PECTIN SUBSTANCES OF SEAWEEDS

XI. AN INVESTIGATION OF SOME PRODUCTS OF THE ENZYMATIC HYDROLYSIS OF ZOSTERIN

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We have shown previously [1, Communication X] that the treatment of zosterin with enzyme preparations from Eulota maackii and Helix pomatia forms fragments of the polysaccharide with high and low molecular weights. From the results of a preliminary study of the low-molecular-weight fraction it can be seen that it includes neutral and acid oligosaccharides, galacturonic acid, and neutral monosaccharides. This mixture was separated in two stages, preliminary separation being performed on columns, with subsequent preparative paper chromatography. Twelve individual neutral oligosaccharides forming three series in agreement with the enzyme preparations used for their production (Table 1) were isolated.

The structures of these oligosaccharides were established on the basis of the results of acid and alkaline hydrolysis, periodate oxidation, exhaustive methylation, and color reactions with triphenyltetrazolium chloride [2] and the "diphenylamine-aniline" reagent [3].

The series of oligosaccharides formed in the treatment of zosterin with various enzyme separations differ little from one another (see Table 1). They form the following sequence of interconnected structures:

Xyl 1→3 Xyl; Xyl 1→3 Xyl 1→3 Xyl; Xyl 1→(3 Xyl)$_2$→3 Xyl. (1)

Xyl 1→3 Xyl 1→3 Rha; Xyl 1→3 Rha 1→2 Rha, (2)

Rha 1→2 Rha. (3)

It has been established previously [4] that in zosterin the rhamnopyranose residues connect fragments of a glacturonan with one another and are the points of branching of the carbohydrate chain of the polysaccharide. The nature of the side chains attached to the rhamnose residues has remained undetermined. On considering the oligosaccharide structures given above, it may be said that carbohydrate chains consisting of 1,3-bound xylopyranose residues are attached to the rhamnose residues by a 1,3 bond. These chains are small and include from 1 to 4 xylose residues.

Thus, the following fragment of a carbohydrate chain forms part of the zosterin molecule:

galacturonan→2 Rha 1→2 Rha 1—galacturonan (4)

Xyl 1→(3 Xyl 1)$_n^{3}$

n=0,1,2,3.

EXPERIMENTAL

The partition paper chromatography (PC) of the monosaccharides and oligosaccharides (hydrolyzates and enzymolyzates) was performed on Filtrak FN-12, 2, and 3 papers, and the chromatography of the methylated sugars on Filtrak FN-15 paper. Type KSK silica gel (270 mesh) was used for thin-layered chromatography. The following solvent systems were used (by volume): 1) butan-1-ol—pyridine—water (6 : 4 : 3), 2) methyl acetate—pyridine—acetic acid—water (5 : 5 : 1 : 3), 3) butan-1-ol—ethanol—water (31 : 11 : 9), 4) benzene—ethyl acetate (7 : 3), 5) benzene—ethyl acetate (5 : 2), 6) chloroform—acetone (3 : 1), 7) chloroform—acetone (5 : 1), 8) chloroform—methanol (9.5 : 0.5), and 9) chloroform—methanol (9 : 1).

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TABLE 1. Oligosaccharides Isolated in the Enzymatic Hydrolysis of Zosterin

Enzyme	Oligosaccharide	R_{Gal}	Deg. of polymerization	Monosaccharide composition	Reducing end	Alkaline treatment	Products of the methanolysis of the methylated oligosaccharides	Structures of the oligosaccharides
E. maackii	A_1	0,93	2	Xylose	Xylose	Xylose	2, 3, 4-tri-, 2, 4-di-O-Me-Xyl	Xyl 1 → 3 Xyl
	A_2	0,67	2	Rhamnose	Rhamnose	—	2, 3, 4-tri-, 3, 4-di-O-Me-Rha	Rha 1 → 2 Rha
	A_3	0,55	3	Rhamnose + xylose (1:2)	-	—	2, 3, 4-tri- / 2, 4-di-O-Me-Xyl	Xyl 1 → 3 Xyl 1 → 3 Rha
	A_4	0,46	3	Rhamnose + xylose (2:1)	-	—	2, 3, 4-tri-O-Me-Xyl; {2, 4-di- / 3, 4-di-O-Me-Rha	Xyl 1 → 3 Rha 1 → 2 Rha
H. pomatia Commercial	B_1	0,94	2	Xylose	Xylose	Xylose	2, 3, 4-tri-, 2, 4-di-O-Me-Xyl	Xyl 1 → 3 Xyl
	B_2	0,55	3	"	-	-	2, 3, 4-tri-, 2, 4-di-O-ME-Xyl	Xyl 1 → 3 Xyl 1 → 3 Xyl
	B_3	0,48	3	Rhamnose + xylose (2:1)	Rhamnose	-	2, 3, 4-tri-O-Me-Xyl; {2, 4-di- / 3, 4-di-O-Me-Rha	Xyl 1 → 3 Rha 1 → 2Rha
	B_4	0,41	Not det.	Xylose + arabinose (2:1)	Not deter.	Not deter.	Methylation not performed	
H. pomatia Moldavian	C_1	0,93	2	Xylose	Xylose	Xylose	2, 3, 4-tri-, 2, 4-di-O-Me-Xyl	Xyl 1 → 3 Xyl
	C_2	0,56	3	"	-	-	2, 3, 4-tri-, 2, 4-di-O-Me-Xyl	Xyl 1 → 3 Xyl 1 → 3 Xyl
	C_3	0,48	3	Rhamnose + xylose (2:1)	Rhamnose	-	2, 3, 4-tri-O-Me-Xyl {2, 4-di- / 3, 4-di-O-Me-Rha	Xyl 1 → 3 Rha 1 → 2Rha
	C_4	0,36	4	Xylose	Xylose	Xylose	2, 3, 4-tri- 2, 4-di-O-Me-Xyl	Xyl 1 → (3 Xyl 1)$_2$→3Xyl

To reveal the spots on the chromatograms we used a solution of aniline hydrogen phthalate in water-saturated butanol at 110°C, an alkaline solution of silver nitrate [5], 10% H_2SO_4 in methanol, and the Bonner reagent [6].

