THE STRUCTURE OF SAPONASIDE D

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We have previously reported the partial structure of a triterpene glycoside, saponaside D, from the roots of Saponaria officinalis [1].

This paper gives the results leading to a definitive structure for this glycoside. To determine the sequence of monosaccharides in the carbohydrate chains we used partial hydrolysis with oxalic acid. A trioside and tetraoside of gypsogenin and also a trisaccharide were isolated. The latter consists of galactose, arabinose, and xylose. Since the arabinose residue is present only in the carbohydrate chain attached to the hydroxyl group of the gypsogenin, this fragment is attached to the glucuronic acid. Hence, the branching in this chain is due to the rhamnose. To determine the points of attachment of the sugars to the glucuronic acid we studied in detail not only the oligosaccharides but also the progenins formed on partial hydrolysis. Smith oxidation [2] of the gypsogenin trioside containing glucuronic acid, xylose, and arabinose, yielded glucuronic acid and glycerol. Consequently, the above-mentioned oligosaccharide is attached to the third, and the rhamnose residue to the fourth, hydroxyls of the glucuronic acid, as was also confirmed by the methylation of the tetraoside containing glucuronic acid, xylose, arabinose, and rhamnose in the carbohydrate moiety. After the cleavage of the permethylated product, the hydrolysate of the tetraoside was found to contain 2-O-methylglucuronic acid, 2,3-di-O-methylxylose, 2,3,4-tri-O-methyl-rhamnose, and 2,3,4-tri-O-methylarabinose. The results of the methylation of the tetraoside show that the arabinose is attached to the fourth, and the galactose, consequently, to the second, hydroxyls of the xylose.

On the basis of these results, the structure of the carbohydrate chain at C_3 of gypsogenin appears as illustrated in the formula of the complete structure of saponaside D.

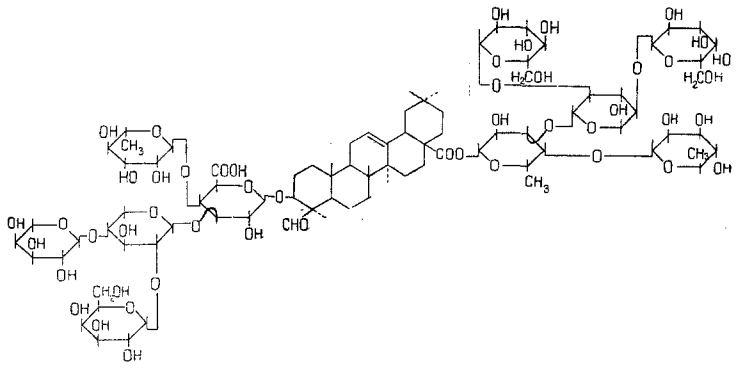
The structure of the carbohydrate chain attached to the carboxyl group of the aglycone was elucidated by means of the Smith degradation of saponaside D. Careful hydrolysis of the polyol formed made it possible to isolate a glycoside containing xylose, fucose, and glucuronic acid. This shows that there is a 1→3 bond between the xylose and the fucose.

Further information on the structure of saponaside D was obtained by treating it with diastase. After 60 hr, the substrate was found to contain a trisaccharide which was isolated in preparative amount.

This fragment consisted of xylose, galactose, and glucose. Since glucose is found only in the chain attached to the carboxyl group of the gypsogenin, it is obvious that this trisaccharide is attached to the third hydroxyl of the fucose. Since 2-O-methylfucose was found among the methylation products of saponaside D, the rhamnose residue is attached to the C_4 hydroxyl of the fucose.

The structure of the saponin was shown finally by a detailed study of the trisaccharide obtained by the action of diastase on saponaside D. When this oligosaccharide was treated with the fungus Rhizopus arrhizus, a glucose residue was split off and a disaccharide consisting of galactose and xylose was formed. The existence of a 1→4 bond between these monosaccharides was confirmed by the methylation of the disaccharide and cleavage of the product, giving 2,3-di-O-methylxylose and 2,3,4-tetra-O-methylgalactose. Furthermore, this type of bond in the disaccharide was additionally confirmed by the reactions with triphenyltetrazolium chloride and with diphenylamine–aniline.

