the structure of its macromolecule is similar to that of the glucuronoxylans of leguminous herbs studied previously. It consists of xylopyranose residues linked to one another by 1→4 bonds. 4-O Methyl-D-glucuronic acid residues are attached in the form of side chains at the C-2 position of some of the xylopyranose residues; to each side chain corresponds not less than 12 xylose residues.

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POLYSACCHARIDES FROM THE INFLORESCENCES OF Matricaria matricarioides

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Fractionation of the polysacchride complex of the inflorescences of pineapple weed has given a galacturonan and a pectic acid. The structures of the galacturonan and pectic acid have been studied by the methods of enzymatic hydrolysis, periodate oxidation, and exhaustive methylation. It has been established that the galacturonan is a linear polysaccharide consisting of D-galacturonic acid residues in the pyranose form with α-1→4-bonds. The main polysaccharide chain of the pectic acid consists of D-galacturonic acid residues in the pyranose form, D-galactose, L-arbinose, and D-xylose residues are covalently bound to carbon atoms 2 or 3 of the main chain of the polysaccharide.

Earlier [1, 2], a water-soluble polysaccharide complex possessing pronounced biological activity [3,4] was isolated from inflorescence of Matricaria matricarioides (pineapple weed, family Asteraceae).

We have studied the structure of the galacturonan and the pectic acid obtained on the fractionation of the initial polysaccharide complex [2].

After the enzymatic hydrolysis of the galacturonan, mono-, di-, tri-, and tetragalacturonic acids were detected [5].

On periodate oxidation [6], the galacturonan consumed 1 mole of sodium metaperiodate per anhydro unit. From the oxidation products was isolated a products was isolated a polyaldehyde with water), which was subjected to further oxidation with 25% nitric acid [7] and was hydrolyzed. The hydrolysis products were investigated by the PC method in system 4. Oxalic and tartaric acids were identified in comparison with authentic samples. The isolation of the tartaric acid in the crystalline state with mp 167-169°C (a mixed melting point with an authentic sample of tartaric acid gave no depression) showed that grouping involving the second and third carbon atoms had been oxidized by the periodic acid. This is possible in the case of a pyranose ring with 1→4-bonds between the galacturonic acid residues. The presence of the pyranose ring was also shown by the resistance of the substance to acid hydrolysis and by its IR spectra with characteristic absorption bands in the 1000-1100 cm^{-1} region.

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A definitive conclusion concerning the structure of the galacturonan was made on the basis of the results of exhaustive methylation. It must be mentioned that the direct methylation of the galacturonan by Hakomori's method [8] did not lead to the desired results, and the methylation of the glacturonan was therefore performed after preliminary esterification with a 1 M solution of sulfuric acid in methanol and the reduction of the esterified carboxy groups with sodium tetrahydroborate to primary alcoholic hydroxyls. As a result, the acid polysaccharide yielded an almost neutral glycan containing 8.1% of galacturonic acid residues and 18.1% of OCH_3 groups, with an ash content of 0.85%.

The galactan was methylated three times by Hakomori's method [8]. This gave the completely methylated polysaccharide with $[\alpha]_D$ + 201° (c 0.5%; chloroform) and 39.9% of OCH_3 groups. Its IR spectrum lacked the absorption band of a hydroxyl. The fully methylated galactan was subjected to methanolysis with a mixture of 72% perchloric acid and methanol (10:1) and to hydrolysis. From the hydrolysate of the permethylate was isolated 2,3,6-Tri-O-methyl-D-galactose, $[\alpha]_D$ + 81° (c 0.3%; water) which, on oxidation with bromine, was converted into 2,3,6-tri-O-methyl-D-galactono-γ-lactam, mp 97-99°C, and very small amounts of 2,3,4,6-tetra-O-methyl-D-galactose, which was identified in the form of the analide, mp 185-187°C.

The results obtained indicated that the polysaccharide contained chains constructed of D-galacturonic acid residues in the pyranose form with α-1→4-bonds, the α configuration being assumed on the basis of the high specific rotation of the polysaccharide [2].

Mono-, di-, tri-, and tetragalacturonic acids, and also galactose, arabinose, and xylose were detected in the products of the enzymatic hydrolysis of the pectic acid.

On the periodate oxidation of the pectinic acid it was established that oxidation was complete after 24 h. The consumption of sodium metaperiodate was 0.79 mole per anhydro unit. Consequently, the polysaccharide does not have a highly branched structure. The oxidation product was isolated from the reaction mixture, $[\alpha]_D$–82° (c 1%; water) and was hydrolyzed. In the hydrolysate PC revealed weak spots of galacturonic acid and rhamnose, and also of arabinose, xylose, and galactose.

