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Structural Characterization of a Tobacco Rhamnogalacturonan

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SUMMARY

A rhamnogalacturonan, extracted with hot water from the aqueous ethanol insoluble residue of flue-cured bright tobacco lamina, was purified by tangential flow ultrafiltration, ion chromatography and gel filtration. It was characterized by chemical and spectroscopic methods. Fractionation revealed that the rhamnogalacturonan consisted of a series of polysaccharides with different amounts of methyl-esterified galactopyranosyluronic acid residues in the backbone and different amounts of neutral sugar residues.

The main pectic polysaccharide fraction has a backbone consisting of 4-linked α-D-galactopyranosyluronic acid residues interspersed with 2-linked L-rhamnopyranosyl residues. Approximately 22% of the galactopyranosyluronic acid residues are methylated. The main chain is branched at C-4 of rhamnose with neutral sugar side chains containing terminal and 4-linked β-D-galactopyranosyl and terminal and 5-linked α-L-arabinofuranosyl residues. The average degree of polymerization of this tobacco rhamnogalacturonan was estimated to be 400.

INTRODUCTION

The pectic polysaccharides are found in the primary cell walls and middle lamella of plant tissues, and may function as an intercellular

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binding material (Dey & Brinson, 1984). Naturally occurring pectins contain galacturonosyl residues as the major constituent and varying quantities of neutral sugar residues (Aspinall *et al.*, 1968). A portion of the galacturonosyl residues are methyl-esterified. Calcium salts of pectins are believed to be present in the primary cell walls (Whistler & Smart, 1953).

The most characteristic physical property of pectin is the ability to form gels (Rees & Welsh, 1977). Pectins are mainly used to prepare jam and jelly products in the food industry (Towle & Christensen, 1973). Pectins are also responsible for the firmness of ripened and processed fruits and vegetables (Van Buren & Peck, 1981; Jarvis, 1984; McFeeters *et al.*, 1985). In the tobacco industry, tobacco pectins are used as a natural binder in making reconstituted sheet materials (Hind & Seligman, 1967, 1968, 1969).

The basic structures of pectins from various sources have been studied and summarized in many papers and reviews (e.g. McNeil *et al.*, 1979; Aspinall, 1980; Darvill *et al.*, 1980; Pilnik, 1981; Selvendran, 1983; Jarvis, 1984; Pressey & Himmelsbach, 1984; Keenan *et al.*, 1985), but little has been published on tobacco pectins. Only three chemical investigations have been reported: the pectic substances from cured stems and uncured leaves (Bourne *et al.*, 1967), a pectin from the midrib of a greenhouse-grown tobacco (Eda & Katō, 1980) and water-soluble pectic polysaccharides from bright tobacco lamina (Siddiqui *et al.*, 1984). The general structural features of tobacco pectic polysaccharides were found to consist of a rhamnogalacturonan main chain carrying side chains composed mainly of arabinose and galactose. We now report the isolation and characterization of a pectin from a single grade of flue-cured bright tobacco lamina.

MATERIALS AND METHODS

Materials

The tobacco lamina used in this study was a single grade of heavy or bodied, field-grown, flue-cured bright tobacco harvested at the upper midstalk position. Prior to extractions, tobacco samples were ground on a Wiley mill to pass a 20-mesh screen. Oxolane (THF) was used shortly after distillation from lithium aluminum hydride. Dimethylsulfoxide was refluxed and distilled from calcium hydride and then stored over molecular sieves.

