POLYSACCHARIDES OF PLANTAGO MAJOR

III. Structure of Degraded Pectic Acid

A. G. Gorin

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As reported previously [1], a polysaccharide complex belonging to the class of pectin substances has been isolated from the leaves of Plantago major L. Since the nondemineralized polysaccharide of the plantain possesses biological activity and has been proposed as a preparation for medical practice [2], it was of interest to establish the main features of the structure of the pectinic acid of the leaves of this plant.

The polysaccharide was subjected to partial hydrolysis with dilute hydrochloric acid. From the hydrolysate a product was isolated in the form of an amorphous white powder sparingly soluble in water. In a number of properties – its solubility in water, specific rotation, equivalent weight by potentiometric titration with alkali, and the amount of 0.01 N solution of iodine consumed per unit weight – this product was similar to the degraded pectic acid obtained from apple pectin and citrus pectin [3, 4]. On subsequent hydrolysis with dilute sulfuric acid, the degraded pectic acid from plantain seeds split off only galacturonic acid. Enzymatic degradation [5] led to the formation of galacturonic acid and oligogalacturonides, which is characteristic for homopolymers. Like other degraded pectic acids, the product of the partial acid hydrolysis of the plantain polysaccharide possesses a high specific dextrorotation (+280°). This gives grounds for assuming that the glycosidic bond between the galacturonic acid residues in the pyranose form has the α-configuration. The presence of a pyranose ring is confirmed by the frequencies of the IR absorption bands at 1025, 1050, and 1080 cm^{-1} (vibrations of pyranose rings and of C–O) [6] and also at 745 and 923 cm^{-1} due to the superposition of the symmetrical and asymmetrical vibrations of the pyranose ring which are displaced in the monomer (D-galacturonic acid) from 775 and 904 cm^{-1}. This displacement is characteristic for polysaccharides with 1–4 bonds between the monosaccharide residues [7].

To show the position of the bond between the galacturonic acid residues, the partially substituted methyl ester of the polygalacturonide was subjected to periodate oxidation [4]. In contrast to the initial polysaccharide, the resulting product possessed a considerable levorotation (–94.5°), reduced Fehling's solution, and gave a positive reaction with aniline phthalate, i.e., revealing itself to be a polyaldehyde. The products of the acid hydrolysis of the polyaldehyde contained only traces of galacturonic acid that had not undergone oxidation. Oxidation of the polyaldehyde with nitric acid gave a very small amount of oxalic and tartaric acids, and also a mixture of three oligomers having R_f values with respect to tartaric acid in the 1-butanol–acetic acid–water (4:1:5) system of 0.00, 0.30, and 0.75, respectively. The mixture of oligomers was hydrolyzed with sulfuric acid to L-tartaric acid, which was the main component of the hydrolysate.

The production of an acid having four carbon atoms as the main product of the periodate–nitric acid oxidation of the polygalacturonide shows that in this case it is the α-diol groupings at the second and third carbon atoms of the galacturonic acid residues that are oxidized. This is possible with pyranose rings only if the links between the sugars are in the 1–4 position.

Thus, it has been established that the galacturonic acid residues in degraded pectic acid have the pyranose form, the α-configuration, and are placed in the 1–4 positions.

Experimental

The paper chromatography was carried out in the following systems: 1) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); and 2) 1-butanol–acetic acid–water (4:1:5).

Isolation of degraded pectic acid. Ten grams of the polysaccharide of the leaves of Plantago major were dissolved in 100 ml of 5% hydrochloric acid and hydrolyzed in the water bath at 85°–90° C for 4 hr. After hydrolysis for 1 hr, a precipitate deposited. After 4 hr, the hydrolysate was centrifuged off and the residue was washed with 3% hydrochloric acid and then with water and alcohol until the reaction for chloride ion was negative. Then the residue was boiled with 80% alcohol for 1 hr, filtered, washed with absolute alcohol and acetone, and dried in vacuum over phosphorus pentoxide.

This gave a white amorphous powder (4.2 g), sparingly soluble in cold water, and forming a highly opalescent solution in the hot, with $[\alpha]_D$ +280.0° (c 0.5; in 0.1 N caustic soda solution). The equivalent weight of the polygalacturonide by potentiometric titration was 180.

Acid hydrolysis. In the manner described previously [1], 0.1 g of the polygalacturonide was hydrolyzed in 5 ml

of 1 N sulfuric acid in the boiling water bath. Only galacturonic acid was found in the hydrolysate by paper chromatography in systems 1 and 2.

Enzymatic hydrolysis. With heating, 0.1 g of the substance was dissolved in 5 ml of distilled water, and the solution was cooled and treated with 0.03 g of pectinase from the fungus Aspergillus niger. Hydrolysis was carried out at 40° C with constant stirring for 96 hr. Only galacturonic acid and oligogalacturonides were found in the hydrolysate by paper chromatography in system 1.

