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THE PECTIC SUBSTANCES OF PIGMENTED ONION SKINS : FORMATION OF GALACTURONE IN HEATED PECTIN AND PECTIC ACID SOLUTIONS

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Abstract. A galacturone was formed on thermal treatment of aqueous solutions of pectin or pectic acid suspensions. Isolation of the galacturone was performed by chromatography on a cellulose column and its characterization was achieved.

Although mannuronic and glucuronic acids are known to form readily crystalline lactones, yet there is no reputable chemical evidence in the literature to date in support of the existence of any crystalline lactone of galacturonic acid. Wlostowska[1] stated that in an aqueous solution of D-galacturonic acid about 5–7% carboxyl groups were present in the form of a lactone. Recently, in a study on garlic pectin, Abdel-Fattah and Khaireldin[2] reported on the possibility of the formation of glacturone in heated water garlic pectin solution.

The present work was undertaken to investigate this important phenomenon in heated aqueous solution of onion pectin and pectic acid as well as D-galacturonic acid. The lactone isolated was also not crystalline, although it was characterised by converting it into crystalline derivatives.

Materials and Methods

Specimen. The outer pigmented skins of onion (*Allium cepa*) used were obtained from El-Nasr Co. Before use they were freed from any foreign substance.

General Experimental Conditions. Partition chromatography was performed on Whatman No. 3 mm paper with the solvent systems given in Table 1. Detection of spots was effected with aniline hydrogen phthalate[7] and hydroxylamine hydrochloride reagents.

Paper electrophoresis was performed using the Labor Hungary horizontal paper electrophoresis apparatus type OE-201. A potential of 750 volts, giving a current of 15 mA at about 4°C with borate buffer of pH 10 (0.05M borax+0.2M NaOH), was applied for 3 hr. The electropherograms were dried and sprayed with aniline hydrogen phthalate. Papers were then heated for 5 min at 105°C.

Uronic acid contents were determined by a modified carbazole reaction.[8] Reaction with orcinol[9] was used for the determination of arabinose and xylose while galactose and rhamnose were determined by reaction with L-cysteine–sulphuric acid.[10,11] Free carboxyl groups were estimated by titration against standard sodium hydroxide (0.1N), while methoxyl content was determined by the method of Myers and Baker.[12] Protein determination was achieved according to the method of Lowry *et al.*[13]

Extraction and Purification of Pectin. The pectin was extracted from onion skins with ammonium oxalate solution according to the method of Abdel-Fattah and Edrees.[14] After treating the pectin solution with ion exchange resins Lewatit S-100 (H^+) and Lewatit MN (OH^-), pure pectin was isolated by treatment with ethanol (Found: $(\alpha)_D = +291°$; methoxyl, 9.47%; protein, 0.0%). On complete hydrolysis with 2N H_2SO_4 for 16 hr at 95°C and quantitative paper chromatography with solvent A, the pectin afforded D-galacturonic acid (96.5%), galactose (1.75%), arabinose (0.22%), xylose (0.22%) and rhamnose (1.31%).

Preparation of Pectic Acid. The pectin was saponified with 0.1N NaOH for 2 hr at room temperature (30°C) and the pectic acid was then isolated by precipitation with HCl. The precipitate was further dissolved in 0.1N NaOH, reprecipitated with HCl and washed with 96% ethanol until free of chloride ions [Found: $(\alpha)_D = +230°$ (in dilute NaOH); galacturonic acid, 98%; methoxyl, 0.0%]. On complete hydrolysis with 2N H_2SO_4, the pectic acid afforded only galacturonic acid (paper chromatography with solvent A).

Thermal Treatment of Pectin Solution. The pectin (3.6 g) in distilled water (1 litre) was boiled under reflux for 24 hr. During that period, 5 ml aliquots were taken, cooled immediately and the free carboxyl groups and methoxyl content were determined. Paper chromatography (solvent B) of the thermally-treated pectin solution was used to follow the course of the reaction. A compound of high *Rf* value (Table 1) giving a positive reaction for lactone (with hydroxylamine hydrochloride reagent) appeared on the chromatograms. After termination of the thermal treat-

ment the pectin solution was filtered and the filtrate freeze-dried.

Thermal Treatment of Pectic Acid Suspension. Pectic acid suspension (0.36%) was boiled under reflux for 24 hr. Paper chromatographic examination of the filtrate showed the presence of the lactone. The solution was filtered and the filtrate freeze-dried.

Isolation of the Lactone. The freeze-dried filtrates from the thermally-degraded pectin or pectic acid (0.5 g) were treated with the least amount of the solvent mixture B and added to a cellulose column (1.5×32 cm). The column was eluted with the solvent B (rate of flow, 0.3 ml/min), 46×10-ml fractions were collected, and each fraction analysed by the phenol–sulphuric acid method.[15] The fractions were each concentrated and examined by paper chromatography using solvent B. Only the fourth and the fifth fractions contained the lactone. These fractions were combined and the lactone further purified by paper chromatography (solvent B). The appropriate bands on unstained chromatograms being cut out and the contents eluted with water. The aqueous eluates were then freeze-dried. The water solution of the isolated lactone showed an initial $(\alpha)_D$ of +100° which changed to a final rotation value of +40° after 48 hr. These values are more or less similar to those of α-D-galacturonic acid.[16]

Esterification of the Lactone, Reduction and Hydrolysis. The freeze-dried galacturone (20 mg) was refluxed for 24 hr with 3% methanolic hydrogen chloride[17] (50 ml). Thereafter, the solution was neutralised with silver carbonate, filtered and the filtrate evaporated in air in an open dish. On cooling, the methyl ester methyl glycoside of D-galacturonic acid crystallized as prisms. The m.p. of the crystalline product and mixed m.p. with authentic material were determined (140°C).[17] The methyl ester methyl glycoside was reduced with sodium borohydride in water for 16 hr in a refrigerator. The solution was then neutralised with acetic acid, treated with Lewatit S-100 (H^+) and Lewatit MN (OH^-) resins, hydrolysed in 1N H_2SO_4 for 4 hr at 100°C. The hydrolysate was subjected to paper chromatography (solvent A) and to paper electrophoresis using galactose as the reference compound in each experiment ($Rf = 0.4$; $MG = 0.95$).