Then the pectic acid was converted into the full methyl derivatives and it was reduced with sodium tetrahydroborate and was methylated by Hakomori's [8] and Purdie's [9] methods. This gave a completely methylated polysaccharide with $[\alpha]_D$ + 195° (c 0.5%; chloroform) in which 40.1% of OCH_3 groups was found. Further methylation did not lead to an increase in the amount of OCH_3. The IR spectrum lacked the absorption band of a hydroxyl.

In an investigation of the products of the degradation of the reduced and methylated pectic acid by the PC method, a complex set of methylated monosaccharides was obtained. From the mixture of methylated monosaccharides on a cellulose column in system 2 were isolated 2,3,6-tri-O-methyl-D-galactose as the main component, and also 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,5-tri-O-methyl-L-arbinose, and 2,3,4-tri-O-methyl-D-xylose. The isolation of 2,3,6-tri-O-methyl-D-galactose as the main hydrolysis product indicated the presence of a skeletal structure consisting of D-galacturonic acid residues linked by 1→4-bonds. The isolation of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,5-tri-O-methyl-L-arabinose, and 2,3,4-tri-O-methyl-D-xylose indicated that the corresponding residues were bound to the second or third atoms of the main chain of the polysaccharide.

EXPERIMENTAL

A standard raw material – inflorescence of pineapple weed – corresponding to the requirements of GOST SSSR [USSR State Standard] 227-53 and the State Pharmacopoeia (Xth ed., Moscow (1968)) was used for the isolation of the polysaccharides.

The partition chromatography of the monosaccharides and oligosaccharides was performed on type M ["slow"] paper of the Volodarskii Leningrad mill and on FN-7 and FN-11 papers. The following solvent systems were used for chromatography (ratios by volume): 1) butan-1-ol–pyridine–water (6:4:3); 2-butan-1-ol–ethanol–water (5:1:4); 3) ethyl acetate–formic acid–water–acetic acid (18:1:4:3); and 4) butan-1-ol–acetic acid–water (4:1:2).

To reveal the monosaccharides and oligosaccharides on the chromatograms we used a solution of aniline hydrogen phthalate in water-saturated butanol at 105-110°C. Organic acids were revealed on the chromatogram with a sulfanilamide reagent consisting of two solutions: a) 0.5 g of α-naphthol and 0.5 g of sulfanilamide in 100 ml of ethanol, and b) a 1% solution of sodium nitrite.

The polysaccharides were dried in vacuum over P_2O_5 (residual pressure 10-12 mm Hg; temperature 40- 50°C.

The IR spectra of the samples of polysaccharides obtained in the form of tablets (1 mg of substance in 200 mg of potassium bromide) were recorded in the 700-3700 cm^{-1} region on a IRG-1 instrument (Japan).

The optical activities of the polysaccharide solutions were determined on EPN-AI instrument, λ = 589 nm, and the amount of methoxy groups by the Zeisel method [10]. The consumption of sodium metaperiodate was determined by the arsenite method [6, 11].

The preparative isolation of the methylated monosaccharides was performed with the use of partition chromatography on a column of cellulose [12].

1. Structure of the Galacturonan. Enzymatic hydrolysis. Samples of the polysaccharide weighing 0.2 g each were dissolved in water with the addition of 2 N sodium hydroxide solution, and 40 mg of the enzyme preparation Pectinase aus Schimmel and a few drops of toluene were added. Enzymatic hydrolysis was performed at pH 3.5 and a temperature of 39°C [5]. The products of enzymatic hydrolysis were investigated by PC in systems 1 and 3.

Esterification. The galacturonan (2 g) was dispersed in 50 ml of a 1 M solution of sulfuric acid in methanol at +2°C with periodic stirring. The esterified acid was filtered off and was washed with ethanol until the reaction for sulfate ion was negative and it was then washed with ether and dried at room temperature; yield 1.75 g $[\alpha]_D$ + 206° (c 0.2% in water in the form of the sodium salt); amount of OCH_3 groups 8.5%.

Oxidation with Sodium Periodate. A solution of 1.5 g of the esterified galacturonan in 50 ml of water was treated with 8 g of sodium metaperiodate in 50 ml of water. Oxidation was carried out in the dark at +4°C. The course drops reaction was monitored by determining the consumption of periodate periodically; in 10 days this amounted to 0.68 mole per anhydro unit. Then the pH of the reaction mixture was brought to 8 and it was left for another day in the dark at +4°C. The consumption of periodate increased to 0.96 mole per anhydro unit. After this, ethylene glycol was added to destroy the periodate. The mineral impurities were removed by successive passage through columns containing the ion-exchangers KU-2 (H^+) and Av-17 (HCO_3^-). The completeness of elimination of the mineral acids was monitored by the reaction with strontium nitrite. This gave 1.4 g of polyaldehyde with $[\alpha]_D$–81° (c 0.5%; water).

