Plant Physiol. (1997) 113: 1265–1272

O-Acetylated Oligosaccharides from Pectins of Potato Tuber Cell Walls[1]

Tadashi Ishii

Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Norin Kenkyu Danchinai, Ibaraki 305, Japan

Acetylated trigalacturonides and rhamnogalacturonan I (RG-I)-derived oligosaccharides were isolated from a Driselase digest of potato tuber cell walls by ion-exchange and size-exclusion chromatography. The oligosaccharides were structurally characterized by fast atom bombardment-mass spectroscopy, nuclear magnetic resonance spectroscopy, and glycosyl-linkage composition analysis. One trigalacturonide contained a single acetyl group at *O*-3 of the reducing galacturonic acid residue. A second trigalacturonide contained two acetyl substituents, which were located on *O*-3 or *O*-4 of the nonreducing galacturonic acid residue and *O*-3 of the reducing galacturonic acid residue. RG-I backbone-derived oligomers had acetyl groups at *O*-2 of the galacturonic acid residues. Some of these galacturonic acid residues were *O*-acetylated at both *O*-2 and *O*-3 positions. Rhamnosyl residues of RG-I oligomers were not acetylated.

Pectin and pectic substances have been investigated minutely for their structural features (O'Neill et al., 1990) and functions within the plant cell wall (Carpita and Gibeaut, 1993). O'Neill et al. (1990) stated that only three pectic polysaccharides have been isolated from the primary cell walls of plants. They are homogalacturonan, RG-I, and RG-II. RG-I contains a repeating sequence of alternating L-rhamnose and D-galacturonic acid residues with a variety of L-arabinosyl and D-galactosyl and L-fucosyl-containing side chains. RG-II has an extremely complex glycosyl-residue and glycosyl-linkage composition, and pectins from some plants carry acetyl groups (O'Neill et al., 1990).

Pectic polysaccharides have been isolated from cell walls of suspension-cultured sycamore cells by treatment with pure EPG (O'Neill et al., 1990). The total amount of material solubilized by the EPG treatment was 13 to 16% (w/w) of the starting cell wall material. An additional approximately 5% (w/w) of the material was solubilized by extraction with 50 mM Na_2CO_3 at 1 and 20°C. The Na_2CO_3-solubilized material was RG-I-like pectic polysaccharides (Ishii et al., 1989). In previous papers we described the characterization of acetylated RG-I-derived oligosaccharides from a Driselase digest of bamboo shoot cell walls (Ishii, 1995a) and reported the presence of acetylated trigalacturonides in the digest of bamboo shoot cell walls (Ishii, 1995b). However, the amount of acetylated trigalacturonides that was present was insufficient to determine the positions of the acetyl groups. Schols and Voragen (1994) reported that a modified hairy region of potato pectin contained acetyl groups. Therefore, potato tuber cell walls were digested with Driselase and acetylated trigalacturonides and RG-I derived oligomers were isolated. Here we report on the linkage positions of acetyl substituents in the oligosaccharides.

MATERIALS AND METHODS

De-starched potato tuber residue (*Solanum tuberosum* L. cv Norin-ichigo), which contained 22% (w/w) uronic acid and 0.5% (w/w) acetic acid, was a gift from Dr. K. Yamamoto (Hokuren, Sapporo, Japan). The residue consisted of (in mol%) Ara (6.0), Fuc (0.4), Rha (1.4), Xyl (1.0), Man (1.0), Gal (14.5), and Glc (75.6) as neutral sugars. Trigalacturonic acid was purchased from Sigma.

