrolyzed with 10% oxalic acid at 75°C for 5 h. Thin-layer chromatography on silica gel in system 1 showed the presence of gypsogenin glucuronoside, compound (II), and also a glycoside (V) containing glucuronic acid and arabinose residues in the carbohydrate moiety.

The methylation of substance (IV) gave 2,3,4-tri-O-methyl-L-arabinose and methyl 2,4-di-O-methyl-D-glucuronate. Reduction of the permethylated glycoside with lithium tetrahydroaluminate followed by remethylation with methyl iodide and silver oxide gave 2,3,4-tri-O-methyl-L-arabinose and 2,4,6-tri-O-methyl-D-glucose.

Smith Degradation of Acutifolioside. The Smith degradation of 10 g of glycoside (I) gave 3 g of the glycoside (VI) with mp 196-198°C and $[\alpha]_D^{20}$ +94° (c 2.0; methanol).

The hydrolysis of compound (VI) with Kiliani's mixture yielded gluceuronic acid, fucose, and xylose. The reoxidation of substance (VI) by Smith's method gave hederagenin fucoside with mp 242-244°C and $[\alpha]_D^{20}$ +24° (c 2.0; methanol).

Enzymatic Hydrolysis of the Oligosaccharide (III). A few milligrams of diastase was added to a solution of 5 g of compound (III) in 200 ml of phosphate buffer. After a day, 2 oligosaccharides (VI and VII) were detected in the substrate, and these were obtained in preparative amounts by paper chromatography. According to paper chromatography, oligosaccharide (VI) consisted of xylose, glucose, and galactose. After reduction with sodium tetrahydroborate followed by acid hydrolysis, glucose, galactose, and xylitol were identified. In the products from the permethylate of (VI), xylose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4,6-tetra-O-methyl-D-galactose were found.

The oligosaccharide (VII) was cleaved with 2% H_2SO_4 (100°C, 5 h) into xylose and glucose, and after reduction with sodium tetrahydroborate and acid hydrolysis it yielded xylitol and glucose.

The oligosaccharide (VII) (40 mg) was methylated by Hakomori's method, and 2,3-di-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-glucose were identified by chromatography in a thin layer of silica gel in system 5.

CONCLUSIONS

The structure of the triterpene glycoside from Gypsophilla acutifolis Fisch. has been established.

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