What has been said above permits the final structure of the carbohydrate chain at the second functional group of the aglycone, and, therefore, of saponaside D as a whole, to be represented in the following way.



EXPERIMENTAL

Chromatography was carried out with type KSK silica gel, alumina, and type "M" paper of the Leningrad no. 2 paper mill, with the following systems of solvents: 1) butan-1-ol–acetic acid–water (4 : 1 : 5), 2) butan-1-ol–ethanol–25% ammonia (7 : 2 : 5), 3) butan-1-ol–pyridine–benzene–water (5 : 3 : 1 : 3), 4) toluene–ethanol (9 : 1), 5) butan-1-ol–ethanol–water (4 : 1 : 5), 6) benzene–acetone (2 : 1), and 7) ethyl acetate–ethanol (25 : 1).

The glycosides were revealed with conc H_2SO_4 and the sugars by spraying the chromatogram with aniline phthalate.

Partial hydrolysis of saponaside D. A solution of 3.0 g of saponaside D in 500 ml of 10% oxalic acid was heated at 75° C for 5 hr. Then the hydrolysate was neutralized with AV-17 anion-exchanger. The aqueous eluate was exhaustively extracted with isoamyl alcohol. By chromatography on silica gel in system 2 the organic layer was found to contain the starting material (I), gypsogenin glucuronoside (II), and glycosides III and IV, and also the saponified glycoside of saponaside D (V). The alcoholic extract was evaporated and chromatographed on silica gel in the same system. This gave 0.2 g of II, 0.3 g of III, 0.5 g of IV, 0.5 g of V, and 0.3 g of I.

The hydrolysis of III with Kiliani's mixture gave gypsogenin, glucuronic acid, xylose, and arabinose [3]. Using Smith's method, 46 mg of III was oxidized. After the cleavage of the polyol with an ammoniacal solution of silver nitrate [4] chromatography on paper in system 3 showed the presence of glucuronic acid and glycerol.

Substance IV (10 mg) was heated with 5 ml of Kiliani's mixture at 100° C for 5 hr. Glucuronic acid, xylose, arabinose, and rhamnose were found in the hydrolysate.

The Smith degradation of IV formed glucuronic acid, glycerol and ethylene glycol. Using Kuhn's method, 100 mg of IV was methylated [5]. After the cleavage of the permethylated product obtained, 2,3,4-tri-O-methylarabinose, 2,3,4-tri-O-methylrhamnose, 2,3-di-O-methylxylose, and 2-O-methylglucuronic acid were identified in the hydrolysate by chromatography on silica gel in system 6 and by gas-liquid chromatography in the presence of reference samples.

By paper chromatography in system 5, the oligosaccharide VI was found in the aqueous layer after extraction with isoamyl alcohol. The hydrolysis of VI gave xylose, arabinose, and galactose. A solution of 10 mg of VI in 3 ml of water was treated with 10 mg of sodium borohydride. After a day, the solution was deionized and hydrolyzed. Galactose, arabinose, and xylitol were detected. The periodate oxidation of VI and subsequent hydrolysis gave xylose and glycerol.

Smith degradation of saponaside D. A solution of 10 g of saponaside D in 150 ml of water was treated with 2.5 g of $NaIO_4$. After the complete consumption of the periodate, the solution was deionized and evaporated. The residue was dissolved in 50 ml of water and treated with 0.5 g of sodium borohydride. After a day the solution was neutralized and the eluate was concentrated. The polyol formed was hydrolyzed with 0.1 N H_2SO_4 at room temperature for a day. Purification of the product gave 0.5 g of the glycoside VII with mp 216–220° C, $[\alpha]_D^{20}$ +15.4° (c 2, 6; methanol). 10 mg of VII was hydrolyzed with Kiliani's mixture. Xylose, fucose, and glucuronic acid were identified. Hederagenin was obtained by chromatography on silica gel in system 7.