General methods

^{13}C NMR spectra were recorded with a Varian XL 300 spectrometer for solutions in D_2O, with sodium 2,2,3,3-tetradeutero-3-trimethylsilyl-propionate as internal or external reference. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. Routine gas chromatography (GC) separation was performed on a Hewlett-Packard model 5880A gas chromatograph equipped with a flame ionization detector, on a fused silica capillary column (30 m × 0·259 mm) coated with 0·25 μm film DB-1 phase (J. & W. Scientific). The oven temperature programs for alditol acetates, partially methylated alditol acetates, and *O*-silylated methylglycosides (or butylglycosides) are: (a) 170°C–200°C at 2°C min^{-1} and 200°C–240°C at 10°C min^{-1}, (b) 150°C for 2 min and 150°C–225°C at 3°C min^{-1}, and (c) 160°C for 2 min, 160°C–200°C at 2°C min^{-1} and 200°C–300°C at 5°C min^{-1}, respectively. Electron impact ionization mass spectra (80 eV) were obtained using a Finnigan MAT 112-S mass spectrometer interfaced with a Varian 3700 GC. The column used in the GC/MS experiment was the identical column described in the routine GC analyses. For liquid chromatography, a Hewlett-Packard model 1084B liquid chromatograph with a UV detector (200 nm) and a reversed-phase C-18 column was used at a flow rate of 2 ml min^{-1}. Colorimetric assays (520 nm) of uronic acid residues were achieved by a *m*-phenyl-phenol method (Blumenkrantz & Asboe-Hansen, 1973). Total carbohydrate contents were detected colorimetrically (485 nm) by a phenol-sulfuric acid method (Dubois *et al.*, 1956). Methoxy content was determined by GC quantitation of methanol generated from reacting the starting material with 1·5 N KOH in 85% ethanol at 25°C overnight (Knee, 1978).

Fractionation of tobacco pectin

The ground bright tobacco lamina (88 g, tobacco dry weight) was Soxhlet-extracted for 16 h with 3·6 liters 80% ethanol and dried in a vacuum at 40°C to give 44·95 g of residue. This residue (25 g) was then extracted with water (1·5 liters) at 100°C for 1 h to yield a water-soluble pectin fraction. The aqueous extract was first passed through a 0·45 μm microporous nitrocellulose membrane filter to eliminate small particles. The filtrate was then sequentially processed through a series of polysulfone membranes with 1 000 000, 100 000, 10 000 and 1000 nominal MW cut-offs using a Millipore tangential flow filtration system to yield, respectively, 1·3%, 36·6%, 12·7% and 17·9% of the extract. The concen-

trated retentate (400 ml) from the 100000 MW cut-off membrane was then cation exchanged with 20 g of Dowex 50W-X8, H^+ resins (20–50 mesh) and lyophilized to give a cation-free crude pectin fraction (3·43 g), which represented a 7·0% yield from tobacco lamina.

The tobacco residue from the water extraction was then extracted with 0·05 M EDTA-Na_2 solution (500 ml × 3) at 100°C. This extract was fractionated by the same procedure of tangential flow filtration used for the aqueous extract. Cations in the retentate of 100000 MW cut-off were also exchanged with Dowex 50W-X8, H^+ resins. After lyophilization, a second crude pectin fraction (5·28 g) was obtained, which represented a 10·8% yield from tobacco lamina (Fig. 1).

Ion chromatography of the crude tobacco pectin

The crude pectin (2 g) obtained from the aqueous extract was dissolved in 0·1 M piperazine buffer at pH 6, applied to a QAE-Sephadex A-50 anion-exchange resin column (17 × 7 cm), and eluted stepwise with 0·1

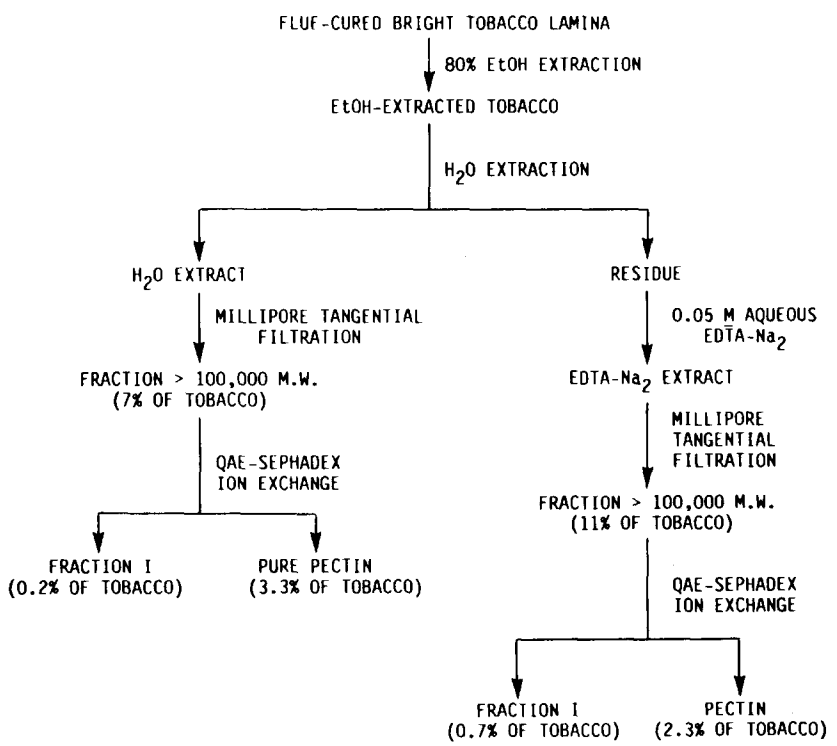

Fig. 1. Fractionation scheme of tobacco pectin.