Isolation and Purification of Acidic Oligosaccharides

De-starched potato tuber residue (20 g) was digested with 5 g Driselase, as previously described (Ishii, 1995a). Driselase digested 52% (w/w) of the residue. The digest was passed through an ultrafiltration membrane (molecular weight cutoff 10,000) and about 5% of the digest remained on the membrane. The filtrate was applied to a DEAE-Sepharose Fast Flow column (2.5 × 40 cm, Pharmacia Biotech, Uppsala, Sweden) that had been equilibrated with 50 mM ammonium formate, pH 7.0. The column was washed with the equilibration buffer (500 mL) to remove the unbound compounds, and then stepwise eluted with 400 mL of 100 mM, 200 mM, and 1 M ammonium formate, pH 7.0. Fractions of 10 mL were collected and assayed colorimetrically for uronic acid by the *m*-hydroxybiphenyl method (Blumenkratz and Asboe-Hansen, 1973). The bound fraction that was eluted with 100 mM of buffer represented about 0.5% of de-starched potato.

[1] This work was supported by a research grant (Biomedia Projects, BMP-97-V-I-1) from the Ministry of Agriculture, Forestry, and Fisheries (Tokyo, Japan).

* E-mail tishii@ffpri.affrc.go.jp; fax 81–298–73–3795.

Abbreviations: EPG, *endo*-polygalacturonase; FAB-MS, fast-atom-bombardment MS; HMBC, heteronuclear multiple bond correlation; HMQC, two-dimensional heteronuclear multiple quantum correlation; HOHAHA, two-dimensional homonuclear Hartmann-Hahn; HPAEC, high-performance anion-exchange chromatography; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; TMS, trimethylsilyl.

Two fractions containing carbohydrates were separately pooled, concentrated to dryness under reduced pressure at 40°C, dissolved in water, and then freeze-dried. Fractions A and B (each 20 mg) were separately dissolved in 200 mM ammonium formate, pH 6.5, and loaded onto a Bio-Gel P-4 column (1.5 × 90 cm, Bio-Rad) and eluted with 200 mM ammonium formate, pH 6.5. Fractions (2 mL) were collected and their uronic acid contents were determined colorimetrically. Each P-4 fraction derived from fraction A was analyzed by FAB-MS. As fractions from 51 to 54 were enriched with oligosaccharides having a molecular weight of 630, which corresponds to di-*O*-acetylated trigalacturonides, these fractions were pooled and freeze-dried (fraction A-1). P-4 fractions derived from fraction B that were enriched with acidic sugars were collected and freeze-dried to give three fractions (fractions B-1, B-2, and B-3). The yields of fractions A-1, B-1, B-2, and B-3 were 0.01, 0.05, 0.05, and 0.1% (w/w), respectively, based on the destarched potato.

HPAEC

HPAEC was performed on a Bio-LC system (Dionex, Sunnyvale, CA), which included a quaternary gradient pump, an eluent degas (He) module, and a CarboPac PA1 column (4.6 × 250 mm, Dionex) with a matching guard column. The effluent was monitored using pulsed amperometric detection with a gold electrode. Potentials of E_1, 0.05 V; E_2, 0.6 V; and E_3, −0.6 V were applied for the duration times of T_1, 0.5 s; T_2, 0.1 s; and T_3, 0.1 s, respectively. The flow rate was 1.0 mL min^{-1}. The gradient was obtained by mixing solutions of 150 mM NaOH and 1 M NaOAc in 150 mM NaOH. After an equilibration step of at least 15 min with 200 mM NaOAc in 150 mM NaOH, 10 μL of the sample was injected, and a linear gradient of 400 mM NaOAc in 150 mM NaOH with 40 min was started. The column was washed for 5 min with 1 M NaOAc in 150 mM NaOH and equilibrated again for 15 min with 200 mM NaOAc in 150 mM NaOH.

Glycosyl-Residue Composition Analysis

Glycosyl-residue compositions were determined by formation of the TMS derivatives of methyl-esterified methyl glycosides and analysis of the derivatives by GLC (York et al., 1985). The derivatives were separated on a DB-1 column (0.25 mm × 30 m, J & W Scientific, Folsom, CA) using a 14A gas chromatograph (Shimadzu, Columbia, MD). Absolute configurations were determined as described previously (Gerwig et al., 1979).

