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STRUCTURE OF THE PECTIC ACID OF Matricaria chamomilla

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A water-soluble polysaccharide complex has previously been isolated from the racemes of Matricaria chamomilla L. (German chamomile) and its mono- and polysaccharide composition has been studied [1-4]. We have now investigated the structure of the pectic acid found in the fractionation of the initial complex [3].

In the first stage of the investigations, the polysaccharide was subjected to enzymatic hydrolysis. The products were found to contain mono-, di, tri-, and tetragalacturonic acids and galactose, arabinose, and xylose.

Periodate oxidation of the pectic acid at +15°C was complete in 24 h. The consumption of sodium metaperiodate was 0.81 mole per anhydro unit. Consequently, the polysaccharide does not have a strongly branched structure. The oxidation product was isolated from the reaction mixture, $[\alpha]_D$ −85° (c 2% in water), and was hydrolyzed. By paper chromatography (PC), weak spots of galacturonic acid and rhamnose, and also of xylogalactose were found in the hydrolyzate.

The partial acid hydrolysis of the pectic acid was then carried out. A polymer was isolated from the hydrolyzate with $[\alpha]_D$ +347° (c 0.2% in water in the form of the sodium salt). The physicochemical properties and IR spectra of the polysaccharide were close to those of the products of partial hydrolysis obtained previously from the pectin substances [5].

The pectic acid was methylated by Hakomori's [6] and Purdie's [7] methods after preliminary esterification with a 1 M solution of sulfuric acid in methanol and reduction of the carboxylic ester groups with sodium tetrahydroborate to primary alcohol groups [8]. Chromatography of the methylated polysaccharide on Al_2O_3 gave only one spot, showing homogeneity. The IR spectra contained no absorption bands in the region of hydroxy groups. This demonstrates that the process of methylation had gone to completetion.

In an investigation of the degradation products from the methylated polysaccharide by PC, a complex set of methylated monosaccharides was obtained, and therefore no further study was continued after they had been separated on a coumn of cellulose [7].

The isolation of considerable amounts of fully methylated L-arabinose and D-xylose indicates that the corresponding sugar residues form a covalent bond with the main skeletal structure of the polysaccharide in the form of individual branches. The same can be said about the D-galactose isolated from the degradation products in considerable amounts.

The isolation of 2,3,6-tri-O-methyl-D-galactose as the main component indicates the presence of a skeletal structure consisting of D-galacturonic acid residues connected by 1→4 bonds. The considerable positive specific optical rotation of the polysaccharide shows the α configuration of the glycosidic bond. In the molecule, the galacturonic acid residues are present in the pyranose form, as is shown by the IR spectrum, which has absorption bands at 1000-1110 cm^{-1} (vibrations of a pyranose ring) [9, 10]. The isolation of 3,4-di-O-methyl-L-rhamnose permitted the assumption that rhamnose is included in the main polysaccharide chain by 1→2 bonds.

Thus, the main polysaccharide chain of the pectic acid from German chamomile consisted of residues of α-D-galacturonic acid in the pyranose form linked by 1→4 glycosidic bonds. Single branchings consisting of the neutral monosaccharides galactose, arabinose, and xylose are possible.

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The rhamnose present in the polysaccharide in very small amounts is apparently included in the main polysaccharide chain.

EXPERIMENTAL

The experiments were performed with a standard raw material (racemes of German chamomile) corresponding to the requirements of the State Pharmacopoeia (Xth edition, Moscow, 1968). Partition PC was performed on type "M" ["slow"] paper of the Volodarskii Leningrad factory in the following solvent systems: 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). The monosaccharides were detected by means of aniline hydrogen phthalate.

The polysaccharides were dried in vacuum over P_2O_5 (residual pressure 8-10 mm Hg, temperature 40-45°C). The IR spectra of specimens of the polysaccharides in tablets with KBr were recorded in the 700-3700 cm^{-1} region on an IRG-1 instrument (Shimadzu, Japan).

The specific rotations were determined on an SPU-M spectropolarimeter (Moscow). Periodate oxidation of the polysaccharides was performed with periodic acid and sodium metaperiodate. The consumption of sodium periodate was determined by the arsenite method [11, 12]. Acid hydrolysis was performed with 1 N sulfuric acid solution at 95-100°C, and enzymatic hydrolysis with the aid of Pectinase aus Schimmel (Ferak, Berlin) [13]. The Zeisel method was used for the quantitative determination of methoxy groups [14]. The methylated monosaccharides were isolated preparatively by partition chromatography in a column of cellulose [15].

The results of the experimental investigations have been given in previous papers [1–4].

Treatment with Pectinase. The pH of a solution of 0.2 g of the material in 20 ml of water was brought with aqueous ammonia to 4.5 [13]. Then the solution was treated with 40 mg of the enzyme preparation at 39–40°C for 40 h. The products of enzymatic hydrolysis were investigated by PC.