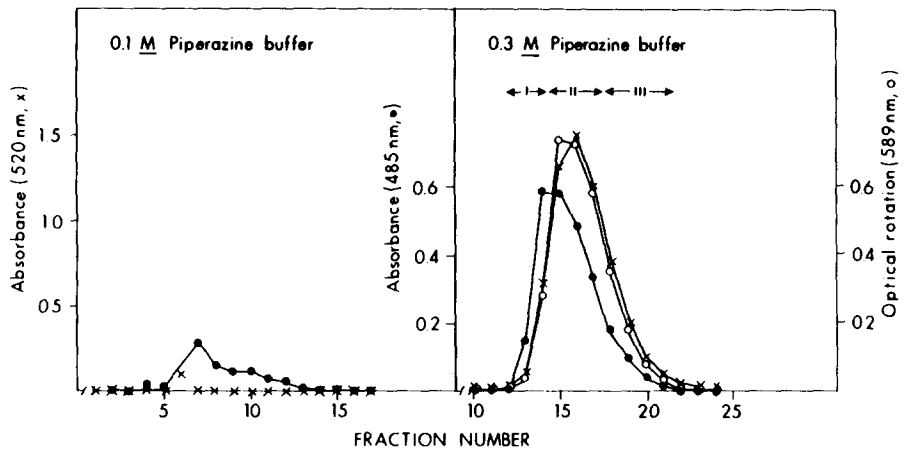

Fig. 2. Ion chromatography of the crude tobacco pectin.

M, 0·3 M, and 0·5 M piperazine buffers. Each fraction (20 ml) collected was assayed for carbohydrates and uronic acid residues using the phenol-sulfuric acid and *m*-phenylphenol colorimetric methods, respectively. Optical rotation was also measured on each fraction. The 0·3 M eluates (Fig. 2) were combined into three fractions, I, II and III, and then dialyzed (12000 daltons cut-off), cation exchanged (Dowex 50W-X8, H^+) and lyophilized. The yields of fractions I (57 mg), II (601 mg) and III (372 mg) represented 0·2%, 2·1% and 1·3%, respectively, of the original tobacco lamina.

Gel filtration of pectin fractions

The pectin fractions I, II and III (50 mg each) eluted from the QAE-Sephadex column were chromated separately on the Fractogel TSK HW-65F columns (40 cm × 2·5 cm × 3) in series with 0·1 M sodium dihydrogen phosphate solution (Fig. 3). Fractions collected (5 ml each) were analyzed for carbohydrates and uronic acid residues by the previously mentioned colorimetric methods. The void volume and included volume of the Fractogel HW-65F column were determined using blue dextran and glucose. The column was further calibrated using dextran standards T10, T70 and T500.

Isolation of pure pectin from the aqueous extract and the EDTA-Na_2 extract

A separate ion chromatography experiment in which 4·1 g of the lyophilized aqueous extract were applied to a QAE-Sephadex A-50 column

