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A PECTIC POLYSACCHARIDE FROM CELL WALLS OF TOBACCO *(Nicotiana tabacum)* MESOPHYLL

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ABSTRACT

A pectic polysaccharide has been purified from the pectin fraction of the cell-wall material of tobacco mesophyll by ion-exchange chromatography and gel filtration, and shown to contain D-galacturonic acid, L-rhamnose, L-arabinose, and D-galactose in the molar ratios 88.2:2.8:3.1:5.9. The polysaccharide contains at least three regions, namely, galacturonan, rhamnogalacturonan, and galactan.

INTRODUCTION

In previous studies of cell-wall polysaccharides of tobacco midrib, pectic polysaccharides[1,2], galactan[3], arabinoxyloglucan[4–6], 4-*O*-methylglucuronoxylan[7], and galactoglucomannan[8] were isolated and characterised. We now report the isolation and structural investigation of a pectic polysaccharide from the cell-wall materials (CWM) of the tobacco mesophyll.

RESULTS AND DISCUSSION

The CWM obtained from tobacco mesophyll contained 30.8% of uronic acids, 38.3% of neutral sugars, 4.0% of lignin, and 10.0% of protein. Rhamnose, arabinose, xylose, mannose, galactose, and glucose were detected in the molar ratios 3.3:6.3:7.4:3.2:10.3:69.4. The CWM was extracted with hot aqueous EDTA to give a pectin fraction, which was eluted from DEAE-cellulose (AcO^- form), stepwise with 0.05, 0.25, 0.5, and 1.0M sodium acetate buffer (pH 6.0), and 0.5M sodium hydroxide. The yields and analyses of some of the fractions are listed in Table I. The major fraction, eluted with 0.5M buffer, contained 92.9% of uronic acid, and gave rhamnose, arabinose, and galactose in the molar ratios 25.3:27.8:47.0 on acid hydrolysis. Gel filtration (Fig. 1) of the major fraction on Sepharose CL-6B revealed a major component (elution volume, 2.2 V) and a minor component at the void volume. The major component was isolated to give a purified pectic polysaccharide (PPS).

Purified PPS had $[\alpha]_D$ +195° (*c* 0.2, 0.1M sodium acetate, pH 6.0), was homogeneous in zone electrophoresis and on column chromatography on DEAE-

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TABLE I

DEAE-CELLULOSE COLUMN CHROMATOGRAPHY OF THE PECTIN FRACTION OF TOBACCO MESOPHYLL

Fraction	*Yield (mg)*	*Uronic acid content (wt.%)*	*Neutral sugar content (wt.%)*	*Neutral sugar composition (Mol%)*					
				Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*
Pectin	2000	85.7	6.0	31.4	22.7	3.0	+	37.9	5.0
0.05M buffer	218	52.4	8.0	16.0	30.8	+	–	41.2	12.0
0.25M buffer	107	56.6	14.1	13.0	37.3	–	–	49.7	–
0.5M buffer	849	92.9	10.9	25.3	27.8	–	–	47.0	–
1.0M buffer	84	78.6	2.0	17.4	35.5	–	–	47.2	–

Sephadex A-50 using a linear gradient of sodium chloride (Fig. 2), and contained D-galacturonic acid, L-rhamnose, L-arabinose, and D-galactose in the molar ratios 88.2:2.8:3.1:5.9. The molecular weight of PPS was estimated to be 16,000 by gel filtration on Sepharose CL-6B (Fig. 1).

PPS was methylated by the Hakomori[11] method, the methylated product (which showed no i.r. absorption for hydroxyl groups) was hydrolysed, and the products were converted into the alditol acetates. G.l.c.–m.s. then revealed derivatives of 3,4-di- and 3-*O*-methylrhamnose, 2,3,5-tri- and 2,3-di-*O*-methylarabinose,