On investigating the oxidation products by the PC method in systems 1-4, a single spot was revealed that had not migrated from the start. Traces of galacturonic acid were detected in the products of acid hydrolysis of the polyaldehyde by PC in system 3.

Oxidation with Nitric Acid. The polyaldehyde (1.2 g) was dissolved in 20 ml of 25% nitric acid and the solution was heated on the boiling water bath for 8 h. The hydrolysate was evaporated to dryness. The nitric acid was removed by repeated distillation with water in vacuum. This gave 1.1 g of the products of the oxidation of the polyaldehyde, which were hydrolyzed with a 1 N solution of sulfuric acid at the boiling point of the reaction mixture for 8 h. The hydrolysate was neutralized with barium hydroxide. The filtrate was evaporated in vacuum and was analyzed by the PC method in system 4.

Isolation of Tartaric Acid. In drops, a 30% solution of potassium hydroxide was added to the concentrated hydrolysate (2 ml) to give pH 8.0, and this was followed by 1.5 ml of glacial acetic acid. The resulting solution was left in the refrigerator for 20 h. The precipitate that had deposited was filtered off and was washed with 50% ethanol and then with acetone. The salt was dissolved in 10 ml of water with heating, and the solution was filtered and was left for crystallization. The crystals that had deposited were separated off and were dried in vacuum over P_2O_5. This gave 0.3 g potassium bitartrate, $[\alpha]_D$ -25° (c 0.5%; water). Of this, 0.25 g was dissolved in 35 ml of water at +40°C. The solution was passed through a column of the cation-exchange resin KU-2 (H+). The eluate was evaporated in vacuum to 3 ml and was investigated by the PC method in system 4. Tartaric acid was identified in comparison with an authentic sample. Then the solution was evaporated in vacuum to dryness and the residue was dissolved in the minimum amount of diethyl ether and the solution was left for crystallization.

Esterification. The galacturonan (3 g) was established in 70 ml of a 1 M solution of sulfuric acid in methanol at +2°C for 30 days. This gave 2.1 g of esterified galacturonan: found, OCH_3 10.2%, ash content, 2.2%.

Reduction. A suspension of 2 g of the esterified galacturonan in 40 ml of 80% aqueous methanol was reduced with sodium tetrahydroborate (1 g). The excess of tetrahydroborate was decomposed with acetic acid, and the solution was dialyzed and was treated with KU-2 anion-exchange resin (H+). The partially reduced polysaccharide was again treated with a 1 M solution gave a polysaccharide containing 8.1% of galacturonic acid and having an ash content of 0.85% in a yield of 0.9 g.

Methylation. The galactan (4 g) was methylated three times by Hakomori's method. This gave a completely methylated polysaccharide with a yield of 1.1 g, $[\alpha]_D$ + 201° (c 0.5%; chloroform); OCH_3 content 39.9%.

Hydrolysis. The methylated polysaccharide (1 g) was dissolved in 24 ml of a mixture of 72% perchloric acid and methanol (10:1). The solution was heated in the boiling water bath for 4 h and was then evaporated to 4 ml, treated with 20 ml of water, and hydrolyzed at 100°C for 5 h. After the reaction mixture had been treated with AV-17 anion-exchange resin (HCO_3^-) it was evaporated to dryness and the residue was dried in vacuum. The mixture was investigated by PC in system 2.

Isolation of the Methylated Monosaccharides. The mixture of methylated monosaccharides (0.2 g) in methanolic solution was deposited on a acellulose column 16 × 400 mm). Elution was performed in system 2, 20-ml fractions being collected. The eluates were analyzed by PC in the same solvent system. The fractions containing individual substances were combined, evaporated to dryness in vacuum, and weighed:

a) 2,3,6-tri-O-methyl-D-galactose (118 mg), colorless syrup, $[\alpha]_D$ + 81° (c 0.3%; water). A solution of 50 mg of the substance in 2 ml of water was treated with 0.1 ml of bromine. Oxidation was carried out in the dark at room temperature for 120 h. This gave 25 mg of 2,3,6-tri-O-methyl-D-galacono-γ-lactone with mp 97-99°C (from a mixture of diethyl ether and petroleum ether); and

b) 2,3,4,6-tetra-O-methyl-D-galactose (12 mg). The sample obtained was dissolved in ethanol, and freshly-distilled aniline (0.1 ml) was added. The mixture was boiled for 8 h and was then evaporated and left for crystallization. On standing, crystals of the anilide of 2,3,4,6-tetra-O-methyl-D-galactose deposited with mp 185-187°C (from ethanol).