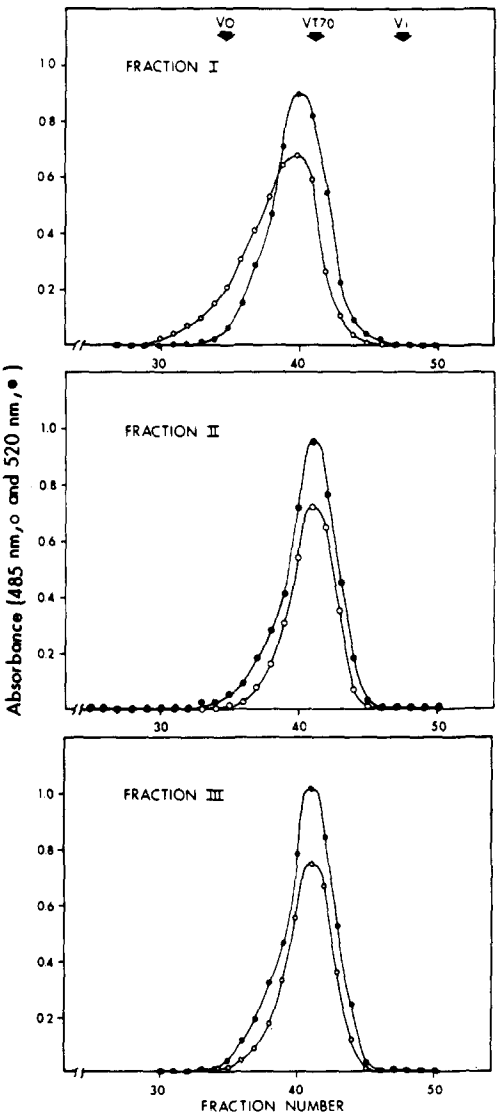

Fig. 3. Gel filtration of pectin fractions I, II and III.

(30 × 7 cm) produced pectin fractions I, II and III, The combined fractions II and III, with a yield of 3·3% from dry tobacco lamina, are referred to as pure pectin in this report.

The pectin-containing retentate (2·0 g) obtained from the tangential flow filtration of the EDTA-Na_2 extract was chromatographed on a QAE-Sephadex A-50 column using the same procedures described previously for the aqueous extract.

De-esterification of the pectin

A sample of the pure pectin (100 mg) in 10 ml of H_2O was saponified with 1 N NaOH (0·6 ml) at pH 12 for 1 h (McCready, 1965). The product was acidified with cation exchange resin (Dowex 50W-X8, H^+) until pH 3 was reached, and then filtered and lyophilized to yield 87 mg of the de-esterified pectin.

Methanolysis and silylation of the pectin

Samples of the pure pectin (1·5–2·0 mg) were dried in a vacuum oven at 40°C overnight and then heated with 4 N HCl in dry methanol (1 ml) at 100°C for 16 h (Pritchard & Todd, 1977). The mixture was neutralized with silver carbonate granules, centrifuged and filtered. Next the methanol was evaporated with a stream of nitrogen. The product was then silylated with 1 ml of trimethylsilylimidazole in pyridine (Tri-Sil Z®, Pierce Chemical Co., Rockford, Illinois, USA) to yield O-silylated methylglycosides.

Neutral sugar analysis of the pectin

The polysaccharide was hydrolyzed with trifluoroacetic acid, according to the procedure of Albersheim *et al.* (1967). Then the hydrolyzed products were reduced with sodium borohydride and acetylated. The resultant alditol acetates were analyzed by GC.

Determination of the absolute configurations of the glycosyl residues

Butanolysis of tobacco pectin with 1 N HCl in R-(−)-2-butanol, S-(+)-2-butanol and (±)-2-butanol separately, using the procedure of Gerwig *et al.* (1979), yielded three mixtures of butyl glycosides. After silylation with Tri-Sil Z® the O-silylated butyl glycosides were separated by GC. By comparison of the three GC profiles, the absolute configurations of D-galacturonic acid, L-rhamnose, L-arabinose, D-galactose and D-glucose were determined.

Methylation analysis of the polysaccharide

The polysaccharide was methylated by a modified version of the Hakomori method (Hakomori, 1964; Standford & Conrad, 1966; Philips & Fraser, 1981) using the potassium salt of methylsulfinyl carbanion. The product was absorbed on a Baker C-18 extraction column, eluted with methanol, and evaporated to dryness with nitrogen.

The methylated polysaccharide was reduced with lithium aluminum deuteride in oxolane (THF) according to the procedure of Standford & Conrad (1966). The methylated, reduced product was hydrolyzed with 2 N TFA (500 μl) at 120°C for 3 h, reduced with sodium borohydride (10 mg) in 1 N ethanolic NH_4OH (500 μl) for 1 h, and then acetylated with acetic anhydride (100 μl) at 120°C for 3 h. The resultant partially methylated alditol acetates were analyzed by GC.

