PECTIN SUBSTANCES OF SEAWEEDS

VI. AN INVESTIGATION OF DEGRADED ZOSTERIN

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In a preceding communication [1] it was stated that in the Smith degradation of zosterin, the pectin of seaweeds of the family Zosteraceae, a fragment is formed which, on hydrolysis, gives galacturonic acid, rhamnose, glycerol, and glycolaldehyde. The rhamnose was separated and strictly identified. Previously [2], in the partial hydrolysis of zosterin with dilute sulfuric acid, we had obtained a galacturonan containing a small amount of monosaccharide identical in behavior on paper chromatograms with rhamnose or apiose. In the present paper we give the results of a study of these fragments.

It was established by gas-liquid chromatography (GLC) and NMR spectroscopy that the fragment obtained by sulfuric acid hydrolysis contained rhamnose. In addition, GLC permitted the detection of rhamnose directly in the hydrolysate from zosterin, although the amount of this monosaccharide was low (about 1%). The specific rotation showed that the rhamnose belonged to the L series.

We called the fragments formed in the Smith degradation of zosterin and on partial hydrolysis rhamnogalacturonans A and B, respectively. They were practically homogeneous, which was shown by gel filtration on Bio-Gels and by chromatography on DEAE-cellulose [3], and also by disk electrophoresis in polyacrylamide gel [4].

The periodate oxidation of rhamnogalacturonan A led to the complete decomposition of the rhamnose residues, while the galacturonic acid was only partially affected. The hydrolysis of the polyalcohol obtained after the reduction of the polyaldehyde with tetrahydroborate formed galacturonic and threonic acids, glycerol, propylene glycol, and glycolaldehyde, which shows the absence of 1,3 bonds and branching in the rhamnose residues in rhamnogalacturonan A. In addition to this, the presence of propylene glycol in the hydrolysate of the polyalcohol showed the attachment of the rhamnose residues by 1,2 bonds. The complete decomposition of the monosaccharide residues in the periodate oxidation of rhamnogalacturonan B led to similar results.

The treatment of rhamnogalacturonan A with pectinase yielded a fragment C containing about 7% of galacturonic acid, in addition to rhamnose, glycerol, and glycolaldehyde. Under the action of pectinase, rhamnogalacturonan B decomposed completely into the component monosaccharides. These results show the linear nature of rhamnogalacturonan B and the presence in rhamnogalacturonan A of branches and of galacturonic acid residues bound directly to the rhamnose. The retention of the rhamnose in the periodate oxidation of zosterin shows the presence of the residues of this monosaccharide at the points of branching of the initial polysaccharide.

The treatment of fragment C with diazomethane with simultaneous tetrahydroborate reduction and subsequent methylation by Purdie's method [5] gave a completely methylated compound in the hydrolysate of which we found 3,4-di-O-methyl-L-rhamnose. The same monosaccharide was formed by the hydrolysis of completely methylated and reduced rhamnogalacturonan B. Consequently, in the polyuronide chains of the polysaccharides under consideration the rhamnose residues are linked by 1,2 bonds. A similar situation has been reported many times previously in studies of the structure of pectin substances [6, 7].

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EXPERIMENTAL

The samples of polysaccharides were hydrolyzed with 2 N sulfuric acid at 100°C for 6 h with subsequent neutralization by barium carbonate and by treatment with Amberlite IR-120 (H^+). The solutions were concentrated in vacuum at 35-45°C.

The methanolysis of the methylated polysaccharides was performed with a mixture of methanol and perchloric acid (10 : 1) with heating in a sealed tube at 95°C for 8-10 h. After treatment with Dowex-1 (HCO_3^-) and evaporation, the mixture of methyl glycosides was used for GLC or was hydrolyzed by being heated with 2 N sulfuric acid to the corresponding methylated sugars, which were studied by paper chromatography (PC) and thin-layer chromatography (TLC).

For PC we used Whatman 3-MM and Filtrak FN-15 papers. The monosaccharides and the products of periodate oxidation were separated in the following solvent systems: 1) butan-1-ol–ethanol–water (31 : 11 : 9) and 2) ethyl acetate–pyridine–water–acetic acid (5 : 5 : 3 : 1); and the methylated sugars were separated in methyl ethyl ketone saturated with aqueous ammonia. For TLC we used KSK silica gel impregnated with sodium dihydrogen phosphate [8]. The methylated sugars were separated in the chloroform–methanol (9 : 1) system. The spots on the chromatograms were revealed with aniline phthalate, Bonner's reagent [9], or an alkaline solution of silver oxide.

