Note

The pectic substances of *Zosteraceae* Part V[1]. Smith degradation of zosterine

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In a previous communication[1], structural studies of an apiogalacturonan produced by pectinase digestion of zosterine were described. Information concerning the remainder of the zosterine molecule was based on the isolation of some oligosaccharides[1] and pectic acid[2]. The present paper is concerned with the Smith degradation[3] of zosterine.

EXPERIMENTAL AND RESULTS

General experimental conditions. — These were described previously[1]. In addition, sugars were examined by g.l.c., as the aldononitrile acetates[4], using columns packed with 4% poly(diethylene glycol succinate) or poly(ethylene glycol adipate) on acid-washed and silanized Chromosorb W (45–60 mesh). The R_{Rha} values in paper chromatography refer to rates of movement relative to that of L-rhamnose in solvent E[1].

The Smith-degradation procedure was carried out as described by Johnson and Percival[5].

First Smith-degradation. — Zosterine (15 g) was oxidised with 30mM sodium metaperiodate (2.8 l) at room temperature and pH 3.6 in the dark. Aliquots (0.5 ml) were withdrawn at intervals, and the uptake of periodate[6] and liberation of formic acid[7] and formaldehyde[8] were measured. The primary oxidation was complete after 48 h, when 1.0 mole of periodate had been reduced and 0.15 mole of formic acid and 0.12 mole of formaldehyde had been liberated for each "anhydro-sugar" unit. The excess of periodate was destroyed with ethylene glycol, and the solution was dialysed against distilled water for 2 days and freeze-dried to yield the polyaldehyde (13.5 g) as a white solid.

To the polyaldehyde (13.5 g) dissolved in 50mM boric acid (1.11 l), sodium borohydride (22 g) was added, and the reduction was allowed to proceed for 48 h at room temperature and pH 9.0. The excess of borohydride was decomposed with acetic acid. After dialysis, the solution was poured into ethanol (1 vol.) to afford polyalcohol **1** (13.6 g) as a white solid. Analysis (by paper chromatography) of a total hydrolysate of a portion of **1** revealed galacturonic acid (R_{Rha} 0.14), threonic

acid (0.52), galactose (0.47), xylose (0.74), rhamnose (1.00), and glycerol (1.15). Ethylene glycol, mono-*O*-methylxylose, glyceraldehyde, and glycolic acid seemed also to be amongst the cleavage products. Apiose and threose or erythrose were not detected. G.l.c. confirmed these results and allowed the detection of threitol and arabinose.

A portion of the total hydrolysate of polyalcohol **1** (1.0 g) was fractionated on Whatman No. 3 paper, and the zone containing rhamnose was eluted with water. Evaporation of the eluate gave rhamnose as a syrup, $[\alpha]_D^{20}$ +8° (water), which was identified by g.l.c. of the TMS derivative and the rhamnononitrile acetate[4] and by n.m.r. spectroscopy, in comparison with an authentic sample.

To a solution of the polyalcohol (13 g) in water (1 litre), Amberlite IR-120 (H^+) resin was added, with stirring, until the pH dropped to 1.8; the stirring was then continued for 24 h at room temperature. The filtered solution was neutralized with ammonia, and degraded zosterine **1** was precipitated with ethanol and separated by centrifugation to yield a white solid (6.9 g), $[\alpha]_D^{19}$ +80° (water) (Found: mol. wt., *ca.* 25,000; galacturonic acid, 36%). Paper-chromatographic examination of an acid hydrolysate revealed galacturonic acid, galactose, xylose, rhamnose, glycerol, and an unidentified product having a higher R_F value. G.l.c. confirmed these results and detected trace amounts of arabinose.

The supernatant solution was evaporated to a thick syrup (2.2 g). Paper chromatography revealed the presence of glycerol and oligosaccharides. Small proportions of galacturonic acid, galactose, and xylose, in addition to glycerol, threonic acid, and unidentified compounds, were detected in the acid hydrolysate of the syrup.

Subsequent Smith-degradations. — The degraded zosterine **1** (4.0 g) was subjected to a further Smith-degradation under the same conditions. The oxidation was complete in 24 h, with a total consumption of 0.32 mole of periodate and liberation of 2 mmoles of formic acid and 15 mmoles of formaldehyde for each "anhydro-sugar" unit. Polysaccharide **2** (3.5 g) and polyalcohol **2** (3.2 g) were recovered as above. A portion of polyalcohol **2** was hydrolysed with M sulphuric acid for 8 h at 95° to afford galacturonic acid, threonic acid, galactose, xylose, rhamnose, and glycerol, together with trace amounts of arabinose.