Glycosyl-Linkage Composition Analysis

Acidic oligoglycoses were reduced with $NaBD_4$ as described previously (Waeghe et al., 1983) to yield the corresponding oligoglycosyl aldonic acids. Solutions of the oligoglycosyl aldonic acids (100 μg) in Me_2SO (500 μL) were methylated with methylsulfinyl methyl potassium and iodomethane as described previously (York et al., 1985). The methyl-esterified and per-*O*-methylated oligoglycosyl aldonic acids were then purified using Sep Pak C-18 cartridges (Waeghe et al., 1983). The methyl-esterified carboxyl groups of the galactosyluronic acid residues and galactonic acid were carboxyl-reduced with lithium triethylborodeuteride (York et al., 1985). The glycosyl-linkage composition of the oligoglycosyl alditols were then determined by GLC-MS analysis of the derived partially *O*-methylated alditol acetates.

FAB-MS

FAB-MS spectra were recorded with a mass spectrometer (HX 110A, JEOL) operating at low resolution (1:1000) in the positive-ion and negative-ion modes with an accelerating voltage of 10 kV and xenon as the bombardment gas. A mixture of glycerol and thioglycerol (1:1, v/v) was used as the matrix.

NMR Spectroscopy

NMR spectroscopy was performed with an ALPHA 500 spectrometer (JEOL). Spectra were obtained (in D_2O) at 500 MHz for ^1H and at 125 MHz for ^{13}C at 25°C. ^1H and ^{13}C chemical shifts (δ) are reported in parts per million relative to external acetone (δ 2.23) and external CD_3OD (δ 49.30) from sodium 3-trimethylsilyl propionic acid in D_2O, respectively. One- and two-dimensional double quantum-filtered correlated spectroscopy, HOHAHA spectroscopy, HMQC, and HMBC were performed with a JEOL ALPHA 500 spectrometer using standard pulse sequences. The HOHAHA spectra were acquired with a phase-sensitive mode (τ_{mix} = 0.1 s). The field-gradient mode was used for HMQC and HMBC (Δ_2, 40 and 100 ms) analyses. The ^1H-NMR spectra of trigalacturonic acid (sodium salt, pH 7.2) were obtained at 500 MHz with a Brucker AMX500 spectrometer (Karlsruhe, Germany) at 25°C.

RESULTS

Driselase solubilized 58% (w/w) of the uronic acid residues from potato cell walls. Eighty percent of galacturonic acid in the Driselase digest was eluted from the DEAE column with a 50 mM washing buffer. The uronic acid content in 100 mM, 200 mM, and 1 M buffer DEAE eluents accounted for 4, 4, and 5%, respectively, of the total uronic acid in the digest. Fractions enriched in two *O*-acetylated oligogalacturonides were isolated from the 0.1 M buffer eluent by gel-permeation and ion-exchange chromatography (see Figs. 1 and 2). HPAEC analysis showed that the major components, fractions A-1 and B-3, co-eluted with authentic trigalacturonic acid. HPAEC was performed under strong alkaline conditions and the esters were removed during analysis. Fraction A-1 was analyzed by FAB-MS and found to consist of two components (monoacetylated and diacetylated trigalacturonides). Fraction B-3 consisted mainly of a monoacetylated trigalacturonide. The mono- and di-*O*-acetylated trigalacturonic acid could not be separated by reversed-phase HPLC or ion-exchange chromatography on a Mono-Q column (data not shown). The nonacetylated trigalacturonic acid was also present (fraction nos. 31–39 in Fig. 1), in addition to mono-*O*-acetylated trigalacturonides. This indicates that Driselase digestion of

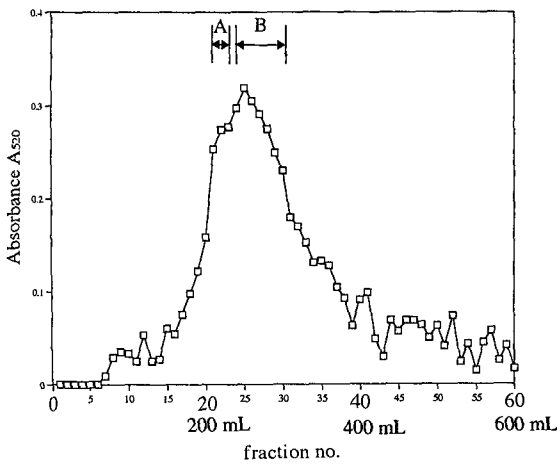


Figure 1. DEAE Sepharose fast-flow chromatography of the Driselase digest of potato tuber cell walls. The digest was eluted with 100 mM ammonium formate, pH 7.0, and 10-mL fractions were collected. The relative amounts of acidic sugars were determined by the *m*-hydroxybiphenyl method. Two fractions that were pooled are indicated as A and B.