Oxidation of the Lactone with Nitric Acid. The method of Heyne and Whistler[18] was used. The m.p. and mixed m.p. of the crystalline product with authentic mucic acid was 214°C. The dibutyl ester of mucic acid was also prepared[19] (m.p. and mixed m.p. 145°C) as well as the acetate derivative of the latter[20] (m.p. and mixed m.p. 112°C).

Results and Discussion

The data recorded in Table 2 indicated that no correlation existed between deesterification (demethoxylation) of pectin and the amount of free carboxyl groups when the thermal treatment continued more than 10 hr. In this respect, the decrease in methoxyl content was accompanied by decrease in free carboxyl groups. Paper chromatography of the thermally degraded pectin solution showed that monogalacturonic acid was released only after 6-hr treatment. As the thermal process proceeded the released galacturonic acid increased and when treatment continued in excess of 10 hr a compound of high *Rf* value (Table 1) giving positive reaction for uronic acid lactones[7] (with hydroxylamine hydrochloride reagent) began to appear on the chromatograms. Furthermore, a parallel correlation between the amount of this compound and that of the released monogalacturonic acid was observed. It is worth mentioning that thermal treatment of pectic acid suspension also afforded the lactone compound. Similar thermal treatment of aqueous solutions of authentic D-galacturonic acid, galactose, arabinose, xylose and rhamnose, followed by paper chromatography, revealed the formation of the lactone in question only in the case of galacturonic acid. In this respect, although considerable amounts of the thermally treated D-galacturonic acid solution were chromatographed, the intensity of the stained spot corresponding to the compound was much fainter than that formed from either pectin or pectic acid. It seemed, therefore, that the acidity of authentic galacturonic acid solution was a limiting factor for the formation of that compound. This might be substaintiated by the fact that when pectin, pectic acid or galacturonic acid solution was treated under strong acid conditions (1N HCl),

TABLE 1. *Rf* VALUES OF THE GALACTURONE IN DIFFERENT SOLVENT SYSTEMS.

Solvent (v/v)	*Rf*
(A) Butyl alcohol–pyridine–water (6:4:3)	0.76
(B) Pyridine–ethyl acetate–water (2:5:7)	0.84
(C) Pyridine–ethyl acetate–water (11:40:6)	0.82
(D) Pyridine–ethyl acetate–acetic acid–water (5:5:1:3)	0.75

TABLE 2. CHANGES IN FREE CARBOXYL AND METHOXYL CONTENTS DURING THERMAL TREATMENT OF PECTIN SOLUTION.

Time (hr)	Free carboxyl (meq/g)	Methoxyl (%)
0.0	3.23	9.47
2	3.78	8.61
3	4.00	8.00
4	4.06	7.77
5	4.06	7.77
6	4.11	6.39
7	4.11	6.39
8	4.17	5.70
10	4.22	5.16
12	3.55	4.83
16	3.33	4.47
20	3.22	4.13
24	3.01	4.13

*meq, milliequivalents.

the lactone in question was not formed. These results suggest that the compound in question is formed from the released galacturonic acid during thermal treatment of pectin or pectic acid solution, and its formation is favoured only under mildly acid conditions (pH 3.0). The decrease in the amount of free carboxyl groups, associated with continuous demethoxylation of pectin and the formation of this compound supports this view. It appears that the released galacturonic acid was not decarboxylated because neither furfural nor reductic acid were formed.

The compound formed from either pectin or pectic acid was isolated by chromatography on a cellulose column followed by paper chromatography, elution and freeze-drying. The isolated product gave positive reaction for uronic acid lactone (hydroxylamine hydrochloride) and for uronic acids.[8] Furthermore, on paper chromatography with the solvent mixtures B and C, specific for uronic acid lactones, the compound moved faster than any of the sugar components in pectin. It is worthy to note that the aforementioned solvents allow for the migration of uronic acid lactones while uronic acids remain on the start. On the other hand, when the aqueous solution of the compound was treated with 0.1N NaOH (pH 8.0), left to stand for 1 hr, then treated with Lewatit S-100 (H^+) resin and examined by paper chromatography with the solvents B and C, it did not move and remained on the start. However, on chromatography of this alkali–resin-treated solution with the solvents A or D it gave only one spot identical in *Rf* and reactions w.th D-galacturonic acid. These results suggest that the compound in question contains a lactone ring and by saponification with alkali and treatment with resin (H^+), free galacturonic acid is obtained. All these results indicate that the compound in question is galacturone.

Esterification of the isolated galacturone with methanolic hydrogen chloride afforded crystalline methyl ester methyl glycoside of galacturonic acid which was identical in m.p. and mixed m.p. with authentic material. Reduction of the methyl ester methyl glycoside with sodium borohydride and hydrolysis of the product gave galactose which was identical in *Rf* (paper chromatography) and *MG* (paper electrophoresis) with authentic sample.

Furthermore, oxidation of the galacturone under investigation with nitric acid gave mucic acid which was further identified as its dibutyl mucate. The latter was also identified as its crystalline acetate derivative.

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