Carbodi-imide reduction of the pure pectin

The pure pectin (120 mg) was reacted with 1-cyclohexyl-3-(2-morpholino-ethyl) carbodi-imide metha-*p*-toluenesulfonate (10 mol eq) in 25 ml) of H_2O at room temperature (Taylor & Conrad, 1972). As the reaction proceeded, the pH of the reaction mixture was maintained at 4·75 with automatic titration of 0·05 N hydrochloric acid using a Metrohm pH-Stat. After the reaction had proceeded for 2 h, the reaction mixture was treated with 15 ml of 2 M aqueous sodium borodeuteride solution at pH 7 over a 2 h period. A drop of octanol was added to prevent foaming. The mixture was dialyzed against distilled water for two days and lyophilized, and then the reaction was repeated. Completion of reaction was indicated by an absence of absorptions for carboxyl carbons in the ^{13}C NMR spectrum and a colorless response from the *m*-phenyl-phenol colorimetric analysis. The reduced pectin was then purified by gel filtration (Fractogel HW-65 F).

Partial hydrolysis of the polysaccharide

The dideuterido, carbodi-imide-reduced pectin (12 mg) was partially hydrolyzed with 1 ml of 88% formic acid for 100 min at 80°C. The mixture thus obtained was reduced with 500 μl of 1·0 N aqueous NH_4OH solution containing 40 mg ml^{-1} $NaBD_4$ and O-methylated by the Hakomori method to give O-methylated di- and oligosaccharide alditols. These derivatives were separated by h.p.l.c. using a C-18 reversed-phase column with CH_3CN and H_2O gradient elution (30% CH_3CN/H_2O for 15 min, 30%–90% CH_3CN/H_2O for 10 min, at a flow rate of 2 ml min^{-1}). Each fraction was further examined by GC/MS using electron impact fragmentation to establish the identities of the derivatives (Kochetkov & Chizhov, 1966).

RESULTS AND DISCUSSION

The flue-cured bright tobacco lamina was Soxhlet-extracted with 80% ethanol to remove low molecular weight sugars and other extractives.

The ethanol-insoluble residue was extracted with water at 100°C and the aqueous extract was fractionated by a Millipore tangential flow filtration system. The largest fraction was the retentate from the 100 000 MW cut-off membrane (Fig. 1). After cations were removed, the crude pectin fraction was separated on a QAE-Sephadex A-50 column using stepwise elution with 0·1 M, 0·3 M, and 0·5 M piperazine buffers. The elution profile is shown in Fig. 2. Small amounts of neutral polysaccharides were eluted with 0·1 M buffer. Pectinaceous material was eluted with 0·3 M buffer. Three fractions (I, II and III) were collected representing, respectively, the beginning, middle and end of a single broad peak. Fraction I had a greater relative content of neutral sugar residues compared to the other two fractions. Only traces of carbohydrate components were detected in the 0·5 M eluates.

Each pectin fraction was chromatographed separately on a Fractogel TSK HW-65F column as shown in Fig. 3. It may be seen for Fraction I that there was a higher relative content of neutral sugar residues in the front of the peak profile. Fraction I also had a greater molecular weight than the other two fractions.

^{13}C NMR spectra of fractions I, II and III (Fig. 4) showed predominant resonances at 71·0, 71·2, 73·3, 81·2, 102·8 and 174·9 ppm, which are in excellent agreement with those obtained on the sodium salt of 4-linked α-D-polygalacturonic acid (Sigma Chemical Co., St Louis, Missouri, USA). The methyl ester form of some of the galacturonic acid residues was detected by ^{13}C resonances at 55·9, 73·5, 81·7, 103·1 and 173·6 ppm. These assignments were confirmed by the lack of methyl ester

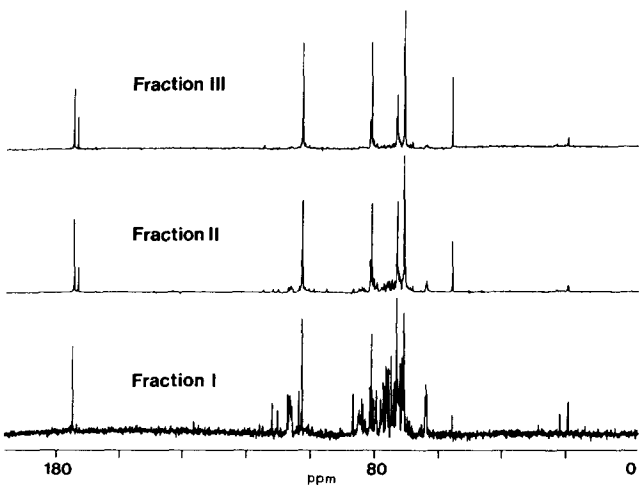

Fig. 4. ^{13}C NMR spectra of pectin fractions.

