PECTIN SUBSTANCES OF SEAWEEDS

VII. ACETOLYSIS OF ZOSTERIN

V. I. Shibaeva, R. G. Ovodova,
and Yu. S. Ovodov

UDC 547.917+541.6

It is known that the acetolysis of a carbohydrate chain leads to results different from those of partial acid hydrolysis [1]. In the acetolysis of zosterin, first apiose is split out and then xylose, galactose, arabinose, and mono-O-methylxylose. It is interesting to note that the galactose is split out practically completely and galacturonic acid is found in the mixture only in traces. In addition to monosaccharides, at least three zosterin fragments differing in molecular weight and qualitative monosaccharide composition are formed; they are readily separated by their different solubilities in water and aqueous ethanol.

The low-molecular-weight fragment, soluble in aqueous ethanol, gives galacturonic acid, apiose, and xylose on complete hydrolysis. The fragment precipitated from aqueous solutions by ethanol contains arabinose in addition to the monosaccharides mentioned. Finally, the polysaccharide insoluble in the reaction mixture during the acetolytic process and sparingly soluble in water after deacetylation includes rhamnose in addition to the monosaccharides named above. Its mean molecular weight of 13,000 permits the assumption of the cleavage, during acetolysis, of pseudoglycuronosidic bonds between residues of rhamnose and galacturonic acid in the polyuronide chain of zosterin.

On partial acid hydrolysis, this heteropolysaccharide forms a galacturonan containing traces of rhamnose and similar to the rhamnogalacturonan B described in the preceding paper [2]. The heteropolysaccharide is eluted by aqueous alkali from DEAE-cellulose in the form of a single peak, which shows the acid nature of the polysaccharide. The elution curve of gel filtration on Bio-Gel P-20 shows two peaks: the fractions corresponding to the two peaks have the same monosaccharide composition. These results show the polydisperse nature of the heteropolysaccharide, which was also confirmed by the results of electrophoresis in polyacrylamide gel: the polysaccharide gave two strong bands with a series of weak bands between them. The autohydrolysis of the heteropolysaccharide splits off apiose and xylose, which shows their terminal nature. The qualitative monosaccharide compositions of the final and initial polysaccharides were identical.

On treatment with pectinase, there was a considerable cleavage of the polyuronide chain with the formation of free galacturonic acid. The hydrolysate of the fragment obtained was shown to contain apiose, xylose, arabinose, and galacturonic acid. Consequently, only part of the galacturonan chain of the heteropolysaccharide has branching in the form of side chains consisting of apiose, xylose, and arabinose residues. The periodate oxidation of the heteropolysaccharide with subsequent reduction by tetrahydroborate formed a polyalcohol in the hydrolysate of which galacturonic and threonic acids, traces of residual monosaccharides, and also glycolaldehyde, ethylene glycol, glycerol, and glyceraldehyde were found.

These results permit the conclusion that the side chains are linear, which is in harmony with the results obtained previously [3].

EXPERIMENTAL

The general experimental conditions are given in the preceding paper [2].

Institute of Biologically Active Substances, Far Eastern Scientific Center, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 249-252, May-June, 1971. Original article submitted February 5, 1971.

© 1973 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for $15.00.

Acetolysis of Zosterin. With stirring, 5 g of freeze-dried zosterin was added to a mixture of acetic anhydride (480 ml) and acetic acid (320 ml). Then 5 ml of 72% perchloric acid was added to the reaction mixture and it was left at room temperature (with stirring) for 4 days.

The substance insoluble in the acetolysis mixture was separated off by centrifuging, deacetylated with 5% sodium methoxide in methanol, and reprecipitated with ethanol from aqueous solution. The residue was dissolved in water, dialyzed, and freeze-dried. This gave a heteropolysaccharide (yield 1.93 g) with $[\alpha]_D^{20}+248°$ (in water), mol. wt. 13,000, containing 58% of galacturonic acid and 13.2% of acetyl groups.

