THE QUESTION OF THE STRUCTURE OF A TRITERPENE GLYCOSIDE FROM SAPONARIA OFFICINALIS

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The isolation from Saponaria officinalis (bouncingbet, fuller's herb) of a triterpene glycoside, saponaside D, the aglycone of which is gypsogenin, has been reported [1]. In this paper the results of experiments giving information on the structure of the substance are reported.

Saponaside D was exhaustively methylated. The resulting product was cleaved with perchloric acid, and the methylated monosaccharides formed were chromatographed on silica gel. Seven individual substances were detected. By chromatography on paper and in a thin layer of silica gel and also by gas chromatography in the presence of reference samples, completely methylated glucose, galactose, arabinose, and rhamnose and also 2-O-methylfucose were identified. From their chromatographic behavior, another two substances may be assigned to monomethylated compounds. When they were demethylated, in the one case xylose and in the other case glucuronic acid were identified.

The xylose fragment is not oxidized in the initial saponin. The dimethylxylose obtained from the methylated saponaside D also undergoes no change under the action of Bonner's reagent [2]. After the hydrolysis of this monosaccharide with sulfuric acid, the product had acquired a capacity for being oxidized by periodic acid. The reaction with triphenyltetrazolium chloride shows the presence in this xylose derivative of a free hydroxyl at position 2. In addition, the substance has a low mobility on paper chromatography in borate buffer, i.e., the formation of a complex with boric acid takes place. Consequently, the compound may be ascribed the structure of 3-O-methylxylose.

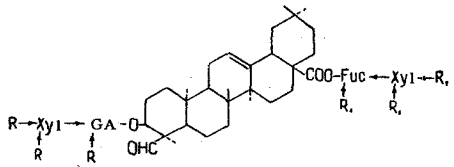
Treatment of the partially methylated glucuronic acid with lithium aluminum hydride gave a monomethyl derivative of glucose. This shows that the carboxyl group cannot be at the position of branching of the carbohydrate chain in the saponin. When saponaside D was oxidized with periodate the glucuronic acid remained unchanged; methyl glucuronoside was revealed by Bonner's reagent. All this permits the assumption that the second substance is the 2-O-methyl or the 4-O-methyl derivative of glucuronic acid. The decision in favor of 2-O-methylglucuronic acid was made on the basis of the fact that on chromatography on paper in borate buffer the substance retains its mobility.

The results of methylation were confirmed by periodate oxidation, in which the saponin absorbed 12 moles of periodate and liberated 6 moles of formic acid. This shows an unusually high degree of branching of the oligosaccharide chains of the saponin.

To elucidate the type of linkage of the monosaccharides with the two functional groups of the aglycone, the methylated saponin was treated with lithium aluminum hydride. In this way it was found that the residues of 2-O-methylglucuronic acid, 3-O-methylglucose, and completely methylated arabinose, rhamnose, and galactose were attached to the hydroxyl of gypsogenin. The carbohydrate chain attached to the carboxyl group contained completely methylated galactose, glucose, and rhamnose, and also 3-O-methylxylose. 2-O-Methylfucitol was identified among the products of aluminum hydride cleavage, and therefore fucose is attached directly to the carboxyl group of gypsogenin. This was also confirmed by the saponification of the saponin with alkali followed by hydrolysis of the fragments obtained. The saponification products of the saponin included glucuronic acid, galactose, xylose, arabinose, and rhamnose.

An acid hydrolysate of the oligosaccharide contained galactose, glucose, xylose, and rhamnose. The absence of fucose from the hydrolysates shows its direct attachment to the carboxyl group of the gypsogenin. According to the literature, such monosaccharides are not preserved on hydrolysis [3]. The glucuronic acid is attached to the $C_{(3)}$ hydroxyl of gypsogenin. This was established by analogy with other triterpene saponins [4,5].

Since no partially methylated sugars other than 2-O-methylfucose, 2-O-methylglucuronic acid, and 3-O-methylxylose were found, it is obvious that one xylose residue is attached to glucuronic acid and the other to fucose. The results obtained make it possible to ascribe the following partial structure to saponaside D:



where R = Rha or Gal or Ara and R_1 = Rha or Gl or Gal.

Previously the most highly branched oligosaccharide fragments known were present in the saponin isolated by Tschesche et al. from Radix sarsaparillae [6]. The degree of branching of saponaside D is greater than this.

EXPERIMENTAL

Chromatography was carried out with silica gel of type KSK, alumina of activity grade II, and paper of types M and S of the "Goznak" Leningrad mill, with the following systems of solvents: 1) benzene–ethanol (10 : 1), 2) methyl ethyl ketone saturated with ammonia, 3) benzene–acetone (2 : 1), 4) butanol–benzene–pyridine–water (6 : 1 : 3 : 3); 5) butanol–ethanol–0.05 M borax solution (5 : 1 : 4), and 6) butanol–ethanol–water (10 : 2 : 5). The spots of the glycosides were revealed with conc H_2SO_4 and those of the sugars with aniline phthalate. The IR spectra were recorded on a UR-10 spectrophotometer.