Polyalcohol **2** (3.2 g) was partially hydrolysed with 0.5M sulphuric acid for 30 h at room temperature to afford degraded zosterine **2** (1.6 g), $[\alpha]_D^{19}$ +40° (water) (Found: mol. wt., *ca.* 16,000; galacturonic acid, 42.4%). The material obtained was hydrolysed with M sulphuric acid to give galacturonic acid, galactose, arabinose, xylose, rhamnose (trace), and glycerol.

The supernatant, alcoholic solution was evaporated to give a syrup **2** (1 g) which contained glycerol and oligosaccharides, and which yielded galacturonic acid, galactose, xylose, glycerol, and threonic acid on acid hydrolysis.

In a separate experiment, polyalcohol **2** (3.0 g) was subjected to autohydrolysis with Amberlite IR-120 (H^+) resin, as above, to furnish polysaccharide material (1.0 g) which afforded, on acid hydrolysis, galacturonic acid, rhamnose, and glycerol.

DISCUSSION

When zosterine was subjected to two successive Smith-degradations, the first degradation gave polyalcohol **1** and degraded zosterine **1**. The considerable uptake of periodate during the first oxidation indicates the presence of a large proportion of (1→4)-linked D-galacturonic acid residues and terminal D-apiose residues. Smith degradation of degraded zosterine **1** afforded polyalcohol **2** and degraded zosterine **2**.

Neither sample of degraded zosterine (**1** and **2**) was homogeneous, as shown by gel filtration. However, all the constituent polysaccharides were acidic and were eluted from DEAE-cellulose with aqueous alkali. Acid hydrolysis showed that polyalcohol **1** contained residues (approximate proportions in parentheses) of galacturonic acid (4+), galactose (+), xylose (+), rhamnose (+), arabinose (trace), glycerol (3+), threonic acid (2+), and some unidentified products.

Mild hydrolysis of the polysaccharide with acid gave degraded zosterine **1** and removed only a part of the ring-cleavage products. More-drastic hydrolysis cleaved the glycosidic linkages and led to extensive degradation of polyalcohol **1**. However, the final material contained glycerol and the other cleavage products noted above. The same phenomenon was observed during hydrolysis of polyalcohol **2**, to an even greater extent, and was used for preparation of polysaccharide material that seemed to be a rhamnogalacturonan.

The presence of unoxidized sugars in the above polysaccharide samples demonstrated the branched nature of the parent molecule and the probable presence of (1→3)-glycosidic linkages.

Almost all the apiose was oxidized in the first Smith-degradation to liberate a considerable amount of formaldehyde. This evidence and the absence of threose or erythrose in the total hydrolysate of the polyalcohol **1** and degraded zosterine **1** indicated the terminal location of the apiose and confirmed the results obtained previously[1]. In addition, the release of formaldehyde may be connected, to some extent at least, with the possible presence of terminal or (1→2)-linked galactofuranose residues.

The trace of rhamnose in the original material increased, relatively, as the other sugars (especially apiose) were oxidized. The isolation of a rhamnogalacturonan suggested that rhamnose residues may be included in the galacturonan chain of zosterine. Similar evidence has been reported for other pectic substances (*cf.* Ref. 9).

The presence of a considerable proportion of free glycerol in each series of fragments was observed. Glycerol could be derived from galactose or arabinofuranose end-groups, from adjacent (1→4)-linked xylose and arabinose residues, and also from (1→2)- and (1→6)-linked galactose residues. The simultaneous liberation of considerable amounts of formic acid may indicate that end-groups of galactose, xylose, and arabinose or (1→6)-linked galactose residues are more probable. In addition to glycerol, trace amounts of threitol were detected in the total hydrolysate of polyalcohol **1**, thereby demonstrating the possible presence of (1→4)- [or (1→5)]-linked galactose residues in the parent molecule. The detection of threonic acid as a com-

ponent of polyalcohol **1** suggested that the original polysaccharide contained (1→4)-[or (1→5)]-linked galacturonic acid residues free of glycosidic bonds with other sugars, as reported previously[1]. The present results permit no extensive deductions about the internal structure of the molecule.

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