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Note

Further structural studies of zosterine*

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Zosterine has[1] a backbone consisting mainly of α-(1→4)-linked D-galacturonic acid residues. Some (1→2)-linked L-rhamnose residues are also present in the backbone[2]. About a quarter of the zosterine molecule is an apiogalacturonan containing D-apiose residues attached to the galacturonan by (1→2)- and (1→3)-linkages[3]. Other, outer chains of zosterine consist of D-xylose, D-galactose, L-arabinose, and 2-*O*-methyl-D-xylose residues[4] attached to the galacturonic acid residues[3] and the rhamnose residues[5] of the backbone.

Acetolysis[6] and enzymic digestion[7] of zosterine afforded a number of oligosaccharides and high-molecular fragments, and we now report on the latter products and on the methylation of zosterine.

Acetolysis[6] of zosterine gave a homogeneous heteropolysaccharide (HPS-A), $[\alpha]_D$ +248° (water), $\bar{M}_n$ 13,000. The product of high molecular weight obtained when zosterine was digested with an enzyme preparation from *Eulota maackii*[7] was subjected to autohydrolysis to furnish a homogeneous heteropolysaccharide HPS-B, $[\alpha]_D$ +77° (water), $\bar{M}_n$ 16,000. Acid hydrolysis of HPS-A and -B afforded D-galacturonic acid, D-xylose, L-rhamnose, D-apiose, and a trace of L-arabinose. Partial hydrolysis of HPS-A and -B with acid gave rhamnogalacturonans A and B, respectively, periodate oxidation of which caused virtually complete cleavage of the sugar residues. For methylation studies, the rhamnogalacturonans were reduced with sodium borohydride to the corresponding rhamnogalactans, which were then permethylated and hydrolysed to furnish 2,3,6-tri-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-L-rhamnose.

Smith degradation of HPS-A and -B resulted in substantial oxidation of the neutral sugar residues. Hydrolysis of the resulting polyalcohols yielded xylose and rhamnose in addition to galacturonic acid.

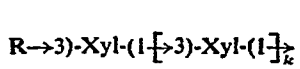
HPS-A and -B were treated with diazomethane, and then permethylated and hydrolysed to afford (after removal of galacturonic acid derivatives), in each case, 2,4-di- and 2-*O*-methyl-D-xylose, 3,4-di- and 4-*O*-methyl-L-rhamnose as the main

*Pectic Substances of *Zosteraceae*: Part XII.

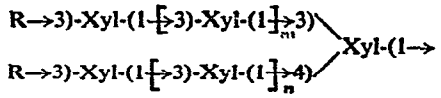
components, small proportions of 2,3,4-tri-*O*-methyl-D-apiose and 2,3,4-tri-*O*-methyl-D-xylose, and a trace of 2,3,4-tri-*O*-methyl-L-arabinose.

Thus, HPS-A and -B appear to possess closely similar structures. The backbone of the heteropolysaccharides is a linear rhamnogalacturonan containing (1→4)-linked D-galacturonic acid residues and a few rhamnose residues involved with (1→2)-linkages. The complete cleavage of the apiose and arabinose residues on Smith degradation of HPS-A and -B indicates the absence of branching and (1→3)-linkages in the side chains comprising these residues. Methylation analysis of HPS-A and -B indicates the terminal position of apiose and arabinose residues, the latter being present in furanoid and pyranoid forms.

The apiose residues are attached to the 2-, 3-, and/or 2,3-positions of the backbone galacturonic acid residues. The other outer-chains of HPS-A and -B consist mainly or solely of xylose residues, and the structural patterns appear to be as shown in **1** and **2**,

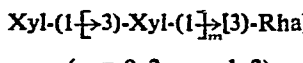


1



2

where R is the terminal residue, and k, m, $n = 0, 1, 2\ldots$. The chains **1** are attached by (1→3)-linkages to rhamnose residues of the backbone, as shown by the isolation of the oligosaccharides **3** after enzymic hydrolysis[5].

