SOME STRUCTURAL FEATURES OF THE PECTIC ACID ISOLATED FROM SUNFLOWER HEADS

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Abstract. Oxidation of sunflower pectic acid with nitric acid afforded mucic acid. Oxidation of the pectic acid by periodate showed that the oxidation was complete after the reduction of about 0.9 mole of oxidant per monomer unit. Chromatographic analysis of the periodate-bromine-oxidised pectic acid hydrolysate revealed the presence of D-(—)tartaric acid and oxalic acid, and the absence of galacturonic acid residues. Esterification and reduction of the pectic acid afforded the galactan. Periodate oxidation of the galactan followed by reduction and hydrolysis afforded threitol. The studies demonstrated that the pectic acid of sunflower heads was a linear galacturonan of α-(1→4)-linked D-galacturonic acid residues.

It has long been known that pectic acid has a linear-chain constituted of α-(1→4)- linked D-galacturonic acid units.[1-3] However, Bouveng[4] reported that linkages other than 1→4 might occur in some galacturonans. Solov'eva *et al*[5] also found that on partial hydrolysis with acid, the pectin of *Panax ginseng* C.A. Mey afforded a galacturonan having a linear chain of (1→4)- and possibly (1→5)-linked α-D-galacturonic acid residues. On the other hand, Zitko and Bishop[6] reported the fractionation of pectic acids from sunflower and other sources into two acidic polysaccharides, a galacturonan to which neutral sugars were attached glycosidically and a galacturonan which yielded only D-galacturonic acid on hydrolysis. Furthermore, Zikto and Bishop[7] investigated the structural features of the latter polyuronide and found that it was a linear polymer of 1→4 linked α-D-galacturonic acid units. The present work deals with the structural features of the pectic acid isolated from local sunflower heads.

Materials and Methods

Specimen. The sunflower (*Helianthus annuus*) heads, obtained from Imbaba locality at Giza, Cairo, were freed from any foreign substance and milled.

General Experimental Conditions. Partition chromatography was performed on Whatman No. 1 paper with the solvent systems: (A) n-butanol-pyridine-water (6:4:3, v/v)[8] and (B) n-butanol-acetic-acid-water (4:1:5, v/v).[9] Detection of spots was effected with aniline-hydrogen phthalate, aniline-xylose and ammoniacal silver nitrate reagents.[10] Methoxyl groups were determined by the method of Myers and Baker.[11]

Complete acid hydrolysis of pectin or pectic acid was done with H_2SO_4 according to the method of Haug and Larsen.[12] Uronic acid contents were determined by a modified carbazole reaction.[13] Reaction with orcinol[14] was used for the determination of arabinose while glucose and rhamnose were determined by reaction with L-cysteine-sulphuric acid.[15,16]

Extraction of Pectin. The pectin was extracted from sunflower heads with 0.3% ammonium oxalate solution at 90° for 1½ hr and the extract was treated with 1 vol. of HCl-acidified ethanol.[17] The isolated pectin was dissolved in water, reprecipitated as before and finally dried (Found: $[\alpha]_D = \pm 250°$; methoxyl, 9.0%). On complete acid hydrolysis and paper chromatography with solvent A, the pectin afforded D-galacturonic acid, glucose, arabinose and rhamnose in the proportions of 93.7, 0.29, 1.92 and 4.09%, respectively.

Preparation and Purification of Pectic Acid. The pectin was saponified with 0.1N NaOH for 2 hr at room temperature and the pectic acid was then isolated by precipitation with HCl. The precipitate was further dissolved in 0.1N NaOH, repeatedly precipitated with HCl and washed with 96% ethanol until free of chloride ions. On complete hydrolysis and paper chromatography with solvent A, the pectic acid afforded only galacturonic acid.

Oxidation of Pectic Acid with Nitric Acid. The pectic acid was oxidised with nitric acid according to Heyne and Whistler[18] and the m.p. of the crystallised product as well as the mixed m.p. with authentic mucic acid were determined.

Preparation of the Dibutyl Ester of the Pectic Acid Oxidation Product. This was done according to the method of Carson[19] using the crystallised product obtained by oxidation of pectic acid as well as authentic mucic acid. The m.p. of the product as well as the mixed m.p. with authentic dibutyl mucate were determined.

Preparation of the Acetate Derivative of the Dibutyl Ester. This was done according to the method of Abdel-Akher and Smith[20] using the dibutyl ester preparation under investigation and also authentic dibutyl mucate. The m.p. of the product and mixed m.p. were determined.