The oligosaccharides were separated preparatively on Filtrak FN-18 and Whatman No. 1 papers in system 1. To determine their monosaccharide compositions, the oligosaccharides were hydrolyzed with 2 N sulfuric acid at 95°C for 3 h. The hydrolyzates were deionized, evaporated, and analyzed by PC in systems 1 and 2, and also by gas-liquid chromatography (GLC), performed on a "Tsvet-6" chromatograph [flame-ionization detector; column a, 3% of HI-EFF-8-BP on Gas-Chrom Q (100-120 mesh)] and a Pye Unicam chromatograph [column b, 5% of neopentyl glycol succinate on Chromosorb W (60-80 mesh)].

For analysis, the monosaccharides were converted into the corresponding aldononitrile acetates [7], and GLC analysis was performed with the programming of the temperature from 175 to 225°C at the rate of 4°C/min on a. The amounts of sugars in the oligosaccharides were determined by using the aldononitrile acetates and taking into account the appropriate correction factors of 1.5 for xylose and 1.0 for rhamnose. The methylated methyl glycosides, and the acetates of the methyl glycosides were analyzed on column b with a programming of the temperature from 125 to 225°C at 5°C/min.

In the determination of the reducing ends, the oligosaccharides (1-2 mg) were treated in aqueous solution with sodium tetrahydroborate (2 mg), deionized, and hydrolyzed; the hydrolyzates were analyzed by PC in system 1, and by GLC. The degree of polymerization of the oligosaccharides was determined by a method described previously [8].

The enzymolysis of the zosterin was performed with enzyme preparations from E. maackii and H. pomatia (commercial and Moldavian) as described previously [1]. A mixture of oligosaccharides was formed (series A, B, and C, respectively).

Separation of the Enzymolyzates of the Oligosaccharides. A. Chromatography on Coal-Celite. A mixture of low-molecular-weight components of an enzymolyzate (0.8-1.0 g) was deposited on a column containing coal-Celite (60 g; 1:1 by weight). The substance was eluted from the column with water, and the combined eluates were evaporated. The yield of a mixture of neutral monosaccharides was 38-45 mg. The elution was performed with increasing concentrations

of aqueous ethanol (3, 5, 7 ... 30%). This gave a series of fractions containing galacturonic acid and a mixture of oligosaccharides.

B. Ion-exchange Chromatography. A mixture of low-molecular-weight components of an enzymolyzate (300-325 mg) was deposited on a column (0.8 × 16 cm) containing Dowex 1 × 2 resin (acetate form). The substances were eluted from the column with water, and the combined eluates were evaporated. The residue consisted of a mixture of neutral mono- and oligosaccharides; yield 112 mg. The acidic components were eluted with 2 M CH_3COOH. Yield 153 mg.

C. Precipitation with Barium Methoxide. A mixture of low-molecular-weight components of an enzymolyzate (300 mg) was dissolved in 10 ml of water, and, with stirring, a 0.1 N solution of barium methoxide was added (until the deposition of precipitates ceased). The mixture was left at 4°C for 2 h, and then the precipitate that had deposited was separated off by centrifuging and was carefully washed with ethanol. The supernatant and the wash-waters were combined, deionized and distilled. A mixture of neutral mono- and oligosaccharides was obtained in a yield of 133 mg.

The residue was dissolved in water, and the solution was treated with Amberlite IR-120 (H^+ form) and evaporated. Yield 158 mg (mixture of galacturonic acid and a series of acid oligosaccharides).

Separation of the Oligosaccharides. The oligosaccharide mixtures were separated by preparative chromatography on paper in system 1. The oligosaccharides were eluted from the paper strip with water, and the eluates were concentrated. The individuality of theoligosaccharides was checked by chromatographing them in system 2. In a number of cases, the fractions contained mixtures of oligosaccharides with very close chromatographic mobilities. To purify the oligosaccharides, it was necessary to repeat the operations several times. The characteristics of the individual oligosaccharides are given in Table 1.

Periodate Oxidation. A 1- to 2-mg sample of oligosaccharide previously reduced with sodium tetrahydroborate was oxidized with a 0.03 M solution of sodium metaperiodate at room temperature in the dark (pH 3.6) for 24 h. After the usual working up, a compound was obtained in a hydrolyzate of which the unchanged monosaccharides were identified by PC and GLC.

Alkaline Hydrolysis. An oligosaccharide (1-2 mg) was treated with 0.005 N NaOH (2 ml) at 74°C for 10 min, and the mixture was then neutralized with Amberlite IR-120 (H^+ form), evaporated, and chromatographed.

CONCLUSIONS

The treatment of zosterin, a seaweed pectin, with enzyme preparations from Eulota maackii and Helix pomatia yielded 12 neutral oligosaccharides, the structures of which have been determined. It has been shown that side chains consisting of 1,3-linked xylopyranose residues are attached to rhamnose residues connecting galacturonan fragments to one another.

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