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Note

The pectic substances of pigmented onion skins
Part II*. Some structural features of the pectin and pectic acid

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In previous papers[1,2], we reported that pectin isolated from pigmented onion skins contains D-galacturonic acid, D-galactose, L-arabinose, D-xylose, and L-rhamnose. We now report on the structural features of the pectin and pectic acid.

Acid hydrolysis of the pectic acid gave only galacturonic acid, and oxidation with periodate resulted in reduction of ~0.999 mole of oxidant per galacturonic acid residue (Table I). The almost complete cleavage of the D-galacturonic acid residues on periodate oxidation indicated the absence of (1→3)-linked residues and branch-points.

TABLE I

PERIODATE OXIDATION OF ONION PECTIN AND PECTIC ACID

Time (days)	*Periodate reduced (mole/sugar residue)*	
	Pectin	*Pectic acid*
1	0.313	0.496
2	0.361	0.595
3	0.519	0.691
4	0.738	0.794
5	0.784	0.824
6	0.784	0.855
7	0.784	0.920
8	—	0.988
9	—	0.999
10	—	0.999

Hydrolysis of the periodate- and bromine-oxidized pectic acid afforded oxalic acid and (−)-tartaric acid. The isolation of the latter acid demonstrated the presence of (1→4)-linked D-galacturonic acid residues in the pectic acid. The absence of galacturonic acid residues in the hydrolysate of the periodate- and bromine-oxidized

*For Part I, see Ref. 1.

pectic acid provided additional evidence of the complete cleavage of D-galacturonic acid residues. Further support for this conclusion was obtained by reduction of the periodate-oxidized pectic acid, followed by acid hydrolysis, which yielded threitol and a trace of glycerol, but no galactose.

Esterification of the pectic acid and subsequent reduction of the product gave the corresponding galactan. Periodate oxidation of the galactan, followed in sequence by reduction with sodium borohydride and acid hydrolysis, afforded glycerol and threitol. These results demonstrated that the galactan contained a linear chain of (1→4)-linked D-galactose residues, and consequently that the galacturonan had a similar linear structure. In addition, the high, positive, specific rotation of the pectic acid suggested a preponderance of α-D interglycosidic linkages.

The pectin consumed 0.784 mole of periodate per sugar residue (Table I). Chromatographic analysis of the hydrolysate of the periodate- and bromine-oxidized pectin revealed the presence of tartaric acid and oxalic acid, but no galacturonic acid. This result, together with the high, positive, specific rotation of the pectin, demonstrates the preponderance of α-(1→4)-linked D-galacturonic acid residues. Since the the pectic polysaccharide contains a linear chain of (1→4)-linked α-D-galacturonic acid residues, the reduction of only 0.784 mole of periodate per sugar residue of the pectin might be due to the presence of other sugars in the latter polymer.

EXPERIMENTAL

General experimental conditions. — Partition chromatography was performed on Whatman 3MM paper with (*A*) butyl alcohol–acetic acid–water[3] (4:1:5, upper layer); (*B*) butyl alcohol–pyridine–water[4] (6:4:3). The detection reagents were (1) aniline hydrogen phthalate[3]; (2) the aniline–xylose reagent[5]; (3) ammoniacal silver nitrate[6]. Uronic acid[7], protein[8], methoxyl[9] contents, and periodate consumption[10] were determined by the appropriate literature procedures. All solutions were concentrated by pervaporation, *i.e.* by placing the solution in a cellophane bag suspended in front of an electric fan.

Pectin. — The outer, pigmented skins of onion (*Allium cepa*) were used as the source of pectin and were obtained from El-Nasr Co. for Dehydrating Agricultural Products. Before use, they were freed from any foreign substance.

Extraction and purification of pectin. — This was isolated by the method of Abdel-Fattah and Edrees[2]. The pectin solution was treated with Lewatit S-100(H^+) and Lewatit MN(HO^-) resins, and the pure pectin, isolated by treatment with ethyl alcohol, had $[\alpha]_D^{20}$ +291° (Found: galacturonic acid, 96.5; methoxyl, 9.47; protein, 0.0%). On hydrolysis with M sulphuric acid for 16 h at 95°, the pectin afforded galacturonic acid (96.5%), galactose (2%), arabinose (trace), and rhamnose (1%) [paper chromatography in solvent (*B*)].