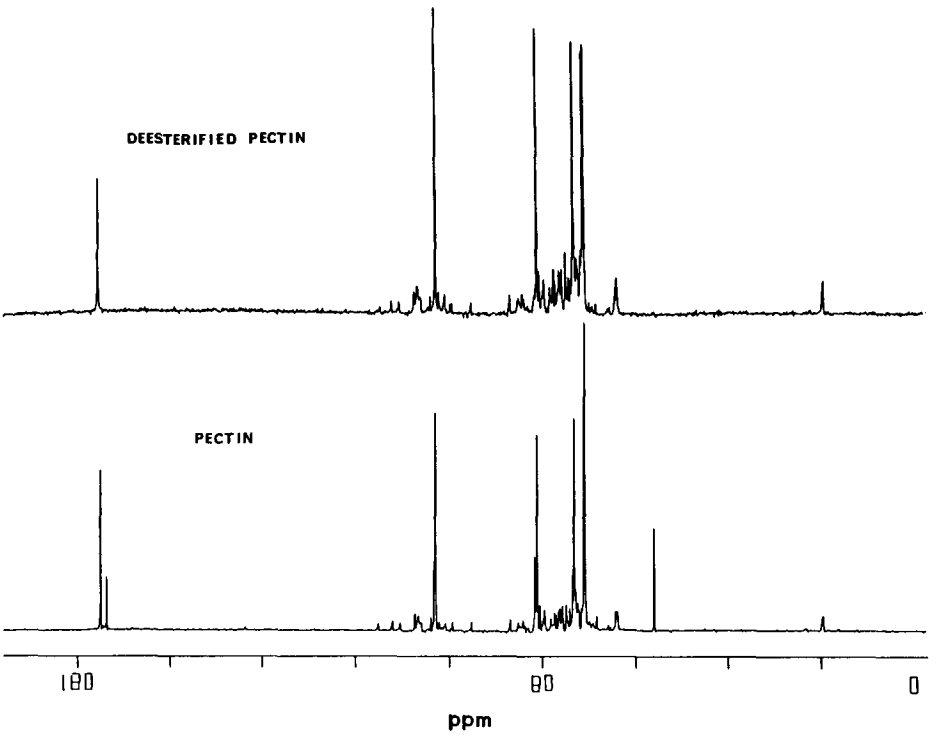

Fig. 5. ^{13}C NMR spectra of pectin and de-esterified pectin.

peaks in the de-esterified pectin (Fig. 5). The two minor absorptions at 19·6 ppm were assigned to the C-6 methyl groups of the rhamnose residues. Absorptions for the primary alcohol carbons of neutral sugars such as galactose and arabinose were observed at 63·6–64·3 ppm. The anomeric carbon (C-1) of galactose absorbed at 106 ppm, which indicate the presence of 4-linked β-D-galactopyranosyl residues (Bradbury & Jenkins, 1984). A minor resonance at 110·4 ppm may correspond to the anomeric carbon of 5-linked α-L-arabinofuranosyl residues (Bradbury & Jenkins, 1984). The presence of a very small amount of acetyl groups was suggested by the methyl carbon signals at 23 ppm. It is evident from the comparison of these three spectra that the ratio of galacturonic acid residues to neutral sugars and the degree of esterification of these pectins are different. Fraction I contained considerably more neutral sugar residues including rhamnose and O-acetyl substituents, but less methyl esterified galacturonic acid than the other two fractions.

On the basis of their ^{13}C NMR spectra and gel filtration profiles, fractions II and III were seen to be similar, but they had slight variations in neutral sugar and methyl ester contents. Gel filtration of the two com-

bined fractions showed a single peak profile and an almost constant ratio of carbohydrates to uronic acid residues, indicating chemical homogeneity. These two combined fractions represent the majority of the pectins present in bright tobacco lamina. A separate large scale ion chromatography experiment produced a pectin fraction containing both fractions II and III with a yield of 3·3% from dry tobacco lamina. This pure pectin fraction had an optical rotation of $[\alpha]_D^{25} + 160°$ ($c = 0{\cdot}1$, H_2O).

The EDTA extract of the tobacco residue left after water extraction was fractionated by the same procedures of tangential flow filtration and QAE-Sephadex ion chromatography. Another pure pectin fraction, identical by ^{13}C NMR spectroscopy, was obtained with a yield of 2·3% from the starting tobacco. The fraction I pectin with high neutral sugar content was obtained in higher yield (0·7%) than from the aqueous extract.