GLC conditions: Tswett-2 chromatograph (OKBA [Experimental Design Bureau for Automation], Dzerzhinsk) with a flame-ionization detector. Columns: 1) 10% of SE-30 on Chromosorb W washed with acid and silanized, and 2) 10% of butanediol succinate on the same support. Column 1 was used for the separation of the products of periodate oxidation in the form of the trimethylsilyl derivatives [10], and column 2 for the monosaccharides in the form of the acetates of the aldononitriles [11].

Gel filtration on Bio-Gels and chromatography on DEAE-cellulose were performed as describedpreviously [3]. Electrophoresis in polyacrylamide gel was carried out as described by Pavlenko and Ovodov [4]. The uronic acid content was determined by decarboxylation [12] and molecular weights by the end-group method [13]. The IR spectra were taken on a UR-20 spectrophotometer and the NMR spectra on a ZKR-60 radiospectrometer.

Rhamnogalacturonan A. Its preparation was described in the preceding paper [1]. For the present investigation a sample was used with $[\alpha]_D^{20} + 73°$ (in water), mol. wt. 13,000, containing 34% of galacturonic acid.

Rhamnogalacturonan B. Zosterin (10 g) was hydrolyzed with 1% sulfuric acid (one liter) at 100°C for 8 h. The precipitate that had deposited was separated off by centrifuging, washed with water, and dried in vacuum. Rhamnogalacturonan B was obtained with a yield of about 2 g, $[\alpha]_D^{20} + 237°$ (in water), mol. wt. 16,000, containing 75% of galacturonic acid. This sample (1 g) was subjected to complete hydrolysis with 2 N sulfuric acid as described above. The hydrolysate was treated with Dowex-1 until the galacturonic acid had been removed completely (monitored by PC). The solution was evaporated to a syrup. L-Rhamnose (20-30 mg), $[\alpha]_D^{20} + 8°$ (in water), was isolated. The NMR spectrum of the sample was identical with that of the authentic material. The corresponding acetate of the aldononitrile had the same retention time as authentic rhamnononitrile acetate.

Periodate Oxidation. a) Rhamnogalacturonan A (100 mg) was oxidized with 0.015 M sodium metaperiodate at room temperature in the dark at pH 3.6. Oxidation was complete after 8 h, the consumption of periodate corresponding to 1 mole per anhydro unit. The solution was dialyzed, and the polyaldehyde was reduced with an excess of sodium tetrahydroborate. After the usual working up, a polyalcohol was obtained which was hydrolyzed completely. The hydrolysate was shown by GLC to contain glycolaldehyde, propylene glycol, and glycerol. PC confirmed these results and showed, in addition, the presence of galacturonic and threonic acids in the hydrolysate.

b) Rhamnogalacturonan B (90 mg) was oxidized similarly. Oxidation was complete in 48 h, the consumption of periodate amounting to 0.9 mole per anhydro unit. The polyaldehyde was reduced to the polyalcohol and the latter was hydrolyzed in the usual way. Only traces of galacturonic acid were found in the hydrolysate, and rhamnose was completely absent.

Treatment of Rhamnogalacturonan A with Pectinase. A solution of 70 mg of the polysaccharide in 6 ml of water was treated with pectinase (12 mg) at room temperature with simultaneous dialysis for 24 h.

Fragment C was isolated in the form of a syrup with a yield of about 50 mg, $[\alpha]_D^{20} + 38°$ (in water), containing 6.8% of galacturonic acid. The hydrolysate was shown by PC to contain galacturonic acid, rhamnose, glycerol, and glycolaldehyde.

Methylation. a) A suspension of 60 mg of fragment C in an ethereal solution of diazomethane was allowed to stand for 48 h. The product obtained was reduced with sodium tetrahydroborate (60 mg) in water (3 ml). The reduced and partially methylated compound was methylated by Purdie's method [5] in methanol (0.1 ml) with methyl iodide (2 ml) in the presence of silver oxide (0.3 g). The reaction mixture was stirred at 70°C for 24 h with the periodic addition of small portions of methyl iodide and silver oxide. The filtrate was evaporated. This gave 50 mg of completely methylated compound. (The IR spectrum contained no hydroxyl absorption band.) By comparison with authentic samples using GLC, the methanolysate was shown to contain methyl glycosides of 3,4-di-O-methyl-L-rhamnose. PC and TLC confirmed the presence of 3,4-di-O-methyl-L-rhamnose in the hydrolysate (spots revealed with Bonner's reagent).

b) Rhamnogalacturonan B (500 mg) was treated similarly. This gave about 500 mg of reduced, completely methylated product in a methanolysate of which GLC showed the presence of methyl glycosides of 3,4-di-O-methyl-L-rhamnose, while a hydrolysate contained the same methylated sugar (TLC).

SUMMARY

It has been shown that the polyuronide chain of zosterin, the pectin of seaweeds of the family Zosteraceae, contains several L-rhamnose residues connected by 1,2 linkages and present at the points of branching of the carbohydrate chain.

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