Pectic acid. — The pectin was saponified with dilute sodium hydroxide, and the pectic acid was isolated by precipitation with hydrochloric acid. The precipitate was dissolved in dilute sodium hydroxide, reprecipitated with hydrochloric acid, and

washed with ethanol–water (24:1) until it was free from chloride ions. The product had $[\alpha]_D^{20}$ +230° (dilute sodium hydroxide) (Found: galacturonic acid, 98; methoxyl, 0.0; protein, 0.0%). On hydrolysis with M sulphuric acid, for 16 h at 95°, the pectic acid afforded only galacturonic acid [paper chromatography in solvent (*B*)].

Periodate oxidation of pectic acid and pectin. — (*a*) Pectic acid or pectin (0.54 g) dispersed in cold acetate buffer (200 ml, pH 3.8) was treated with 50mM sodium metaperiodate (200 ml) and the reaction mixture was left, with occasional shaking, for 7–10 days in the dark at 2°. Aliquots (5 ml) were withdrawn at intervals and the periodate consumed was determined[10].

(*b*) The pectic acid or pectin (3 g/100 ml of water) was added to sodium metaperiodate (15.8 g/100 ml of water), and the mixture was stored at room temperature for 24 h with continuous shaking. The product was then precipitated with *tert*-butyl alcohol (750 ml), filtered off, dissolved in water, and deionised by dialysis. Thereafter, strontium carbonate (15 g) and bromine (5 ml) were added and the mixture was immediately stirred for 24 h at room temperature. After removing excess of bromine by aeration, the reaction mixture was treated with 3M sulphuric acid (60 ml) and the precipitate removed by filtration. The filtrate was then dialyzed for 3 days and concentrated, and the residue was hydrolysed in 25mM sulphuric acid for 36 h at 100°. After removal of sulphate with barium hydroxide, the hydrolysate was chromatographed using solvent (*A*), with authentic (−) tartaric acid and oxalic acid as markers. Detection of spots was achieved with aniline–xylose[5] and aniline hydrogen phthalate[3] spray reagents.

The acid potassium salt of (−)-tartaric acid, prepared by the method of Levene and Kreider[11], had $[\alpha]_D^{30}$ −24° (*c* 0.24, water).

(*c*) In a separate experiment, when the controlled periodate oxidation of pectic acid was complete, ethylene glycol was added to the reaction mixture. After deionisation, the solution was treated with sodium borohydride (0.5 g in 20 ml of water) for 24 h, and then deionised and concentrated. The residue was hydrolysed with M sulphuric acid for 10 h at 100°. The hydrolysate, after removal of sulphate ions with barium carbonate and treatment with Lewatit S-100(H^+) and Lewatit MN(HO^-) resins, was concentrated and examined by paper chromatography [solvent (*B*)]. As reference substances, threitol and glycerol were also chromatographed. Detection of spots was achieved with aniline hydrogen phthalate[3] and ammoniacal silver nitrate[6].

Esterification and reduction of pectic acid. — Following the method of Jones and Perry[12], the pectic acid (4 g) was moistened with methanol and then treated with ethereal diazomethane at room temperature. After the reaction had reached completion, the residue was filtered off, washed with ether, dissolved in water (150 ml), and reduced with sodium borohydride (2 g/50 ml of water). Thereafter, the reaction mixture was kept overnight at 4°, neutralized with acetic acid, dialyzed, treated with Lewatit S-100(H^+) resin, and evaporated to dryness. Methanol was twice distilled from the residue. The esterification and reduction procedures were repeated to give the galactan.

The galactan (0.3 g) was oxidized with 50mM sodium metaperiodate (100 ml)

for 3 days in the dark at 10°. Thereafter, the solution was deionised, reduced with sodium borohydride (0.2 g) for 24 h, deionised again, and evaporated. The residue was hydrolysed in 0.5M sulphuric acid for ~7 h and examined by paper chromatography [solvent (*B*)] with threitol and glycerol as reference compounds. Detection was achieved with aniline hydrogen phthalate[3] and ammoniacal silver nitrate[6].

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