Similar glycosyl compositions were determined for the pure tobacco pectin by two separate methods (Table 1): (i) GC quantitation of O-sily-

TABLE 1
Glycosyl Composition of the Tobacco Pectin

Glycosyl residue	*Glycosyl composition*[a]	
	A (%)	*B (%)*
Galacturonic acid	~ 80	79·8
Rhamnose	3·7	5·2
Arabinose	4·5	4·8
Xylose	0·3	—
Galactose	10·0	10·1
Glucose	1·5	0·1

[a]Glycosyl composition determined by the *m*-phenylphenol method for uronic acid residues and GC quantitation of alditol acetates for neutral sugars (A), and by GC after methanolysis and silylation (BP).

lated methyl-glycoside derivatives, using correction factors for the individual components, and (ii) combining values from the neutral sugar analysis and the *m*-phenyl-phenol procedure for uronic acid residues. Saponification of the pectin generated 3·1% methanol indicating a degree of esterification of 21·7%.

In the pure pectin the galactose and galacturonic acid residues were found to be in the D-configuration and the rhamnose and arabinose residues were in the L-configuration. Methylation analysis (Table 2) revealed

TABLE 2
Glycosyl Linkage Patterns of the Pure Pectin

Glycosyl residue	*Positions of O-methyl groups*	*RRT × 1000 of methylated alditol acetate*	*Structural units*
Arabinosyl	2,3,5	389	Ara(f)→
Arabinosyl	2,3	531	→5-Ara(f)→ or →4-Ara(p)→
Rhamnosyl	2,3,4	435	Rha(p)→
Rhamnosyl	3,4	540	→2-Rha(p)→
Rhamnosyl	3	651	→2,4-Rha(p)→
Galactosyl	2,3,4,6	615	Gal(p)→
Galactosyl	2,3,6	706	→4-Gal(p)→
Galactosyl	2,4,6	727	→3-Gal(p)→
Galactosyl	2,4	899	→3,6-Gal(p)→
Galactopyranosyluronic acid[a]	2,3,4	783	GalA(p)→
Galactopyranosyluronic acid[a]	2,3	867	→4-GalA(p)→

[a]Residues produced by the reduction of galactopyranosyluronic acid residues to galactosyl residues.

the glycosyl linkage patterns of the pure pectin. The incorporation of two D atoms at C-6 in the 2,3-di-O-methyl and 2,3,4-tri-O-methyl galactitol derivatives indicated that these were derived from terminal and 4-linked galacturonic acid residues, respectively. Rhamnosyl residues were found in terminal, 2- and 2,4-linked forms, arabinosyl residues in terminal, 3- and 5-linked forms, and galactosyl residues in terminal, 3-, 4- and 3,6-linked forms. However, the derivatives, 2,3,4-tri-O-methylrhamnitol and 2,3,4-tri-O-methylgalactitol are most likely produced by the degradation of rhamnogalacturonan via β-elimination due to the basic methylation conditions. The molar percentage of the partially methylated alditol acetates obtained from methylation of pectin, de-esterified pectin and reduced pectin in comparison with polygalacturonic acid (sodium salt, Sigma grade II) are reported in Table 3. Because of the degradation of pectin during methylation the results were considered to be qualitative, rather than quantitative.

The carbodi-imide-reduced pure pectin contained 78·8% galactose, 14·8% rhamnose and 6·4% arabinose as determined by GC quantitation after methanolysis and silylation. The increase in rhamnose and arabinose contents from 5·2% and 4·8%, respectively, in the starting material, indicated that a significant quantity of galacturonic acid residues was degraded during the reduction. This neutral polysaccharide, which was

TABLE 3
Glycosyl Linkage Compositions of Reduced Pectins

Glycosyl linkage	*Product compositions (mol %)*			
	A	*B*	*C*	*D*
Ara(f)→	9·8	1·4	1·6	—
→5-Ara(f)→(or→4-Ara(p)→)	5·5	4·7	2·8	—
Rha(p)→	5·4	1·7	1·3	—
→2-Rha(p)→	3·0	5·7	8·9	6·2
→2,4-Rha(p)→	3·9	7·2	6·8	1·6
Gal(p)→	9·5	7·0	6·8	3·8
→4-Gal(p)→	16·7	12·0	47·9	7·5
→3-Gal(p)→	5·6	6·6	4·5	—
→6-Gal(p)→	12·7	14·6	4·0	19·3
→4,6-Gal(p)→	19·6	28·2	—	61·6
→3,6-Gal(p)→	8·3	10·9	7·5	—
→2,3,6-Gal(p)→	—	—	7·9	—

