pH 8.6. The reaction was stopped by heating to 100° for 2 min. Almost 95% of the cGMP activity was lost. The formation of 5'-GMP was detected by TLC (Si gel, C_6H_6–EtOAc–MeOH, 1:1:3).

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ENZYMATIC EXTRACTION AND LINKAGE ANALYSIS OF PECTIC POLYSACCHARIDES FROM ONION

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Key Word Index—*Allium cepa*; Liliaceae; onion; cell walls; pectic polysaccharides; extraction; methylation analysis.

Abstract—Pectin lyase was superior to polygalacturonase for the extraction of onion cell wall pectic polysaccharides. Exhaustive treatment of onion tissue with pectin lyase solubilized 89% of the total uronides of the tissue. The galacturonides released from the tissue were separated into three fractions (10.7, 5.3 and 84%, in order of MW) by gel filtration on Sephadex G-100. The low MW fraction was a mixture of oligogalacturonides. High and intermediate MW fractions were purified by DEAE-Sephadex column chromatography. The intermediate MW fraction was a rhamnogalacturonan II type component which contained 3- and 3,4-linked rhamnose. Methylation analysis showed that the pectic polysaccharides of onion resembled those of potato tuber.

INTRODUCTION

Homogalacturonans or homogalacturonan regions of pectic polysaccharides in the cell wall contribute to tissue coherence by the ability of their chains to form bundles [1]. If galacturonan chains are greatly modified by insertion of rhamnosyl residues and branching, the cell walls lack cohesion [2]. Darvill *et al.* [3] isolated highly branched and complex pectic polysaccharides (rhamnogalacturonan II, RG-II) from the cell wall of suspension-cultured sycamore cells. Recently Ishii [4] has isolated a RG-II type component from potato tuber cell walls by purified polygalacturonase (PG) treatment of the tissue. It was characterized by the existence of 3- and 3,4-linked rhamnosyl residues.

The cell walls of onion are known to contain pectic polysaccharides [5, 6]. It is of interest to know whether monocotyledonous plants contain RG-II type components in the cell wall.

RESULTS AND DISCUSSION

Pectin lyase (PL) solubilized 62.5% of the total uronides from onion tissue in 6 hr at 30°, while PG only solubilized 18%. Under the same condition 1.3% of the tissue uronides were released without enzyme. It has been demonstrated that susceptibility of plant tissue to enzymatic attack depends largely on enzyme specificity but not on enzyme concentration [7]. Therefore, PL which is specific for methyl-galacturonide linkages is essential for the extraction of

Table 1. Glycosyl linkage compositions of high MW and intermediate MW fractions isolated from soluble products released from onion by pectin lyase

Parent sugar	Position of methyl groups	Mode of linkage	Relative mol %	
			High MW fraction	Intermediate MW fraction
Galacturonic acid	2,3,4,6	Terminal	0.2	4.7
	2,3,6	4-Linked	9.7	27.3
	2,6	3,4-Linked	0	4.7
Rhamnose	2,3,4	Terminal	0	2.5
	3,4	2-Linked	1.4	4.0
	2,4	3-Linked	0	4.9
	3	2,4-Linked	1.4	0.3
	2	3,4-Linked	0	3.4
Arabinose	2,3,5	Terminal(furanose)	0.7	8.8
	2,3,4	Terminal(pyranose)	0	2.0
	2,3	5-Linked	1.2	0.1
	3	2,5-Linked	0	9.7
	2	3,5-Linked	0	8.3
Galactose	2,3,4,6	Terminal	5.4	6.3
	2,3,6	4-Linked	69.7	4.0
	3,6	2,4-Linked	0.9	6.4
	2,6	3,4-Linked	1.2	2.6
	2,3	4,6-Linked	8.2	0

onion cell wall pectic polysaccharides. Exhaustive extraction of onion pectic polysaccharides was carried out by subjecting the tissue to the action of PL. Three successive PL treatments released 73.0, 9.8, and 6.0%, respectively, of the total uronides of the tissues. The soluble products of the three treatments were combined and lyophilized. The lyophilized sample was dissolved and the solution placed on a Sephadex G-100 column. The galacturonides released from the tissue were separated into three fractions with respect to MW: they consisted of 10.7% of high MW, 5.3% intermediate MW and 84% low MW galacturonides (Fig. 1).