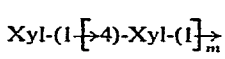


($m = 0$–2, $n = 1, 2$)

3

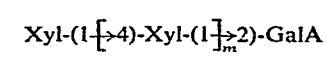
Zosterine was treated with diazomethane and then permethylated, and the product, $[\alpha]_D$ +20° (chloroform), was hydrolysed with acid. The following methylated sugars were obtained: 2,3,5- and 2,3,4-tri-*O*-methyl-L-arabinose; 2,3,4-tri-*O*-methyl-D-apiose; 2,3,4-tri-, 2,3- and 2,4-di-, and 2-*O*-methyl-D-xylose; 3,4-di- and 4-*O*-methyl-L-rhamnose; 2,3,4,6-tetra-, 2,4,6-tri-, and 2,4-di-*O*-methyl-D-galactose; and 2,3-di- (main), 2-, and 3-*O*-methyl-D-galacturonic acid. D-Galacturonic acid was also detected.

Thus, zosterine contains terminal residues of D-apiofuranose, L-arabinose (furanose and pyranose), D-xylopyranose, and D-galactopyranose. Also present are (1→3)- and (1→4)-linked D-xylose and (1→2)-linked L-rhamnose residues. Some of the L-rhamnose residues have side chains attached to position 3. The presence of (1→4)-linked D-xylose residues demonstrates the presence of linear side-chains (**4**) in the zosterine molecule.



($m = 0, 1, 2\ldots$)

4



5

The isolation of oligosaccharides **5** after pectinase digestion of zosterine[3] confirms this suggestion and demonstrates the occurrence of (1→2)-linkages between these chains and the backbone.

Thus, zosterine appears to possess a block structure composed of galacturonan, apiogalacturonan, and heteroglycanogalacturonan, interlinked by residues of galacturonic acid and rhamnose.

EXPERIMENTAL

Zosterine was isolated[4] from fresh eel-grass, *Zostera marina* L., collected at the sublittoral of the Sea of Japan.

Acetolysis[6], enzymic digestion[7], and methylation[8] of zosterine, as well as methylation[8] of HPS-A and -B, were carried out as described previously. Methyl glycoside methyl ethers were partially separated by chromatography on silica gel, using gradient elution with methanol–chloroform. Fractions were demethylated with boron tribromide[9] in order to identify the parent sugars. Methylated sugars were determined by g.l.c. as the methyl glycosides, methyl glycoside acetates, and alditol acetates. General experimental conditions are given in the preceding papers of this Series.

REFERENCES

1 R. G. Ovodova and Yu. S. Ovodov, *Carbohyd. Res.*, 10 (1969) 387.
2 L. V. Mikheyskaya, R. G. Ovodova, and Yu. S. Ovodov, *Khim. Prir. Soedin.*, (1971) 246.
3 Yu. S. Ovodov, R. G. Ovodova, O. D. Bondarenko, and I. N. Krasikova, *Carbohyd. Res.*, 18 (1971) 311.
4 R. G. Ovodova, V. E. Vaskovsky, and Yu. S. Ovodov, *Carbohyd. Res.*, 6 (1968) 328.
5 V. I. Shibaeva, R. G. Ovodova, and Yu. S. Ovodov, *Khim. Prir. Soedin.*, (1974) 289.
6 V. I. Shibaeva, R. G. Ovodova, and Yu. S. Ovodov, *Khim. Prir. Soedin.*, (1971) 249.
7 V. I. Shibaeva, R. G. Ovodova, and Yu. S. Ovodov, *Comp. Biochem. Physiol.*, 46B (1973) 561.
8 L. V. Mikheyskaya, R. G. Ovodova, and Yu. S. Ovodov, *Khim. Prir. Soedin.*, (1973) 3.
9 T. G. Bonner, E. J. Bourne, and S. McNally, *J. Chem. Soc.*, (1960) 2929.