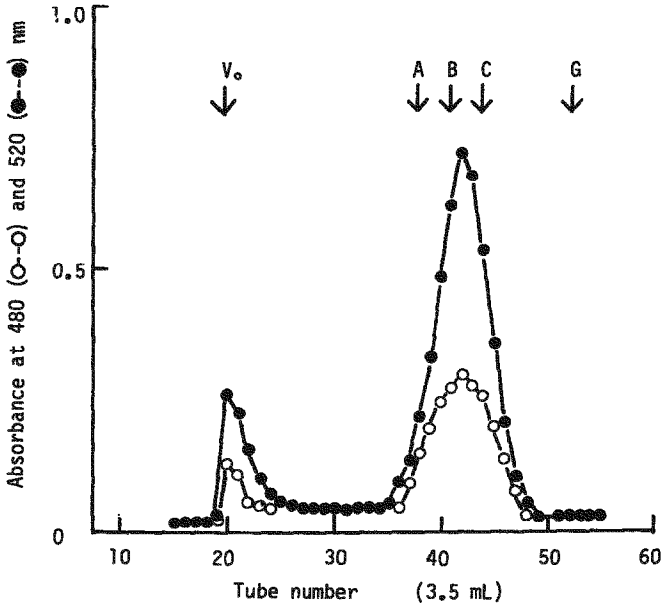


Fig. 1. Elution profile on Sepharose CL-6B of crude pectic polysaccharide. Fractions 37–48 contained PPS. Tube numbers at which Blue Dextran (V_0), standard dextrans T-70 (A), T-40 (B), T-10 (C), and galactose (G) appeared are indicated by arrows. Fractions (3.5 mL) were analysed for total sugars (—○—) and uronic acids (—●—) by the phenol–sulfuric acid method[9] and 3-hydroxybiphenyl method[10], respectively.

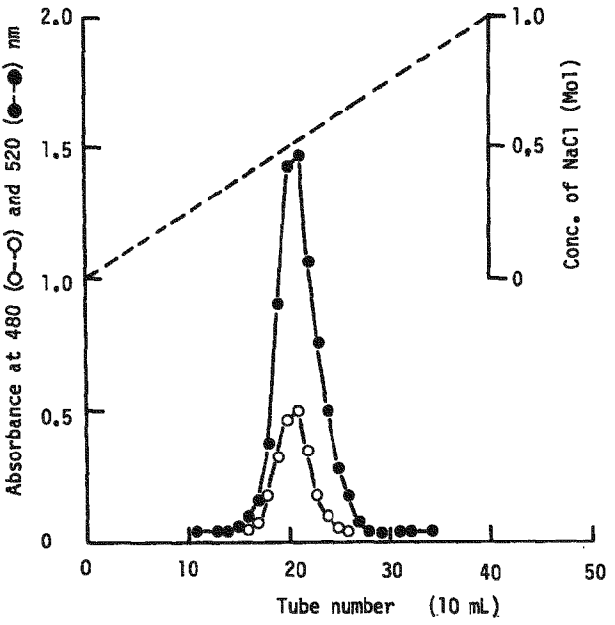


Fig. 2. Elution profile on DEAE-Sephadex A-50 of PPS. A solution of PPS (20 mg) in 20 mL of 50mM potassium phosphate buffer (pH 7.0) was applied to a column (2 × 10 cm) of DEAE-Sephadex A-50 equilibrated with 50mM potassium phosphate buffer (pH 7.0). The column was washed with the buffer, and the absorbed PPS was eluted with a linear sodium chloride gradient (200 mL of the buffer and 200 mL of the buffer containing M sodium chloride). The contents of total sugars (—○—) and uronic acids (—●—) were measured colorimetrically.

and 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methylgalactose (Table II). Methylation analysis of the carboxyl-reduced PPS (PPS-R) gave 2,3,6-tri-*O*-methylgalactose as a prominent product together with small proportions of the above methylated sugars (Table II). These results suggested the rhamnosyl residues to be (1→2)-linked, and about one-third to be substituted at position 4. The arabinosyl residues were (1→5)-linked and the galactosyl residues were (1→4)-linked. The presence of (1→4)-linked galactosyluronic acid residues was also suggested by the increased proportion of 2,3,6-tri-*O*-methylgalactose on methylation analysis of PPS-R.

Partial acid hydrolysis of PPS afforded an insoluble degraded polysaccharide (DPS) together with neutral and acidic fragments. DPS contained only D-galacturonic acid, and the carboxyl-reduced DPS gave 2,3,6-tri-*O*-methylgalactose on methylation analysis, indicating DPS to be a (1→4)-linked galacturonan.