Product	*Starting material*	*Reactions prior to alditol acetate formation*
A	Tobacco pectin	Methylation; LAD reduction
B	Tobacco pectin	Saponification; methylation; LAD reduction
C	Tobacco pectin	Carbodi-imide reduction; methylation
D	Polygalacturonic acid	Methylation; LAD reduction

suitable for determination of the linkage patterns, was then partially hydrolyzed with 88% formic acid. The oligosaccharide mixture thus obtained was reduced and methylated. The resultant derivatives were separated using C-18 reversed-phase h.p.l.c. and then hydrolyzed for structural elucidation (Table 4). The fragments, GalA→2Rha and Rha→4GalA, showed that rhamnose was linked at C-1 and C-2 to galacturonic acid residues. The indentification of Gal→4Rha unambiguously demonstrated that 4-linked galactose residues are covalently linked to the rhamnogalacturonan through C-4 of the rhamnopyranosyl residues. The methylation analysis yielded no evidence of branching at galactopyranosyluronic acid residues. The presence of 5-linked arabinan and 4-linked galactan side chains was supported by the formation of their di- and trisaccharides during partial hydrolysis. No oligosaccharides were found that contained both arabinose and galactose.

CONCLUSIONS

The preceding results suggest that the pure tobacco pectin has a main chain consisting of 4-linked α-D-galactopyranosyluronic acid residues

TABLE 4
Di- and Tri-Saccharide Fragments Identified by Electron-Impact Mass-Spectral Fragment Ions

Disaccharide fragments	*Electron-impact mass-spectral fragment ions*[a]			
	aJ_2	aJ_1	bA_1	bA_2
b a				
GalA→4-GalA→	238	298	221	189
Gal→4-Gal→	236	296	219	187
Ara→5-Ara→	192	252	175	143
Rha→4-GalA→	238	298	189	157
Gal→4-Rha→	206	266	219	187
GalA→2-Rha→	206	266	221	189

Trisaccharide fragment	aJ_2	aJ_1	abJ_2	abJ_1	cbA_1	cbA_2	cA_1	cA_2
c b a								
Ara→5-Ara→5-Ara→	192	252	352	—	335	—	175	143

[a]The nomenclature developed by Kochetkov and Chizhof (1966) is used in describing EI mass spectra.

interspersed with 2-linked L-rhamnopyranosyl residues. Approximately 22% of the galactopyranosyluronic acid residues are methyl-esterified. The main chain is branched at C-4 of L-rhamnose with neutral sugar side chains containing 4-linked β-D-galactopyranosyl residues. 5-Linked α-L-arabinofuranosyl residues are also present in the side chains. However, it has not yet been determined whether the 5-linked arabinose is attached at C-4 of rhamnose in the main chain, or to galactose in the side chain or both. Small amounts of 3- and 3,6-linked galactopyranosyl residues were also found, suggesting some degree of branching of the 4-linked β-galactan side chains, but the exact locations are not known. The proposed structural features are illustrated in Fig. 6. This structure is

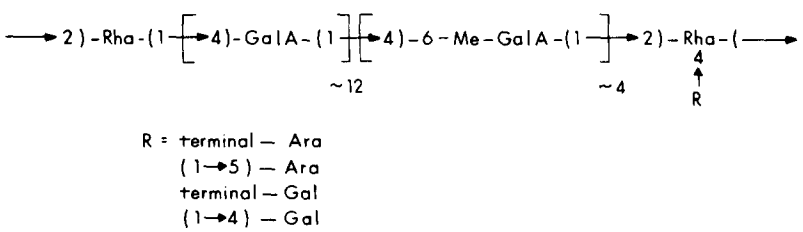

Fig. 6. Structure of tobacco rhamnogalacturonan.

consistent with the basic structural elements found in pectin from other sources. This pectin, which represents the majority of the pectin in a single grade of flue-cured bright tobacco lamina, is similar to, but not identical with, the pectin isolated by Eda & Katō (1980) from uncured tobacco stems.

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