OLIGOSACCHARIDES OF SAPONARIA OFFICINALIS

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Khimiya Prirodnykh Soedinenii, Vol. 5, No. 6, pp. 469–473, 1969

UDC 547–597

From a methanolic extract of the roots of Saponaria officinalis L. (bouncingbet; fullers' herb) we have isolated two triterpene glycosides, derivatives of gypsogenin and of gypsogenic acid [1]. A study of the extract by paper chromatography has shown that it contains three oligosaccharides (A, B, and C) with R_f values of 0.17, 0.06, and 0.03, respectively. The main component of the mixture proved to be oligosaccharide C, which we have called saponarose (12% of the weight of the extract). The amount of oligosaccharide B was small, and its R_f value is close to that of oligosaccharide C. Consequently, it was impossible to isolate this oligosaccharide in the pure state. Oligosaccharide A amounted to only 1.5% of the mixture; it differed in polarity from the two preceding compounds and could therefore, be isolated in the individual state. The preparation of the combined oligosaccharides, their separation from triterpene glycosides, and their separation into the individual components was carried out by partition chromatography on a column of cellulose. They were freed from mineral impurities either by gel filtration on Sephadex G-25 or by dialysis.

Oligosaccharide A consists of a crystalline compound decomposing into D-glucose on being heated with dilute acids. In its constants, chromatographic behavior, and elementary analysis, it was completely identical with gentiobiose.

Saponarose, forming the bulk of the mixture, is an amorphous powder which is hydrolyzed by mineral acids forming D-glucose and D-galactose in a ratio of 4 : 1. A determination of the molecular weights of the oligosaccharide and its acetate showed that it is a pentasaccharide. It did not reduce Fehling's solution, did not form a phenylosazone, and underwent no change under the action of dilute alkalis, which shows the presence of a 1–1 bond in it.

The full methyl ether of saponarose was obtained by treating it with dimethyl sulfate and water with subsequent methylation by means of methyl iodide in dimethylformamide. The IR spectrum of the product obtained lacked the absorption bands of free hydroxyl groups. After its hydrolytic decomposition, 2,3,4,6-tetramethyl-D-glucose, 2,4,6-trimethyl-D-galactose, and 2,3,4,6-tetramethyl-D-galactose were identified. Consequently, the sugar chain has a straight structure, the glucose occupying a terminal position, and the galactoses are connected with one another by 1–3 bonds. The latter is also confirmed by the results of periodate oxidation. Thus, the action of potassium periodate on the compound and subsequent heating with dilute mineral acids gave a hydrolysate containing galactose (the glucose had completely disappeared). The fact that the glucose is attached to a chain consisting of galactose by a 1–1 bond is shown by the results of mild partial hydrolysis. We have succeeded in showing that when saponarose is heated in aqueous solution with KU-2 ion-exchange resin at 50–60° C glucose is the first of the monosaccharides split out.

Heating with dilute acetic acid led to a more far-reaching decomposition of the molecule of the oligosaccharide and from the reaction mixture it was possible to isolate a product corresponding in R_f value and elementary analysis to a trisaccharide. This compound consisted only of galactose, it reduced Fehling's solution, and its full methyl ether decomposed on hydrolysis into 2,4,6-trimethyl-D-galactose and 2,3,4,6-tetramethyl-D-galactose.

The configuration of the glycosidic centers was calculated by means of Klyne's rule. It was found that the calculated value of the molecular rotation is in good agreement with that found experimentally if one β-bond and four α-bonds are present in the oligosaccharide (see table).

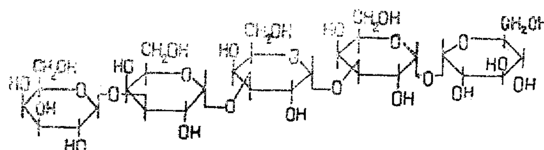
The presence of α- and β-glycosidic centers in the oligosaccharide is also shown by the characteristic frequencies at 845 cm^{-1} (α-configuration) and 890 cm^{-1} (β configuration) in the IR spectrum [4].

As can be seen from the table, calculation according to Klyne does not make it possible to determine the position of the β-linkage, since close values of Δc are obtained when it is present in any position. However, the fact of the predominant formation of the trisaccharide in the hydrolysis of saponarose with dilute acetic acid permits the β-linkage to be assigned to the end of the galactose chain. In this case the trisaccharide may be formed by the rupture of

the weak 1–1 and β-linkages at the ends of the chain.