2. Structure of the Pectic Acid. Enzymatic Hydrolysis. Samples of the polysaccharide weighing 0.2 g each were subjected to enzymatic hydrolysis as described above. The products of enzymatic hydrolysis were investigated by PC in systems 1 and 3.

Periodate Oxidation. The oxidation of 0.3 g of pectic acid was performed with sodium metaperiodate as described above. The consumption of sodium metaperiodate was 0.79 mole per anhydro unit.

From the oxidation products was isolated 0.2 g of a polyaldehyde with $[\alpha]_D$–82° (c 0.2%; water). The polyaldehyde (0.1 g) was hydrolyzed with 1 N solution of sulfuric acid in a sealed tube in the boiling water bath for 20 h. The hydrolysis products were investigated by PC in systems 1 and 3.

Esterification. The pectic acid (8 g) was esterified with a 1 M solution of sulfuric acid in methanol as described above. This gave 6.5 g of esterified pectic acid; found OCH_3 9.8%, ash content 1.7%.

Reduction. The esterified pectic acid (6 g) was suspended in 100 ml of 80% aqueous methanol and was reduced with sodium tetrahydroborate (3 g).

The partially reduced polysaccharide was again treated with a 1 M solution of sulfuric acid in methanol and was reduced with sodium tetrahydroborate. This gave 4.5 g of a glycano-galactan containing 3.5% of galacturonic acid and having an ash content of 1.5%.

Methylation. The glycanogalactan (4.2 g) was methylated three times by Hakomori's method. As a result, an only partially methylated polysaccharide was obtained in a yield of 3.3%; found OCH_3 33.8%.

Supplementary Methylation by Purdie's Method. The partially methylated glycanogalactan (3 g) was dissolved in 100 ml of absolute methyl iodide, and 35 g of freshly prepared silver oxide was added in small portions. The mixture was carefully boiled with stirring for 10 h. Then it was filtered and the precipitate was washed with chloroform. The combined solutions were evaporated in vacuum to dryness. This gave 1.8 g of the fully methylated polysaccharide

with $[\alpha]_D$ + 195° (c 0.5%; chloroform); found OCH_3 40.1%.

Hydrolysis. The completely methylated polysaccharide (1.6 g) was dissolved in 40 ml of a mixture of 72% perchloric acid and methanol (10:1). Methanolysis and hydrolysis were performed as described above. This gave 1.3 g of a mixture of methylated monosaccharides.

Chromatography on a Cellulose Column. The mixture of sugars (1.3 g) in methanolic solution was deposited on a column containing cellulose (25 × 600 mm) and was eluted with system 2. The fractions were investigated by the PC method.

2,3,4-Tri-O-methyl-D-xylose (60 mg), colorless syrup, $[\alpha]_D$ + 17.6° (c 0.2%; water).

2,3,5-Tri-O-methyl-L-arabinose (50 mg). Demethylation gave L-arabinose with mp 157-158°C; $[\alpha]_D$ + 104° (c 0.2%; water).

2,3,4,6-Tetra-O-methyl-D-galactose (87 mg), $[\alpha]_D$ + 108° (c 0.2%; water). The substance was indentified in the form of 2,3,4,6-tetra-O-methyl-D-galactose, mp 185-187°C (from ethanol).

2,3,6-Tri-O-methyl-D-galactose (490 mg), $[\alpha]_D$ + 93° (c 0.3%; water). The substance was identified in the form of 2,3,6-tri-O-methyl-D-galactono-γ-lactone, mp 96-98°C (from a mixture of diethyl ether and petroleum ether).

SUMMARY

1. On the basis of the results of enzymatic hydrolysis, IR spectroscopy, periodate oxidation, and exhaustive methylation it has been established that the galacturonan of the inflorescences of the pineapple weed is a linear of polysaccharide consisting of D-galacturonic acid residues in the pyranose form with α-1→4 bonds.

2. The main features of the structure of the corresponding pectic acid have been determined. The main polysaccharide chain of the pectic acid consists of D-galacturonic acid residues in the pyranose form with α-1→4-bonds.

The isolation of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,5-tri-O-methyl-L-arabinose, and 2,3,4-tri-O-methyl-D-xylose indicates that the corresponding sugar residues are covalently bound to the second or third atoms of the main chain of the polysaccharide.

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