The neutral fragments were fractionated by gel filtration on Bio-Gel P-2 (Fig. 3). Fraction 1 contained L-rhamnose, L-arabinose, and D-galactose in the molar ratios 1:85:4. Fraction 2 contained *O*-*α*-D-galactopyranosyl-(1→4)-D-galactose as a main component which was identified by t.l.c. using the authentic sample[3]. Fraction 3, eluted at the void volume, gave only D-galactose on acid hydrolysis, and methylation analysis gave 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose in the molar ratio

TABLE II

METHYLATION ANALYSES OF PECTIC POLYSACCHARIDE (PPS) AND CARBOXYL-REDUCED PPS (PPS-R)

Methylated sugar[a]	T_1[b]	T_2[b]	*Mole percent*		*Mode of linkage*
			PPS	*PPS-R*	
3,4-Rha	0.92	0.87	17.6	3.6	→2)-L-Rha*p*-(1→
3-Rha	1.05	1.19	8.8	2.0	→2,4)-L-Rha*p*-(1→
2,3,5-Ara	0.74	0.68	4.2	0.8	L-Ara*f*-(1→
2,3-Ara	0.91	0.99	15.3	4.9	→5)-L-Ara*f*-(1→
2,3,4,6-Gal	1.00	1.00	2.9	0.4	D-Gal*p*-(1→
2,3,6-Gal	1.11	1.21	49.0	87.1	→4)-D-Gal*p*-(1→
2,3-Gal	1.34	2.13	2.2	1.2	→4,6)-D-Gal*p*-(1→

[a]3,4-Rha = 3,4-di-*O*-methylrhamnose, *etc.* [b]Retention times of the derived alditol acetates on an OV-101 capillary column programmed at 2°/min from 150 to 220° (T_1) and on an SP-1000 capillary column programmed at 2°/min from 150 to 220° (T_2), relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol.

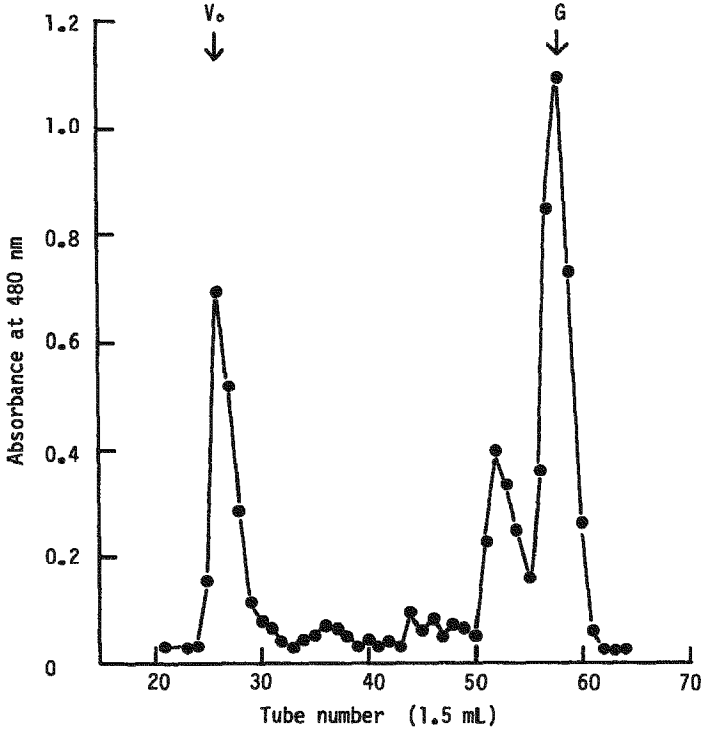


Fig. 3. Gel filtration of the neutral fraction of the partial acid hydrolysate of PPS. A solution of the neutral fraction (8 mg) in water (1 mL) was applied to a column (1.6 × 90 cm) of Bio-Gel P-2 and eluted with water: fraction 1, 56–61; 2, 51–55, 3, 25–30. Tube numbers at which Blue Dextran (V_0) and galactose (G) appeared are indicated by arrows. Fractions (2.6 mL) were analysed by the phenol–sulfuric acid method.