Substance	M	[α] (water), degrees		Most probable linkage	$[M]_D = \frac{M \cdot [\alpha]_D}{100 \text{ deg.}}$
		α	β		
Methyl D-galactopyranoside [2]	194.2	196.6	−0.42	4α *Gal* + 1β *Gl*	1460.8
Methyl D-glucopyranoside [3]	194.2	158.8	−34.2	($1\beta + 3\alpha$) *Gal* + 1α *Gl*	1452.9
Saponarose	828.7	167		—	1382.8

On the basis of what has been said above, the complete structural formula of saponarose may be represented in the following way:



EXPERIMENTAL

Chromatography was carried out with cellulose powder obtained from type M Leningrad chromatographic paper and on Sephadex G-25 (coarse). The following solvent systems were used: 1) butan-1-ol saturated with water, 2) butan-1-ol-acetic acid-water (4 : 1 : 5), 3) benzene-butan-1-ol-pyridine-water (10 : 50 : 30 : 30), 4) butan-1-ol-ethanol-water (5 : 1 : 4) and 5) toluene-butan-1-ol-water (60 : 38 : 2).

Separation and purification of oligosaccharides A and C. A methanolic extract of the roots of Saponaria officinalis (45 g) was dissolved in water and the solution was extracted with butan-1-ol. The aqueous solution was evaporated to small volume, transferred to a column containing 1 kg of cellulose powder, and eluted with systems 1 and 2, 500-ml fractions being collected. The separation of the fractions was monitored by paper chromatography in system 2.

The 1st and 2nd fractions contained gypsoside (9.3 g), the 3rd and 4th gentiobiose (2.7 g), the 5th–7th a mixture of oligosaccharides A, B, and C (4 g), and the 7th–10th saponarose (23.5 g).

Gentiobiose. The unpurified product obtained in the previous experiment (2.7 g) was transferred to a column containing 100 g of Sephadex G-25 and was eluted with water, the eluate being collected in fractions of 25–30 ml. The 3rd–6th fractions contained 2.4 g of oligosaccharide C which, after drying in vacuum over P_2O_5, had mp 190° C, $[\alpha]_D$ + + 6° (c 1.3; water).

Found, %: C 41.83; H 6.15. Calculated for $C_{12}H_{22}O_{11}$, %: C 42.10; H 6.43.

When it was heated with 5% HCl, the hydrolysate was found by paper chromatography in system 2 and 3 to contain D-glucose. The melting point of the phenylosazone from the hydrolysate was 208° C.

Saponarose. 1) The unpurified product (3.5 g) was transferred to a column containing 100 g of Sephadex G-25 and was eluted with water, 50-ml fractions being collected. Fractions 3–6, by reprecipitation from methanolic solution with acetone and drying in vacuum over P_2O_5, yielded 2.9 g of saponarose, mp 272–275° C, $[\alpha]_D^{20}$ + 167 ± 3° (c 1.2; water); mol. wt. 780 (cryoscopy). The oligosaccharide did not reduce Fehling's solution.

2) The unpurified product (5 g) was placed in a cellophane bag (two layers) and dialyzed in flowing water for 10 days. Then the contents of the bag were evaporated to dryness and reprecipitated from methanolic solution with acetone. This gave 0.4 g of the product described above.

Saponarose acetate. The substance (0.25 g) was dissolved in a mixture of 2 ml of acetic anhydride, 6 ml of pyridine, and 1 ml of dimethyl sulfoxide and left at room temperature for 2 days. Then the reaction mixture was

poured into water and the precipitate was recrystallized from 60% ethanol giving the acetate with mp 105–107° C, $[\alpha]_D^{20}$ + + 154 ± 3° (c 2.4; chloroform), mol. wt. 1475 (cryoscopy).

Found, %: C 49.90; H 5.90. Calculated for $C_{64}H_{86}O_{43}$, %: C 49.80; H 5.57.

Hydrolysis of saponarose. The substance (0.12 g) was heated with 7 ml of 5% HCl in the water bath for 2 hr. Then the reaction mixture was diluted to 25 ml. D-Galactose and D-glucose (4 : 1) were identified by paper chromatography in system 3 using Zaitseva and Afanas'eva's method [5].

Methyl ether of saponarose. A solution of 3.5 g of saponarose in 45 ml of water was treated with 45 ml of dimethyl sulfate. With cooling to 0° C, 67 ml of 40% aqueous alkali was added over 8 hr and stirring was continued for another 15 hr. Then 75 g of caustic soda was added and 110 ml of dimethyl sulfate was run in over 8 hr with cooling and stirring. The precipitate that deposited was filtered off, the reaction mixture was extracted with chloroform (5 × × 100 ml), and the solvent was evaporated to dryness. The residue (3 g) was dissolved in 50 ml of dimethylformamide, 15 g of barium oxide was added, and the mixture was stirred for 3 hr. Then 15 ml of methyl iodide was added and the mixture was heated and stirred for 6 hr, another 2–3 g of barium oxide and 3–5 ml of methyl iodide being added every 2 hr. The reaction mixture was poured into a saturated solution of sodium thiosulfate and extracted with chloroform (5 × 100 ml), and the solvent was evaporated to dryness. The oily product (3.2 g) was transferred to a column of silica gel (2 × 25 cm). Elution with chloroform yielded 1.5 g of liquid methyl ether with $[\alpha]_D^{20}$ + 132° (c 5.0; chloroform).

The IR spectrum lacked the absorption band of an OH group.