The supernatant liquid was poured into a cooled aqueous solution of sodium carbonate and was extracted with chloroform (4 × 50 ml). The chloroform extracts were washed with sodium carbonate solution and then with water to neutrality and were dried over calcined potassium carbonate. After filtration, the solution was evaporated, and the resulting syrup was treated with 2-4 ml of 0.1 N sodium methoxide in absolute methanol, and the mixture was left at +5°C for 6 h. Then the mixture was treated with Amberlite IR-120 (H^+) and evaporated in vacuum. This gave a mixture of monosaccharides in the form of a syrup with a yield of 0.56 g. By means of PC, apiose, xylose, arabinose, galactose, mono-O-methylxylose, and only traces of galacturonic acid were detected.

The solution remaining after chloroform extraction was treated with Amberlite IR-120 (H^+), dialyzed, and evaporated. The product obtained was deacetylated as described above. The polysaccharides were dissolved in water and fractionated with ethanol. The polysaccharide that precipitated was hydrolyzed with 2 N sulfuric acid for 6 h. By PC, the hydrolysate was shown to contain galacturonic acid and apiose and traces of xylose and arabinose. The yield of polysaccharide was 1.67 g.

The fragment soluble in aqueous ethanol was dialyzed against distilled water and freeze-dried. Yield 0.9 g. On hydrolysis it gave galacturonic acid, apiose, and traces of xylose (PC).

Partial Hydrolysis of the Heteropolysaccharide. A mixture of 0.1 g of the substance and 10% sulfuric acid in a sealed tube was heated in the boiling water bath for 3 h. The precipitate that deposited was separated off, washed with water, and dried. The yield of rhamnogalacturonan was about 60 mg. On hydrolysis, this product gave galacturonic acid and traces of rhamnose.

Autohydrolysis of the Heteropolysaccharide. A solution of 0.15 g of the substance in 50 ml of water was heated in the boiling water bath for 3 h. The solution was evaporated to small bulk and poured into ethanol. The precipitate that deposited was separated off, washed with ethanol, and dried. This gave a fragment which decomposed on hydrolysis into the same monosaccharides as the initial heteropolysaccharide. The ethanolic filtrate was evaporated and chromatographed. Apiose and xylose were detected.

Enzymatic Hydrolysis of the Heteropolysaccharide. A solution of 0.1 g of the substance in 10 ml of water was treated with 4 mg of pectinase at 37°C for two days. The solution was evaporated to small bulk and poured into ethanol. The precipitate was separated off, washed with ethanol, and dried. This gave a fragment with a yield of about 45 mg, $[\alpha]_D^{20}+216°$, containing 52% of galacturonic acid. PC of the hydrolysate showed the presence in it of apiose, xylose, arabinose, and galacturonic acid. The alcoholic filtrate was shown on chromatograms to contain galacturonic acid and only traces of apiose and xylose.

Periodate Oxidation of the Heteropolysaccharide. In the dark at room temperature and pH 5.4, 50 mg of the substance was oxidized with a 0.015 M solution of sodium metaperiodate (6 ml). Oxidation was complete after 8 h. The consumption of periodate was 0.76 mole per anhydro unit. After the usual working up, a polyaldehyde was isolated which was reduced with potassium tetrahydroborate for 5 h. The excess of tetrahydroborate was decomposed with acetic acid, and the solution was dialyzed and evaporated several times with methanol. This yielded a polyalcohol giving, on hydrolysis, galacturonic and threonic acids (PC), traces of rhamnose, arabinose, and xylose (GLC of the acetates of the aldononitriles and PC), glycerol, glyceraldehyde, ethylene glycol, and glycolaldehyde (GLC of the acetates of the polyols and of the aldononitriles and PC).

SUMMARY

The acetolysis of zosterin has given a fragment based upon a linear rhamnogalacturonan chain with side chains consisting of xylose, arabinose, and apiose residues attached to it.

LITERATURE CITED

1. K. Matsuda, U. Watanabe, and K. Aso, Tohoku J. Agr. Res., 12, 352 (1961).
2. L. V. Mikheiskaya, R. G. Ovodova, and Yu. S. Ovodov, Khim. Prirodn. Soedin., 7, 246 (1971) [in this issue].
3. Yu. S. Ovodov, R. G. Ovodova, O. D. Bondarenko, and I. N. Krasikova, Carbohydrate Res., 18, 311 (1971).