Periodate Oxidation. A solution of 300 mg of the pectic acid in 40 ml of water was treated with 40 ml of 0.1 M $NaIO_4$ solution. Then the volume was brought up to 100 ml with water. Oxidation was carried out in water with pH 5.5 at +15°C. After every 4 h, 5-ml samples were analyzed for their $NaIO_4$ content. The amount of periodate absorbed by the polysaccharide was calculated in moles per anhydro unit.

After the end of oxidation, the mineral impurities were removed by the successive passage of the solution through columns containing KU-2 (H^+) and AV-17 (HCO_3^-) ion-exchange resins. This yielded 200 mg of polyaldehyde with $[\alpha]_D$–85° (c 2% in water), and the hydrolyzate was shown by PC to contain galacturonic acid and rhamnose, and traces of galactose and xylose.

Preparation of Degraded Pectic Acid. The pectic acid (2 g) was hydrolyzed with 1 N sulfuric acid solution (1 : 20) for 6 h. The precipitate was separated from the liquid and was washed with 1% sulfuric acid and with water, ethanol, and acetone. Yield 1.2 g, $[\alpha]_D$+347° (c 0.2% in water in the form of the sodium salt); its IR spectrum was identical with those of degraded acids of known pectins [5]. Galacturonic acid was found by PC in the products of complete hydrolysis.

Methylation. A. Reduction. Since the methylation of pectic acid takes place with great difficulty, it was previously methoxylated with a 1 M solution of sulfuric acid in methanol and was reduced with sodium tetrahydroborate to the corresponding glycanogalactan [8].

B. Methylation of the Glycanogalactan. This process was carried out by Hakomori's [6] and Purdie's [7] methods. The fully methylated compound was obtained with $[\alpha]_D$+196°C (c 0.5% in chloroform). Found: OCH_3 40.06% (the IR spectrum showed no hydroxyl absorption).

C. Methanolysis of the Methylated Glycanogalactan. The polysaccharide (2 g) was boiled for 6 h in a solution containing 50 ml of methanol and 50 ml of 2 N hydrochloric acid. After the end of the reaction, the methanol was eliminated by vacuum distillation and the solution was diluted with water until the concentration of hydrochloric acid was 1 N and was then heated in a sealed tube at 100°C for 5 h.

In the investigation of the products of the degradation of the methylated glycanolactan by PC, a complex set of methylated monosaccharides was detected, and therefore for subsequent study they were first separated on a cellulose column.

D. Chromatography. The mixture of sugars (1.6 g) was dissolved in the minimum amount of chloroform deposited on a column of cellulose, and eluted with system 2. After reseparation on a cellulose column under the same conditions, 2,3,4-tri-O-methyl-D-xylose (80 mg), 2,3,5-tri-O-methyl-L-arabinose (60 mg), 2,3,4,6-

tetra-O-methyl-D-galactose (115 mg), 3,4-di-O-methyl-L-rhamnose (20 mg), and 2,3,6-tri-O-methyl-D-galactose (980 mg) were obtained. These substances were finally purified by preparative chromatography on paper and were identified in the form of known crystalline derivatives [7, 15].

SUMMARY

The main features of the structure of the pectic acid from the racemes of German chanomile have been established. The main polysaccharide chain consists of D-galacturonic acid residues in the pyranose form with α-1→4 bonds. Isolated single-unit branches of the neutral monosaccharides galactose, arabinose, and xylose are possible. L-rhamnose, which was isolated from the degradation products of the permethylated pectic acid only in the form of 3,4-di-O-methyl-L-rhamnose is apparently included in the main polysaccharide chain.

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THE STRUCTURE OF THE GLUCOMANNAN FROM THE TUBEROUS ROOTS OF Eremurus altaicus

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We have previously described the physicohemical properties of the polysaccharide of fraction I obtained by the fractionation of the initial water-soluble polysaccharide with 96% ethanol [1, 2]. Since the polysaccharide of fraction I is homogeneous, contains only glucose and mannose, and makes up the bulk of the water-soluble polysaccharide, we have investigated the chemical structure of this fraction of the glucomannan.

The glucomannan – a white amorphous powder, viscosity η_{rel} =27.3 (c 0.6%), $[\alpha]_D^{22}$ −41.7° (c 0.6; H_2O) – gives a red coloration with iodine. Gel filtration and sedimentation show that the glucomannan is homogeneous. The ratio of D-glucose and D-mannose in it (GLC) is, as in the purified polysaccharide [1], 1 : 2.6.

The weight-average molecular weight of the glucomannan determined from the sedimentation constant [3] is 120,000 ± 10% which is close to the molecular weight of 108,000 obtained in the gel filtration of the glucomannan through a column of Sephadex G-200 (Fig. 1).

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