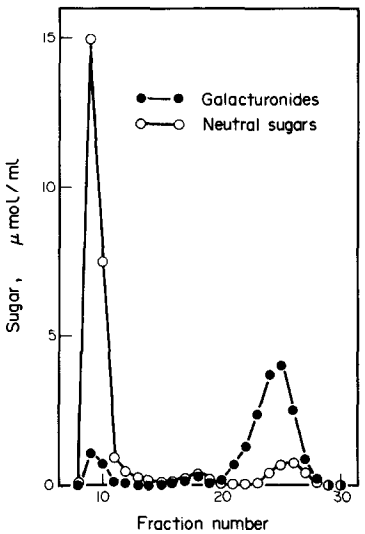

Fig. 1. Gel filtration pattern of products solubilized from onion tissues by PL on Sephadex G-100. The lyophilized sample was dissolved in 2 ml of 0.1 M ammonium acetate buffer (pH 4.5), and the solution was placed on a Sephadex G-100 column (1.9 × 98 cm). 10 ml fractions were collected and each fraction was assayed for galacturonides by the *m*-hydroxydiphenyl method and for neutral sugars by the anthrone method.

Low MW fractions (Nos. 20–28) were a mixture of oligogalacturonides with a variety of esterification patterns. This indicated that over 84% of the galacturonides in the cell wall may form homogalacturonans or homogalacturonan regions of pectic polysaccharides.

High MW (Nos. 8–11) and intermediate MW fractions (Nos. 16–19) were further purified by DEAE-Sephadex column chromatography as described previously [4]. The glycosyl linkage compositions of both purified high MW and intermediate MW fractions are given in Table 1. The intermediate MW fraction was characterized by the existence of 3- and 3,4-linked rhamnose. It also contained branched galacturonosyl, arabinosyl, and galactosyl residues. Thus, the fraction is a RG-II type component. The high MW fraction was structually similar to fractions I and II [4] isolated from the cell wall of potato tuber by PG, except that they did not contain 4,6-linked galactose and most of rhamnosyl residues in these fractions were 2,4-linked.

These results showed that the sugar components and their linkages in the cell wall pectic polysaccharides of onion resemble those of potato tuber.

EXPERIMENTAL

Enzymatic extraction of pectic polysaccharides. Sliced tissues (0.5 mm thick) of onion bulb were repeatedly washed with 50 mM ammonium acetate buffer (pH 5.5). After excess buffer soln was removed with filter paper, 2.5 g of the tissues were placed in 100 ml Erlenmeyer flasks containing 25 ml of 50 mM ammonium acetate buffer, pH 5.5 (for PL) or pH 4.5 (for PG) and 20 units of PL or 500 units of PG ex. *Aspergillus japonicus* [7]. For exhaustive extraction 5 g of tissue in 100 ml of 50 mM ammonium acetate buffer, pH 5.5 were treated with 50 units of PL at 30°. After 7 hr the reaction mixtures were filtered and the residue repeatedly washed with buffer. The tissues were then suspended in 100 ml of buffer and incubated with an additional 25 units of PL for another 7 hr. The solubilized material was removed from the tissue by filtration and washing. The third extraction was carried out in the same manner with a further 25 units of PL.

Analysis of pectic polysaccharides. The galacturonide content was estimated by the *m*-hydroxydiphenyl method of ref. [8]. Total uronides were determined by the method of ref. [9]. The neutral sugar content was estimated by the anthrone method [10] using galactose as standard.

Glycosyl linkage compositions were determined by GC/MS analysis of $NaBD_4$-reduced pectic polysaccharides as described previously [4].

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INOSITOL ANGELATES FROM *INULA CAPPA*

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Key Word Index—*Inula cuppa*; Compositae; inositol and myoinositol esters.

Abstract—The aerial parts of *Inula cappa* afforded four inositol tetra angelates.

From the aerial parts of *Inula cappa* DC. some unusual flavones have been isolated [1]. A re-investigation afforded in addition to the thymol and isothymol derivatives **1–4**, β-farnesene, squalene and caryophyllenepoxide, four angelates, which could only be separated with difficulty. Careful ^{1}H NMR investigation of these esters (**6** and **7** could not be separated completely) led to the structures **5–8** (Table 1). The presence of tetra angelates clearly followed from the ^{1}H NMR signals, as did the presence of two free hydroxyls. As the molecular formula of each compound was the same, **5–8** were isomeric compounds. Spin decoupling allowed the assignment of the sequences of the neighbouring oxygen functions, as the signals of those protons under the ester function were clearly shifted downfield. The stereochemistry was deduced from the observed couplings. Obviously **5** and **6** were derivatives of inositol, while **7** and **8a** were isomeric tetra angelates of myoinositol. Saponification of **5** afforded L-inositol, which seems to be widespread in the Compositae [2], while esters have not been reported. *I. cappa* belongs to the group of species, which lacks sesquiterpene lactones. These seem to be replaced by thymol derivatives [3, 4].