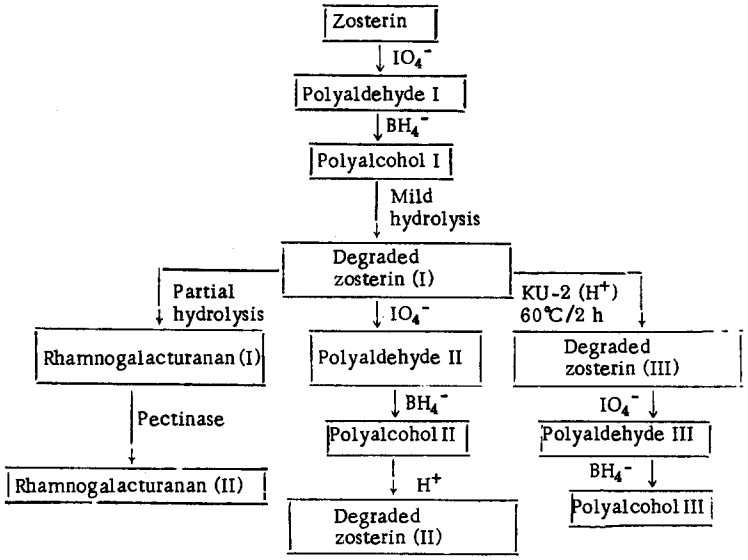
PECTIN SUBSTANCES OF SEAWEEDS

IX.* A STUDY OF DEGRADED ZOSTERIN (III)

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

UDC 547.917 + 541.6

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:



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

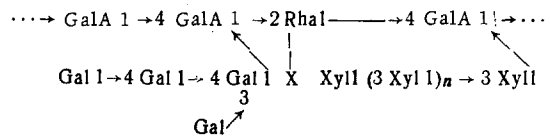
*For Communication VIII, see O. D. Bondarenko and Yu. S. Ovodov, Khim. Prirodn. Soedin., 385 (1972).

Institute of Biologically Active Substances, Far-Eastern Scientific Center, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 3-6, January-February, 1973. Original article submitted April 25, 1972.

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):



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 (HCO_3^-) and evaporation, the mixture of methyl glycosides was used for GLC or was hydrolyzed with 2 N sulfuric acid to the corresponding methylated sugars, 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 (31:11:9), 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), Dzherzhinsk] with a flame-ionization detector using a column containing 8% of butanediol succinate on Chromosorb W that had been acid-washed and silanized.

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 (I) (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.

Methylation. A suspension of 1.3 g of degraded zosterin (III) in an ethereal solution of diazomethane was kept in the refrigerator for 10 days. The resulting product was methylated by Purdie's method [3] in methanol (10 ml) with methyl iodide in the presence of silver oxide (10 g). The reaction mixture was stirred at 40°C with the periodic addition of new portions of methyl iodide and silver oxide. The methylated product was extracted with chloroform, giving 0.47 g of the fully methylated compound (the IR spectrum lacked the absorption band of a hydroxyl).

SUMMARY

The composition of degraded zosterin (III) with mol. wt. ≈ 17,000 has been studied and a probable schematic structure has been proposed for it.

LITERATURE CITED

1. Yu. S. Obodov, L. V. Mikheiskaya (Mikheyskaya), R. G. Ovodova, and I. N. Krasikova, Carbohyd. Res., 18, 319 (1971).
2. L. V. Mikheiskaya, R. G. Ovodova, and Yu. S. Ovodov, Khim. Prirodn. Soedin., 246 (1971).
3. T. Purdie and J. C. Irvine, J. Chem. Soc., 83, 1021 (1903).
4. V. I. Shibaeva, L. A. Elyakova, and Yu. S. Ovodov, Comp. Biochem. Physiol., 36, 183 (1970).
5. Yu. S. Ovodov, R. G. Ovodova, O. D. Bondarenko, and I. N. Krasikova, Carbohyd. Res., 18, 311 (1971).
6. V. M. Easterwood and B. J. L. Huff, Svensk Papperstidn., 72, 768 (1969).
7. Yu. S. Ovodov and M. N. Adamenko, Khim. Prirodn. Soedin., 203 (1969).
8. D. M. W. Anderson, S. Garbutt, and S. S. H. Zaidi, Anal. Chim. Acta, 29, 39 (1963).
9. M. Unrau and F. Smith, Chem. Ind. (London), 330 (1957).