Found, %: C 52.78; H 7.88. Calculated for $C_{47}H_{86}O_{26}$, %: C 52.90; H 8.07.

Hydrolysis of the methyl ether of saponarose and separation of the methylated sugars. A solution of 0.5 g of the substance in 50 ml of 2% methanolic HCl was heated in the water bath for 6 hr. Then the mixture was diluted twofold and heated for another 3 hr. The hydrolysate was neutralized with AV-17 anion-exchanger and evaporated to dryness. The syrup obtained was used for the subsequent separation. By paper chromatography in system 4 with an authentic reference sample, 2,3,4,6-tetramethyl-D-glucose was identified. The hydrolysate (0.5 g) was transferred to a column of powdered cellulose (1 : 50) and eluted, 15-ml fractions being collected.

The separation of the fractions was checked by paper chromatography (system 4). Eight fractions were collected. Fraction 2 (0.05 g) consisted of pure 2,3,4,6-tetramethyl-D-galactose with $[\alpha]_D^{20}$ 90 ± 3° (c 2; ethanol). According to the literature: $[\alpha]_D^{20}$ + 118° [6].

A small portion of the sample (0.01 g) was heated with 0.5 ml of 48% HBr for 5 min in the boiling water bath. Then the mixture was diluted with water and extracted with benzene. D-Galactose was identified in the hydrolysate by paper chromatography in system 3 with a reference sample.

Fractions 3–8 contained as unresolved mixture. It was deposited in 0.03-g portions on sheets (58 × 100 cm) of Schleicher und Schull paper and chromatographed in system 4. Narrow strips of the chromatograms, cut from the edges and from the centers of the sheets, were treated with aniline phthalate and from these the zones on the main sheets were found. They were cut out and extracted from ethanol in a Soxhlet apparatus for 3 hr. In addition to the above-described 2,3,4,6-tetramethyl-D-galactose, 2,4,6-trimethyl-D-galactose was isolated in the form of a syrup with $[\alpha]_D^{20}$ + 80 ± 3° (c 1; ethanol). Literature: $[\alpha]_D$ + 92° [7]. On demethylation by heating with 48% of hydrobromic acid by the method described above, D-galactose was obtained.

Periodate oxidation of saponarose. A solution of 0.07 g of saponarose in 5 ml of water was treated with 150 ml of KIO_4 and 25 ml of acetate buffer with pH 3.6 (28.4 ml of acetic acid and 2.8 g of sodium acetate in 500 ml of water) and was left in the dark at room temperature for 2 days. Then a few drops of ethylene glycol were added and the solution was evaporated to dryness in vacuum. The residue was treated with 10 ml of 5% HCl and the mixture was heated for 6 hr. D-Galactose was identified in the hydrolysate by paper chromatography in system 3.

Partial hydrolysis of saponarose. A) A solution of 0.2 g of saponarose in 20 ml of water was heated with 2 g of KU-2 ion-exchanger in the H-form at 50–60° C, samples being taken after 5, 15, 30, and 60 min. The samples were deposited on strips of chromatographic paper and chromatograms were run in system 3. D-Glucose was identified in the second sample and D-glucose and D-galactose in the subsequent ones.

B) The oligosaccharide (0.7 g) was heated with 30 ml of 1 M acetic acid in the boiling water bath for 3 hr. Then the reaction mixture was evaporated to dryness in vacuum and the residue was transferred to a column (2.5 × 30 cm) of cellulose and was eluted with system 2, 20-ml fractions being collected. Fractions 10–14 contained 200 mg of a trisaccharide. After reprecipitation from 90% aqueous methanol with acetone and drying over P_2O_5 in vacuum, mp 183–186° C, $[\alpha]_D^{20}$ + 144° (c 1.26; water).

Found, %: C 42.36, 42.66; H 6.80, 7.02. Calculated for $C_{18}H_{32}O_{16}$, %: C 42.85; H 6.35.

The product (0.01 g) was heated with 5% HCl. D-Galactose was identified in the hydrolysate by paper chromatography in system 3.

The trisaccharide (0.025 g) was methylated by being heated in a tube with 0.25 g of barium oxide and 1.5 ml of methyl iodide in 1 ml of dimethylformamide at 90° C for 9 hr. After the usual working up process, the methyl ether obtained was hydrolyzed with 2% methanolic HCl. 2, 3, 6-Trimethyl-D-galactose and 2, 3, 4, 6-tetramethyl-D-galactose were identified by paper chromatography in system 4.

CONCLUSIONS

It has been found that the roots of Saponaria officinalis L. contain three oligosaccharides.

Two of them have been isolated: gentiobiose and the pentasaccharide saponarose. It has been shown that the latter is O-β-D-galactopyranosyl-(1 → 3)-O-α-D-galactopyranosyl-(1 → 3)-O-α-D-galactopyranosyl-(1 → 3)-O-α-D-galactopyranosyl-(1 → 1)-α-D-glucopyranoside.

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17 June 1968

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