Periodate Oxidation of Pectic Acid Under Controlled Conditions. This was performed by dispersing 0.4 g in 200 ml of cold acetate buffer (pH 3.8) followed by the addition of 200 ml 0.05 M $NaIO_4$ and the reaction mixture was left, with occasional shaking, in the dark at 2° for 6 days. During that priod 5 ml aliquots were withdrawn at definite time intervals and the consumed periodate was determined according to the method of Fleury and Lange.[21]

Periodate Bromine Oxidation of Pectic Acid. The pectic acid (3 g/100 ml water) was added to sodium metaperiodate (15.8 g/100 ml water), and the mixture was stored at room temperature for 24 hr with continuous shaking. The product was then precipitated with t-butanol (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 strirred for 24 hr at room temperature. After removing excess of bromine by aeration, the reaction mixture was treated with 6 N H_2SO_4 (60 ml) and the precipitate removed by filtration. The filtrate was then dialysed for 3 days, concentrated and hydrolysed in 0.05N H_2SO_4 for 36 hr at 100°. After removal of sulphate with $Ba(OH)_2$ the hydrolysate was chromatographed using solvent B with authentic (—)- tartaric acid and oxalic acid as markers. Detection of spots was achieved with aniline xylose and aniline hydrogen phthalate spray reagents.[10]

Esterification and Reduction of Pectic Acid. Following the method of Jones and Perry,[22] 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 water). Thereafter, the reaction mixture was kept overnight at 4°C, neutralized with acetic acid, dialysed, 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 was oxidised with 50 mm sodium metaperiodate (100 ml) for 3 days in the dark at 10°. Thereafter, the solution was deionised, reduced with sodium borohydride (0.2g) for 24 hr, deionised again, and evaporated. The residue was hydrolysed in 0.5M H_2SO_4 for about 7 hr and examined by paper chromatography with solvent A. Threitol and glycerol were also chromatographed as markers. Detection of spots was effected with aniline hydrogen phthalate and ammoniacal silver nitrate.[10]

Results and Discussion

Oxidation of pectic acid with nitric acid resulted in the production of mucic acid which was identical in m.p. with authentic sample (215). The dibutyl ester of the produced mucic acid was also identical in m.p. (145) with authentic dibutyl mucate. Similarly the m.p. and mixed m.p. of the acetate derivative of the dibutyl ester were identical (112). These results suggest that the pectic acid of sunflower heads is composed of galacturonic acid residues.

The data in Table 1 indicates that the oxidation of pectic acid by periodate reached completion after the reduction of about 0.9 mole oxidant per monomer unit. However, it is known that an α-(1→4)-galacturonan should consume 1.0 mole of periodate per monomer unit. Thus, the reduction of only 0.9 mole of periodate per monomer residue of pectic acid might be due to the presence of trace chromatographically undetectable amounts of other sugar residues in the latter polyuronide. Such a behaviour was also noted with the pectin isolated from pigmented onion skins, which consumed 0.784 mole periodate per sugar residue due to the presence of other sugars.[3] It is worthy to note that Zitko and Bishop[7] found that the carboxyl-reduced pectic acid of sunflower heads consumed only 0.82 mole of periodate per monomer unit.

TABLE 1. PERIODATE OXIDATION OF SUNFLOWER PECTIC ACID.

Time (hr)	20	40	60	80	100	120	144
Periodate reduced (mole/sugar residue)	0.69	0.77	0.83	0.88	0.89	0.90	0.90

The periodate-oxidised pectic acid was further oxidised with bromine. Chromatographic analysis of the periodate bromine oxidised pectic acid hydrolysate revealed the presence of D-(—)-tartaric acid and oxalic acid. It is noteworthy that no galacturonic acid residues were found in the hydrolysate, indicating the absence of (1→3)-linked residues and branch points. On the other hand, the detection of D-(—)-tartaric acid and the high, positive, specific rotation of the pectin, demonstrate the preponderance of α-(1→4)- linked D-galacturonic residues in the pectic acid of sunflower heads.

A substantial evidence for the constitution of pectic acid was provided by performing esterification and reduction. Thus, esterification of the pectic acid and subsequent reduction of the product gave the corresponding galactan. Periodate oxidation of the produced galactan, followed in sequence by reduction with sodium borohydride and acid hydrolysis afforded threitol. These results demonstrate that the galactan contains a linear chain of (1→4)- linked D-galactose residues, and consequently that the galacturonan had a similar linear structure. These results are in agreement with those reported for the pectic acid of sunflower heads and also other plant materials.[2,3,7,23]

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