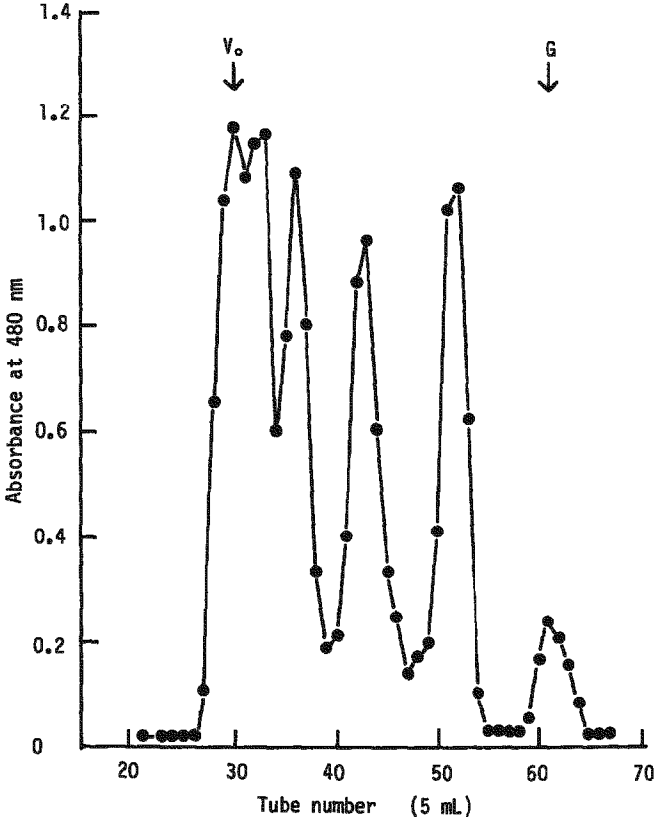


Fig. 4. Gel filtration of the acidic fraction of the partial acid hydrolysate of PPS. A solution of the acidic fraction (30 mg) in 50mM sodium acetate buffer (pH 6.0) was applied to a column (2.6 × 80 cm) of Bio-Gel P-2 and eluted with the buffer to give fractions 1, 59–64; 2, 50–54; 3, 41–47; 4, 35–39; 5, 32–34; 6, 27–31. Tube numbers at which Blue Dextran (V_0) and galacturonic acid (G) appeared are indicated by arrows.

~1:10. The ^{13}C-n.m.r. spectrum showed a signal at 105.4 p.p.m. for anomeric carbon, indicating[12] the presence of β-D-galactopyranosyl residues. Thus, fraction 3 was a (1→4)-β-D-galactan with a d.p. of ~10.

The acidic fragments were separated into six fractions by gel filtration on Bio-Gel P2 (Fig. 4). Fraction 1 contained only D-galacturonic acid. P.c. of each fraction in Fig. 4 revealed that fraction 2 contained two components with R_{GalA} 1.20 and 0.89 (oligosaccharides **1** and **2**, respectively), and that fractions 3–6 contained one main component (oligosaccharides **3**–**6**, respectively). These components were purified by ion-exchange column chromatography on DEAE-Sephadex A-25, exemplified in Fig. 5 which shows the elution profile of fraction 2. The purified oligosaccharides were homogeneous in p.c. and zone electrophoresis, and their yields and properties are summarised in Table III.

Oligosaccharide **1**, which contained L-rhamnose and D-galacturonic acid in

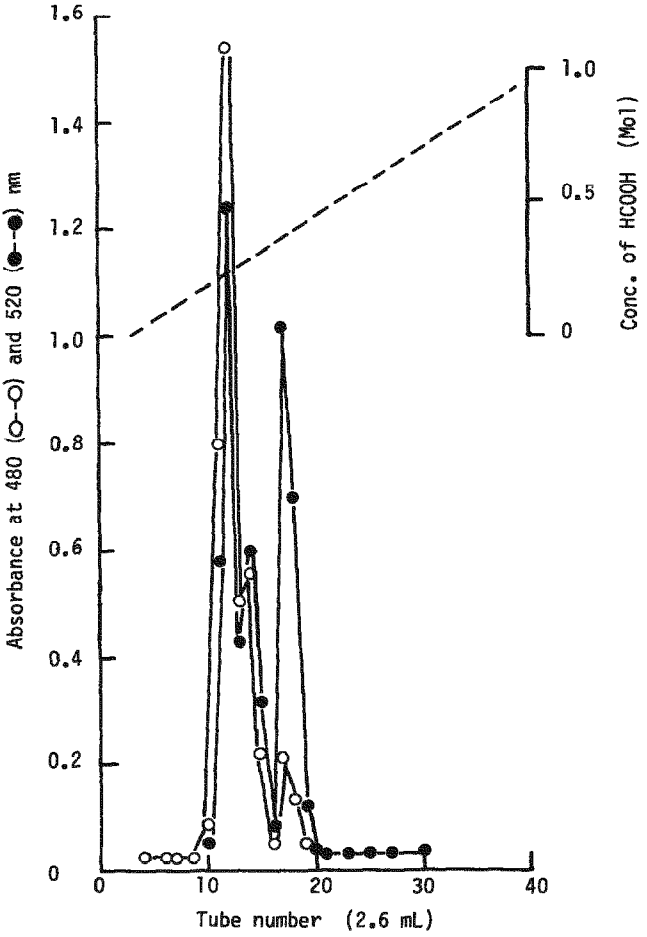


Fig. 5. Elution profile of fraction 2 on DEAE-Sephadex A-25. A solution of fraction 2 in water (1 mL) was applied to a column (0.8 × 50 cm) of DEAE-Sephadex A-25 equilibrated with 50mM formic acid. The column was washed with water, and the absorbed oligosaccharides were eluted with a linear formic acid gradient (50 mL of water and 50 mL of M formic acid). Fractions (2.6 mL) were analysed colorimetrically. Fractions 11–13 contained oligosaccharide **1**, and 17–19 contained oligosaccharide **2**.

