PECTIN SUBSTANCES OF SEAWEEDS

IX.* A STUDY OF DEGRADED ZOSTERIN (III)

L. V. Mikheiskaya, R. G. Ovodova, and Yu. S. Ovodov

We have shown [1] that in the Smith degradation of zosterin, a seaweed pectin, even in the first stage of oxidation there is complete degradation of the D-apiose and the O-methyl-D-xylose residues with the formation of fragments of molecules in accordance with the following scheme:

\[
\text{Zosterin} \xrightarrow{\text{IO}_4^-} \text{Polyaldehyde I} \xrightarrow{\text{BH}_4^-} \text{Polyalcohol I} \xrightarrow{\text{Mild hydrolysis}} \text{Degraded zosterin (I)} \xrightarrow{\text{KU-2 (H}^+\text{)}} \text{60°C/2 h} \xrightarrow{\text{Pectinase}} \text{Rhamnogalacturanan (II)} \xrightarrow{\text{Partial hydrolysis}} \text{Polyaldehyde II} \xrightarrow{\text{BH}_4^-} \text{Polyalcohol II} \xrightarrow{\text{H}^+} \text{Degraded zosterin (II)}
\]

The structures of some of the fragments obtained have been established previously [2].

The present paper gives the results of a study of the structure of degraded zosterin (III), formed by the mild acid hydrolysis of degraded zosterin (I). After fractionation and purification, we isolated a homogeneous sample giving one peak in gel filtration on Bio-Gels and in chromatography on DEAE-cellulose. The molecular weight of the compound obtained (about 17,000) shows that a considerable part of the zosterin molecule is resistant to periodate oxidation.

The complete acid hydrolysis of degraded zosterin (III) formed D-galacturonic acid (about 60%), and also D-galactose, D-xylose, and L-rhamnose in an approximate molar ratio of 2 : 3 : 1.

The periodate oxidation of the heteroglycan obtained with subsequent reduction of the polyaldehyde gave the corresponding polyalcohol III, a considerable decomposition of the galacturonic acid residues being observed, and the ratio of galactose, xylose, and rhamnose being 3 : 6 : 1. In the polyalcohol III, the residues of the neutral monosaccharides remained unchanged, which shows the presence of branchings of 1,3-glycosidic links between them. The relatively high proportion of xylose residues in the polyalcohol shows the


© 1975 Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $15.00.
resistance to periodate oxidation of this monosaccharide in degraded zosterin (III). The decomposition of the galactose residues is probably connected with the presence in the degraded zosterin (III) of linear sections of a galactan chain having no 1,3-glycosidic linkages.

These results are confirmed by those of the exhaustive methylation of degraded zosterin (III), which was achieved by the repeated treatment of the substance with diazomethane and subsequent methylation by Purdie's method [3]. The hydrolyzate of the completely methylated heteroglycan (the IR spectrum lacked the absorption band of a free hydroxyl) was shown by gas-liquid chromatography (GLC) to contain 2,3,4,6-tetra-, 2,3,6-tri-, and 2,6-di-O-methyl-D-galactoses and also 2,3,4-tri- and 2,4-di-O-methyl-D-xyloses.

In view of the results obtained previously on the presence in the zosterin molecule of sections consisting solely of galactose residues [4] or solely of xylose residues [5], the following schematic structure may be proposed for degraded zosterin (III):

![Schematic structure](image)

where GalA represents galacturonic acid, Gal represents galactose, Xyl represents xylose, Rha represents rhamnose, and X represents a carbohydrate chain of undetermined monosaccharide composition.

**EXPERIMENTAL**

The samples of the polysaccharides were hydrolyzed with 2 N sulfuric acid at 100°C for 6 h, with subsequent neutralization using barium carbonate and treatment with KU-2 (H⁺) cation-exchange resin. The solutions were concentrated in vacuum at 35-40°C.

Methanolysis of the methylated polysaccharides was performed with a mixture of methanol and perchloric acid (10:1) with heating in sealed tubes at 95°C for 10-12 h. After treatment with Dowex-1 (HCO₃⁻) and evaporation, the mixture of methyl glycosides was used for GLC or was hydrolyzed with 2 N sulfuric acid to the corresponding methylated carbohydrates, which were used for thin-layer chromatography (TLC). Paper chromatography (PC) was performed on Filtrak FN-15 and FN-3 papers with the following systems of solvents: 1) ethyl acetate–pyridine–water–acetic acid (5 : 5 : 3 : 1), 2) butan-1-ol–ethanol–water (31 : 11 : 95), and 3) butan-1-ol–pyridine–water (6 : 4 : 3).

TLC was performed on KSK silica gel impregnated with sodium dihydrogen phosphate. The methylated sugars were run in the chloroform–methanol (9 : 1) system. The spots on the chromatograms were revealed with aniline phthalate and an alkaline solution of silver oxide.

GLC conditions: Tswett-2 chromatograph [OKBA (Experimental Design Bureau for Automation), Dzerzhinsk] with a flame-ionization detector using a column containing 8% of butanediol succinate on Chromosorb W that had been acid-washed and silylated.

For GLC, the monosaccharides were previously converted into the acetates of the corresponding aldonitriles [6] or of the methyl glycosides. Quantitative determination was performed by using the acetates of the aldonitriles and the appropriate correction factors of 1.3 for galactose, 1.5 for xylose, and 1.0 for rhamnose. Gel filtration on Bio-Gels and chromatography on DEAE-cellulose were performed as described previously [7]. The amounts of uronic acids were determined by decarboxylation [8] and molecular weights by the end-group method [9]; the IR spectra were taken on a UR-20 spectrophotometer.

Preparation of the Degraded Zosterin (III). Degraded zosterin (D) (1.3 g) was dissolved in 65 ml of water (60°C), KU-2 [H⁺] cation-exchange resin was added to bring the pH to 1.8, and the mixture was heated at 60°C for 2 h. After cooling, the resin was filtered off and the hydrolyzate was neutralized with aqueous ammonia. Then it was evaporated, and the degraded zosterin (III) was precipitated with ethanol and separated by centrifuging. This gave a white powder (yield 0.82 g, galacturonic acid content 60%, mol. wt. 17,000). Monosaccharide composition: D-galacturonic acid, D-galactose, D-xylose, and L-rhamnose (determined by PC and GLC).

Periodate Oxidation of (III). The degraded zosterin (III) (0.6 g) was oxidized with a 0.03 M solution of sodium metaperiodate at room temperature in the dark (pH 3.6). Oxidation was complete after 25 h, the consumption of periodate amounting to 0.89 mole per anhydro unit. After the usual working up, the polyalcohol (III) was obtained, and a hydrolyzate of this was shown by GLC to contain galactose, xylose, and rhamnose and by PC to contain galacturonic acid, as well as the monosaccharides mentioned.