Methylation of saponaside D. A solution of 5.0 g of the glycoside in 75 ml of dimethylformamide was treated with 35 ml of methyl iodide, and then 25.0 g of barium oxide and 0.8 g of barium hydroxide were added with stirring. After this the reaction product was methylated further with methyl iodide in the presence of silver oxide (5 times). The degree of methylation was checked by IR spectroscopy from the disappearance of the peak in the 3500 cm^{-1} region, and also by chromatography in a thin fixed layer of silica gel in system 1. The product obtained, I, was purified on a column of silica gel in the same system.

Found, %: C 58.32; H 8.28; OCH_3 33.20. Calculated for $C_{113}H_{190}O_{49}$, %: C 58.13; H 8.21; OCH_3 34.14.

Methanolysis of the permethylated glycoside. 3.0 g of product I was heated with 50 ml of a mixture of 72% perchloric acid and methanol (1 : 2) in a sealed tube at 100° C for 5 hr. After the end of the reaction, the mixture was diluted with water and the precipitate that deposited was filtered off. After neutralization with IR-45 anion-exchanger (OH^- form) and subsequent evaporation, the mixture was chromatographed in a thin layer of silica gel in system 3. The plates were sprayed with H_2SO_4; seven methyl glycosides were detected.

Separation and identification of the methylated sugars. The mixture of methyl glycosides was dissolved in 200 ml of water and extracted exhaustively with chloroform. On chromatography on silica gel in system 3, it was seen that the aqueous layer contained the partially and the chloroform layer the completely methylated monosaccharides. These extracts were evaporated to minimum volume and chromatographed on silica gel in system 3. Seven individual methyl glycosides II–VIII, numbered in the order of increasing polarity, were obtained.

When the methylated substances II–V were demethylated with boron trichloride [7], galactose, glucose, arabinose, and rhamnose were identified by chromatography on paper in system 4. Compounds II–V remained unchanged under the action of methyl iodide. Fully methylated galactose (II), glucose (III), arabinose (IV), and rhamnose (V) were identified by gas chromatography in the presence of reference samples. Among the partially methylated products, substance VII was detected and identified by chromatography on paper and in a thin layer of silica gel and by gas-liquid chromatography in the presence of reference samples as 2-O-methylfucose. On demethylation, substance VI gave glucuronic acid. On treatment with lithium aluminum hydride, VI was converted into a monomethylglucose. The glucuronic acid was not decomposed in the initial saponin while the methyl glucuronoside was decomposed under the action of Bonner's reagent. When chromatographed on paper impregnated with 0.05 M borax in system 5, substance VI retained its mobility. On treatment with boron trichloride, substance VIII gave xylose. The xylose was preserved in the initial saponin. Substance VII underwent no change under the action of periodic acid, and on being heated with H_2SO_4 with subsequent detection by means of Bonner's reagent, it underwent oxidation.

When treated on paper with triphenyltetrazolium chloride, substance VII gave a red coloration. This compound forms a boric complex on chromatography on paper in system 5.

Aluminum hydride cleavage of methylated saponaside D. To 1.0 g of I in 100 ml of absolute tetrahydrofuran was

added 0.5 g of lithium aluminum hydride. The mixture was heated to the boil for 10 hr. After the reaction mixture had been worked up [8], the reduced glycoside IX and an oligosaccharide X were obtained. Methanolysis and the subsequent separation of the methyl glycosides in a thin layer of silica gel, and also gas-liquid chromatography permitted the identification in compound IX of completely methylated residues of rhamnose, arabinose, and galactose, and also the monosaccharides VI and VIII.

In the reduced oligosaccharide X, the completely methylated methyl glycosides of rhamnose, glucose, and galactose, and compound VIII and 2-O-methylfucitol were found.

Alkaline saponification of saponaside D. A mixture of 0.5 of the substance and 25 ml of 10% aqueous ethanolic caustic potash was heated at 80° C for 5 hr. After the alkali had been eliminated, the solution was exhaustively extracted with isoamyl alcohol. After purification of the ethanolic layer on a column in system 6, the substance was subjected to acid hydrolysis. Galactose, arabinose, xylose, glucuronic acid, and rhamnose were identified.

The substance from the aqueous layer was deposited on a column of silica gel and chromatographed in system 6 and was then eluted with pure methanol. The methanolic fractions were combined and evaporated and the residue was hydrolyzed as described above. Galactose, glucose, xylose, and rhamnose were identified.

Preparation of gypsogenin glucuronoside. A mixture of 1.0 g of saponaside D and 20 ml of 2% H_2SO_4 was heated at 80° C for 5 hr. The precipitate that deposited was filtered off, washed with water to neutrality, and dissolved in methanol, and the methanolic solution was decolorized with activated carbon; then the product was repeatedly recrystallized from methanol and dried in vacuum; mp 203–205° C (decomp.).

Found, %: C 63.85; H 8.35. Calculated for $C_{36}H_{54}O_{10} \cdot 2H_2O$, %: C 65.04; H 8.49.

CONCLUSIONS

1. It has been shown that saponaside D is a decaoside of gypsogenin.

2. It has been established that the carbohydrate moiety attached to the hydroxyl group of the aglycone comprises galactose, arabinose, xylose, rhamnose, and glucuronic acid and that attached to the carboxyl group of gypsogenin comprises galactose, glucose, xylose, fucose, and rhamnose.

3. It has been shown that glucuronic acid is attached directly to the hydroxyl of the gypsogenin by a β-glycosidic linkage and a fucose residue is attached directly to the carboxyl group.

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