the molar ratio ~1:1, was reduced and methylated. G.l.c.–m.s. of the resulting methylated alditol (*T* 26.3 min) gave the pertinent ions listed in Table IV. The presence of ions at *m/z* 233 and 205 suggested that **1** was a hexuronosyl-deoxyhexitol (Scheme 1). The linkage between hexuronic acid and deoxyhexose was deduced as (1→2) from the relative intensities of the ions at *m/z* 147 and 103, which are diagnostic[13] for methylated deoxyhexitol substituted at position 2. The ^{13}C-n.m.r. spectrum of reduced **1** showed a signal for an anomeric carbon at 98.6 p.p.m. ($J_{C\text{-}1,H\text{-}1}$ 170 Hz) which was assigned to that of α-D-Gal*p*A. Thus, **1** was *O*-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnose.

Oligosaccharide **2** contained D-galacturonic acid only. G.l.c.–m.s. of the

TABLE III

YIELDS AND PROPERTIES OF ACIDIC OLIGOSACCHARIDES (1–6) DERIVED FROM PPS BY PARTIAL ACID HYDROLYSIS

Oligo-saccharide	*Yield (mg)*	R_{GalA}[a]	M_{Glc}[b]	$[\alpha]_D$ *(degrees)*	*Sugar composition (Mol%)*	
					GalA	*Rha*
1	7	1.20	0.58	+80	52	48
2	2	0.89	0.89	+118	100	—
3	5	0.62	0.64	+135	55	45
4	5	0.40	0.67	+120	53	47
5	3	0.19	0.69	+107	51	49
6	7	n.d.	0.71	+99	48	52

[a]Mobilities in p.c., relative to D-galacturonic acid, with ethyl acetate–acetic acid–formic acid–water (18:3:1:4). [b]Mobilities in zone electrophoresis, relative to D-glucose, with 0.1M sodium borate (pH 9.2) at 1600 V for 40 min.

derived methylated alditol (*T* 32.2 min) gave ions at *m/z* 233 and 249 (Table IV), suggesting **2** to be a hexuronosyl-hexuronic acid (Scheme 1). The linkage between two galacturonic acid residues was deduced as (1→4) from the relative intensities of the ions at *m/z* 409, which are diagnostic[13] of methylated GalA-OH substituted at position 4. The ^{13}C-n.m.r. spectrum of **2** contained three signals for anomeric carbons at 100.9 ($J_{C\text{-}1,H\text{-}1}$ 170 Hz), 97.3, and 99.3 p.p.m. The first signal was assigned to α-D-Gal*p*A and the last two to non-reducing terminal D-GalA. Thus, **2** was *O*-(α-D-galactopyranosyluronic acid)-(1→4)-D-galacturonic acid.

Oligosaccharide **3** contained L-rhamnose and D-galacturonic acid in the molar ratio ~1:1. F.d.-m.s. of **3** gave an $[M + Na]^+$ ion with *m/z* 685 as the base peak, indicating it to be a tetrasaccharide consisting of two rhamnose and two galacturonic acid residues. Methylation analysis of the reduced **3** revealed 2-substituted rhamnitol and 2-substituted rhamnose residues, and indicated that one of the rhamnose residues in **3** was present at the reducing terminal and that the other was internal. Methylation analysis of carboxyl-reduced **3** gave 2,3,6-tri- and 2,3,4,6-tetra-*O*-methylgalactose in addition to the above derivatives of rhamnose, suggesting that the non-reducing terminal of **3** was GalA. The sequence of rhamnose and galacturonic acid residues was deduced from the m.s. fragmentation pattern of the methylated alditol derivative of **3**. The mass spectrum (Table IV) contained aA (*m/z* 233, 201, and 169) and dA (*m/z* 205, 173, and 141) series of ions, also suggesting the presence of non-reducing terminal D-galacturonic acid and terminal rhamnitol residues, respectively. The rhamnitol was inferred to be linked to the internal galactosyluronic acid residue from the relative abundance of the ion at *m/z* 483 (bcdJ). The terminal galactosyluronic acid group was thought to be linked to the internal rhamnosyl residue from the relative abundance of the ions of baA (*m/z* 407 and 375) series. Thus, the sequence of the four sugar residues of **3** is as shown in Scheme 2. The ^{13}C-n.m.r. spectrum of **3** contained five signals for anomeric carbons