Acetylation of the polygalacturonide. With stirring, 1 g of the degraded pectic acid was acetylated with acetic anhydride in pyridine at 40° C for 48 hr. The reaction mixture was poured into 60 ml of 3% hydrochloric acid. The precipitate that deposited was washed with alcohol and ether. The substances was dissolved in alcohol and acetone and the part that did not dissolve was separated off. The acetone solution was poured into ether, and the precipitate was filtered off and dried, $[\alpha]_D$ +225.0° (c 0.55; in acetone).

Found, %: $COCH_3$ 33.60, 33.45.

Titration of the polygalacturonide with iodine solution. Samples of the degraded pectic acid (from 0.01 to 0.1 g) were dissolved in 2 ml of water, treated with an equivalent amount of 0.1 N caustic soda solution, and titrated with 0.01 N iodine solution as described by Lacko and Malek [8]. The average consumption of 0.01 N iodine solution was 45 ml per gram of substance.

Periodate–nitric acid oxidation of the polygalacturonides. A. Partial methylation of the carboxy groups of the polyuronide. A suspension of 4 g of the substance in 60 ml of a 3.6% solution of dry hydrogen chloride in absolute methanol was boiled in the water bath with stirring for 8 hr. The solid matter was separated off, washed free from acid with methanol, and dried. The substance obtained (2.5 g) was dissolved in the minimum amount of water and centrifuged. The clear solution was treated with 5 volumes of ethanol, and the precipitate formed was filtered off, washed with alcohol and acetone, and dried in vacuum. This gave 2.0 g of a white powder, $[\alpha]_D$ +207.00° (c 0.58; in water). The equivalent weight by potentiometric titration was 678.

B. Oxidation with periodic acid. A solution in 50 ml of water of 2 g of the polygalacturonides partially substituted at the carboxy groups was treated with 12.5 g of crystalline periodic acid in 70 ml of water. Oxidation was carried out in the dark at 20° C with periodic stirring for 60 hr. The mineral acids were quantitatively eliminated by treating the solution with an approximately threefold excess of AB-17 anion exchanger (HCO_3^-) and the solution was then evaporated in vacuum at 45° C to dryness. This gave 1.6 g of a polyaldehyde in the form oa a light yellow amorphous powder. The polyaldehyde actively reduced Fehling's solution and reacted with aniline phthalate on paper forming a yellow spot; $[\alpha]_D$ −94.5° (c 3.3; in water).

In a sealed tube, 0.1 g of the polyaldehyde was hydrolyzed with 1 N sulfuric acid on a boiling water bath for 15 hr. Only traces of galacturonic acid were found in the neutralized hydrolysate.

C. Oxidation of the polyaldehyde with nitric acid. A solution of 1.5 g of the polyaldehyde in 15 ml of concentrated nitric acid (d = 1.19) was heated on a water bath (85°–90° C) for 3 hr. The nitric acid was evaporated to dryness in a dish and the last traces of it were eliminated by repeated evaporation with water in vacuum. Chromatography in system 2 showed the presence of oxalic and tartaric acids and a mixture of three acidic oligomers. The mixture of oligomers was additionally hydrolyzed with 1 N sulfuric acid at the boiling point for 8 hr. The sulfuric acid was quantitatively neutralized with 0.2 N barium hydroxide solution and the precipitate was filtered off. The filtrate was concentrated to 4 ml, and then 30% caustic potash solution was added to give pH 8.0 using universal indicator solution, after which about 1.5 ml of acetic acid was added. The crystals of potassium hydrogen tartrate that deposited were filtered off, washed with 50% alcohol and acetone, and dried. Yield 0.8 g. After crystallization from hot water the substance had $[\alpha]_D$ −24.8° (c 0.24 in water).

Found, %: K 20.85. Calculated, %: K 20.78.

The potassium hydrogen tartrate was dissolved in water and treated with KU-2 ion exchanger (H^+). The solution of the acid was evaporated to dryness and recrystallized from a mixture of alcohol and ether; mp 169°–170° C, $[\alpha]_D^{20}$ −12.0° (c 2; water), equivalent weight by titration 75.

The IR spectra of the polysaccharides were obtained by I. P. Kovalev (Khar'kov Chemical and Pharmaceutical Scientific–Research Institute) on a UR-10 spectrophotometer, using potassium bromide tablets.

Summary

1. The partial hydrolysis of the polysaccharide of the leaves of Plantago major L. has given degraded pectic acid.

2. A study of the physicochemical properties and IR spectra of the polygalacturonide and the products of its periodate–nitric acid oxidation has shown that the galacturonic acid residues in degraded pectic acid are in the pyranose

form and are linked by $\alpha-0-1-4$ bonds.

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