Substance VII (0.4 g) was cleaved by Smith's method as described above. Chromatography on silica gel in

system 2 yielded 0.2 g of the glycoside VIII with $[\alpha]_D^{20}$ −21° (c 0.96; methanol). When VIII was subjected to acid hydrolysis, fucose and glucuronic acid were identified.

Enzymatic hydrolysis of saponaside D with diastase. A solution of 0.5 g of saponaside D in 50 ml of phosphate buffer was treated with a few milligrams of diastase and the mixture was kept at 30° C for 60 hr. The substrate was found to contain the oligosaccharide IX, which was isolated by chromatography on carbon–Celite (1 : 1), being eluted with mixtures of water and ethanol with the concentration of the latter increasing gradually to 16%. The separation of the fractions was checked by paper chromatography in system 3. The fractions containing the individual oligosaccharide were combined and evaporated. Yield 300 mg, $[\alpha]_D^{20}$ +43.3° (c 3.25; water).

Substance IX (10 mg) was hydrolyzed (1% H_2SO_4, 100° C, 2 hr), and galactose, glucose, and xylose were identified. 10 mg of IX was reduced with sodium borohydride and cleaved as described above. Galactose, glucose, and xylitol were found.

Enzymatic cleavage of the oligosaccharide IX by the fungus Rhizopus arrhizus. A solution of 200 mg of IX in 100 ml of phosphate buffer was treated with the fungus Rhizopus arrhizus and was shaken at 30° C. After 100 hr, in addition to the starting material and glucose, the oligosaccharide X was detected, and this was isolated in the same way as IX. The acid hydrolysis of X gave xylose and galactose. 25 mg of X was subjected to Kuhn methylation. The cleavage of the permethylated product gave 2, 3, 4, 6-tetra-O-methylgalactose and 2, 3-di-O-methylxylose.

With triphenyltetrazolium chloride [6], the disaccharide X formed a red spot on a white background. Compound X gave a positive reaction with diphenylamine–aniline [7].

Table

Comparison of the Molecular Rotations of the Progenins and Oligosaccharide in Order to Determine the Configurations of the Sugars in the Saponin

Methyl glycosides of the monosaccharides	$[M]_D^{20}$, deg		Glycoside	$[M]_D^{20}$, deg	Δc, deg	Form of the bond
	α	β				
Methyl D-galactopyranoside	+380	0	The pentaoside V	+474	+615	α
			The tetraoside IV	−141		
Methyl L-rhamnopyranoside	−111	+170	The tetraoside IV	−141	−252	α
			The trioside III	+111		
Methyl L-arabopyranoside	+ 28	+ 402	The trioside III	+111	− 29	α
			The bioside XI*	+140		
Methyl D-xylopyranoside	+253	−108	The bioside XI	+140	+ 31	β
			The monooside II	+109		
Methyl D-glucopyranosiduronic acid	+307	− 62	The monooside II	+109		
			Gypsogenin	+433	− 324	β
Methyl D-fucopyranoside	+351	− 29	The bioside VIII	−174		
			The monooside II	+109	−283	β
Methyl D-xylopyranoside	+253	−108	The tetraoside VII	+174		
			The trioside XII** (calculated)	+111	+ 63	β
Methyl D-galactopyranoside	+380	0	The oligosaccharide IX	+220		α
Methyl D-glucopyranoside	+309	− 66	--	—	—	β
Methyl D-xylopyranoside	+253	−108	--	—	—	β
Calculated:						
Methyl β-xylopyranoside + methyl galactoside + methyl glucoside						
α α				+581		
α β				+206		
β α				+201		
β β				−174		
Methyl L-rhamnopyranoside	−111	+170	Saponaside D	+637		
			and the nonaoside XIII*** (calculated)	+651	− 14	α

*Obtained by the saponification of the glycoside VII.

**The bioside XI + methyl β-fucopyranoside.

***The sum of $[M]_D^{20}$ for the aglycone and $[M]_D^{20}$ for methyl glycosides of known configuration.

The configurations of the glycosidic bonds were determined from Klyne's rule [8] (table).

CONCLUSIONS

The complete structure of the triterpene glycoside saponaside D has been established.

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