TABLE IV

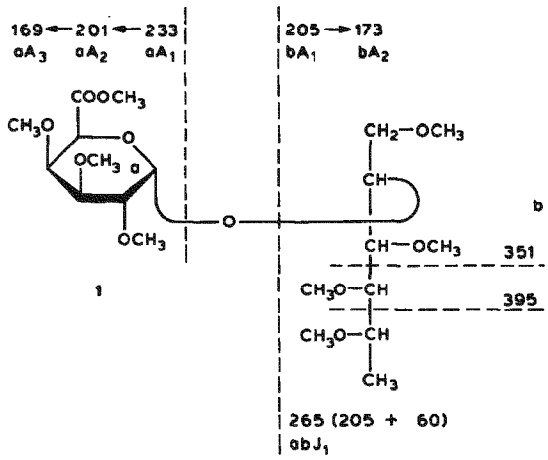
DIAGNOSTIC IONS OBTAINED ON M.S. OF PERMETHYLATED ALDITOLS DERIVED FROM OLIGOSACCHARIDES **1–3**

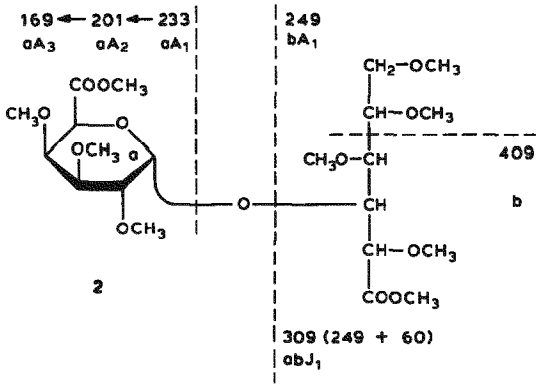
Diagnostic ion (m/z)	*Relative abundance*		
	1	**2**	**3**
45	23.0	75.3	17.0
59	100.0	39.3	77.9
71	56.1	—	38.4
75	27.3	—	36.4
85	15.5	—	25.3
88	29.0	95.2	87.0
89	—	45.5	12.4
101	52.9	100.0	91.4
103	24.9	—	32.0
111	8.5	—	23.9
115	10.2	—	11.5
129	—	21.2	11.9
133	15.3	12.7	14.0
141	11.0	14.9	17.1
145	12.7	—	17.7
147	3.1	—	—
169	3.1	6.0	16.8
173	6.6	9.1	12.5
201	26.2	39.3	63.8
205	70.1	6.9	100.0
233	29.7	24.2	79.4
249	—	35.3	—
265	2.6	—	—
309	—	13.8	—
351	1.0	—	—
375	—	—	9.5
395	0.6	—	—
407	—	—	4.3
409	—	5.4	—
483	—	—	1.5
657	—	—	0.9
711	—	—	0.2

at 100.2 ($J_{C\text{-}1,H\text{-}1}$ 172 Hz), 99.8 ($J_{C\text{-}1,H\text{-}1}$ 170 Hz), 99.5 ($J_{C\text{-}1,H\text{-}1}$ 170 Hz), 94.7, and 92.4 p.p.m., which were assigned to α-L-Rha*p* (110.2), α-D-Gal*p*A (99.8 and 99.5), and reducing terminal L-Rha (94.7 and 92.4), respectively. Thus, **3** was α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→2)-L-Rha.

Both oligosaccharides **4** and **5** contained L-rhamnose and D-galacturonic acid in the molar ratio ~1:1 (Table III). According to the graphical method[14] of mobilities in p.c., **4** and **5** were hexa- and octa-saccharides, respectively. Partial acid hydrolysis of **4** and **5** gave mainly **1** which was detected by p.c. and also by g.l.c.–m.s. of its methylated alditol. Thus, **4** and **5** are hexa- and octa-saccharides, respectively, containing the repeating unit **1**.

Oligosaccharide **6** also contained L-rhamnose and D-galacturonic acid in the



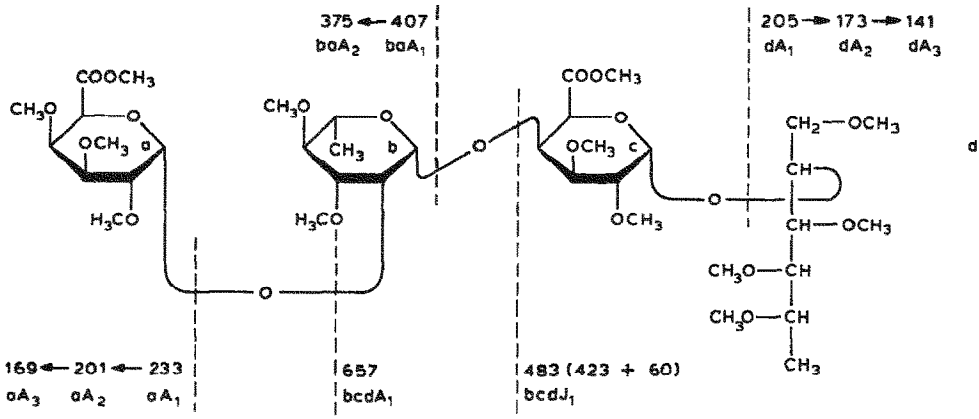


Scheme 1. Mass fragmentation patterns of the permethylated alditols of **1** and **2**.

molar ratio ~1:1 (Table III), and was estimated to be larger than an octasaccharide. Partial acid hydrolysis of **6** gave mainly **1**, indicating that **6** also has a repeating unit of **1**.

Thus, the pectic polysaccharide (PPS) from the tobacco mesophyll contains acidic and neutral moieties with the former comprising galacturonan and rhamnogalacturonan. The rhamnogalacturonan region is thought to consist of repeating units of *O*-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnose, and the neutral material is a (1→4)-β-D-galactan.

The pectic polysaccharides reported so far have been thought to consist of "smooth regions" (blocks of homogalacturonan) and "hairy regions" (blocks of rhamnogalacturonans carrying side chains of oligo- and poly-saccharides composed of arabinose, galactose, xylose, and/or glucose residues)[15,16]. PPS also appears to



Scheme 2. Mass fragmentation pattern of the permethylated alditol of 3.

contain two such blocks and, in the hairy regions, a (1→4)-β-D-galactan appears to be linked to the rhamnogalacturonan backbone at O-4 of rhamnosyl residues.

EXPERIMENTAL

The general methods have been reported previously[1,8,18].

Preparation of CWM from tobacco mesophyll. — *Nicotiana tabacum* L., cv. Bright Yellow, was grown in a greenhouse and harvested at an early stage of flower breeding. Mesophyll (intercostal region of the leaves) was cut from the leaves with a razor blade and stored in a freezer until use.

CWM from the tobacco mesophyll was prepared[8] according to the method of Ring and Selvendran[19]. Fresh mesophyll (1 kg) gave 20 g of dried CWM.

Isolation of the pectic polysaccharide. — CWM (8.8 g) was extracted with 50mM EDTA-Na_2 in 50mM sodium acetate buffer (pH 6.0, 3 × 1 L) at 100° for 1 h. The combined extracts were dialysed and freeze-dried to give a pectin fraction (2.77 g), a solution of a portion (2.5 g) of which in 50mM sodium acetate buffer (pH 6.0) was applied to a column (30 × 5 cm) of DEAE-cellulose (AcO^- form). The column was eluted stepwise with 0.05, 0.25, 0.5, and 1.0M sodium acetate buffer (pH 6.0), and 0.5M sodium hydroxide. The main fraction eluted with the 0.5M buffer was dialysed and freeze-dried to give a crude pectic polysaccharide (850 mg), a solution of which in 0.5M sodium acetate buffer (pH 6.0) containing 5mM EDTA was eluted from a column (2.6 × 90 cm) of Sepharose CL-6B with the same buffer. The eluate was dialysed and freeze-dried to give a purified pectic polysaccharide (PPS, 780 mg).

Partial acid hydrolysis of the polysaccharide. — The polysaccharide (550 mg) was treated with 0.05M H_2SO_4 at 100° for 1 h. Insoluble polysaccharide was separated by centrifugation and re-treated with 0.25M H_2SO_4. The insoluble residue was collected by centrifugation, suspended in water, dialysed, and freeze-dried, to

give a degraded polysaccharide (DPS, 350 mg). The combined aqueous hydrolysates were neutralised with barium carbonate, filtered, treated with Dowex 50W (H^+) resin to remove barium ions, concentrated, and freeze-dried. A solution of the resulting degraded product (50 mg) in water was separated on a column (2 × 20 cm) of DEAE-Sephadex A-25 ($HCOO^-$ form) into neutral (10 mg, eluted with water) and acidic (30 mg, eluted with 5M HCO_2H) fractions. An aqueous solution of each fraction was applied to a column (1.6 × 90 cm) of Bio-Gel P-2. Fractions were analysed by the phenol–sulfuric acid method[9]. Some of the acidic oligosaccharides in each fraction was purified by ion-exchange column chromatography on DEAE-Sephadex A-25 ($HCOO^-$ form), using an HCO_2H gradient. The purities of the oligosaccharides were checked by p.c. with ethyl acetate–acetic acid–formic acid–water (18:3:1:4) and by zone electrophoresis in 0.1M sodium borate (pH 9.2) at 1600 V.

Carboxyl-reduction of acidic polysaccharides. — PPS and DPS were reduced[20] with $NaBH_4$. Three treatments achieved ~70% reduction of the uronic acid, as checked by the 3-hydroxybiphenyl method[10].

Sugar analyses of acidic oligosaccharides. — Each oligosaccharide (~1 mg) was hydrolysed with aqueous 3% H_2SO_4 (1 mL) in a sealed tube at 120° for 1 h. The neutral sugars and uronic acids[21] in the hydrolysate were converted into the alditol acetates and analysed by g.l.c.

Methylation of acidic oligo-alditols. — Each oligosaccharide (~1 mg) was reduced with $NaBH_4$, and then methylated by the Hakomori[11] method. The resulting methylated oligo-alditols were subjected to g.l.c.–m.s., using a glass capillary column (0.28 × 50 m) coated with OV-101 at 240°, or to direct m.s.

Partial acid hydrolysis of oligosaccharide. — Each oligosaccharide (~1 mg) was heated with 0.5M trifluoroacetic acid at 100° for 1 h. The acid was evaporated and the residue was converted into the methylated alditols as described above.

REFERENCES

1 S. Eda and K. Katô, *Agric. Biol. Chem.*, 44 (1980) 2793–2801.
2 S. Eda, K. Katô, A. Ishizu, and J. Nakano, *Agric. Biol. Chem.*, 46 (1982) 1783–1790.
3 S. Eda and K. Katô, *Agric. Biol. Chem.*, 42 (1978) 2253–2257.
4 S. Eda and K. Katô, *Agric. Biol. Chem.*, 42 (1978) 351–357.
5 M. Mori, S. Eda, and K. Katô, *Agric. Biol. Chem.*, 43 (1979) 145–149.
6 M. Mori, S. Eda, and K. Katô, *Carbohydr. Res.*, 84 (1980) 125–135.
7 S. Eda, F. Watanabe, and K. Katô, *Agric. Biol. Chem.*, 41 (1977) 429–434.
8 S. Eda, Y. Akiyama, K. Katô, R. Takahashi, I. Kusakabe, A. Ishizu, and J. Nakano, *Carbohydr. Res.*, 131 (1984) 105–118.
9 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
10 N. Blumenkrantz and D. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.
11 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
12 P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.
13 B. J. H. Stevens and R. R. Selvendran, *Phytochemistry*, 23 (1984) 107–115.
14 D. French and G. M. Wild, *J. Am. Chem. Soc.*, 75 (1953) 2612–2616.
15 R. W. Stoddart, A. J. Barret, and D. H. Northcote, *Biochem. J.*, 102 (1967) 194–204.

16 J. A. DE VRIES, A. G. J. VORAGEN, F. M. ROMBOUTS, AND W. PILNIK, *Carbohydr. Polym.*, 1 (1981) 117–127.
17 J. A. DE VRIES, A. G. J. VORAGEN, F. M. ROMBOUTS, AND W. PILNIK, *Carbohydr. Polym.*, 2 (1982) 25–33.
18 S. EDA, Y. AKIYAMA, K. KATŌ, A. ISHIZU, AND J. NAKANO, *Carbohydr. Res.*, 137 (1985) 173–181.
19 S. G. RING AND R. R. SELVENDRAN, *Phytochemistry*, 17 (1978) 745–752.
20 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry*, 11 (1972) 1383–1388.
21 Y. SONE, M. KAKUTA, AND A. MISAKI, *Agric. Biol. Chem.*, 42 (1978) 417–425.