the potato cell walls gave nonacetylated trigalacturonide or that deacetylation occurred during separation. However, the amount of nonacetylated trigalacturonic acid from the digest was insufficient for further analysis. Thus, commercially available (nonacetylated) trigalacturonic acid was used as the reference compound for NMR analysis (Tables I and II).

Characterization of Monoacetylated Trigalacturonide Present in Fraction B-3

Glycosyl-residue composition analysis showed that fraction B-3 contained only D-galacturonic acid. Glycosyl-linkage composition analysis of NaBD4-reduced and carboxyl-reduced diglycosyl alditol gave 6,6′-dideuterio derivatives of 4-linked galactitol (27 mol%), 4-linked Gal (36 mol%), and nonreducing Gal (36 mol%). The negative-ion mode FAB-MS spectrum of fraction B-3 contained an abundant ion at *m/z* 587 $(M-H)^-$, which corresponds to an oligogalacturonide composed of three galacturonic acid residues and one acetyl group. The negative-ion mode linked-scan FAB-MS spectrum of the quasimolecular ion at *m/z* 587 gave an intense daughter ion at *m/z* 369 and weak ions at *m/z* 411, 235, and 193 (these ions correspond to either "Y" or "C" ions, described by Domon and Costello [1988]). The formation of a "Y" or "C" ion with *m/z* 235 suggested that acetylation occurred either at the reducing end or the nonreducing end (Fig. 3A).

The ^1H chemical shifts of the monoacetylated trigalacturonic acid in fraction B-3 were assigned completely by one-dimensional and two-dimensional $\{^1H\text{-}^1H\}$ spectra (see Table I). The singlet at δ 2.05 was assigned to the methyl protons of the *O*-acetyl substituent. The doublets at δ 5.30 ($J_{1,2}$ = 3.1 Hz) and 4.64 ($J_{1,2}$ = 7.8 Hz) correspond to H-1 of the α and β anomers of the reducing galacturonic acid. The signals at δ 4.96 and 4.97 ($J_{1,2}$ = approximately 3.8 Hz) are the resonance of the H-1 of the internal and terminal galacturonic acid residues, respectively (Lo et al., 1994). The chemical shift value of H-1s of the nonreducing and internal sugar residues and the magnitude of their coupling constants (approximately 3.8 Hz), show that the D-galacturonosyl residues are α-linked. Ring proton signals were assigned by two-dimensional HOHAHA spectrometry.

The signals at δ 5.14 and 4.91 were assigned to the H-3s of the reducing α- and β-galacturonic acids. In contrast, the H-3 signals of the reducing α- and β-galacturonic acids of the nonacetylated trigalacturonic acid have chemical shifts of δ 3.96 and 3.72, respectively, which is in good agreement with the published values (Lo et al., 1994). Thus, the H-3 resonances of the α- and β-reducing galacturonic acids of fraction B-3 are shifted downfield by 1.18 and 1.19 ppm, respectively, suggesting that O-3 of the reducing galacturonic acid is the site of the acetylation. The ^{13}C-NMR spectrum of fraction B-3 was assigned by HMQC and HMBC spectroscopy (Table II). The C-6 (C=O carboxyl) signals of the galacturonic acid residues were assigned by the $^2J_{CH}$ coupling to H-5 of the same residues by HMBC experiments. The location of the acetyl carbonyl-carbon signals at δ 173.49 and 174.27 were correlated with the H-3 signals at δ 4.91 and

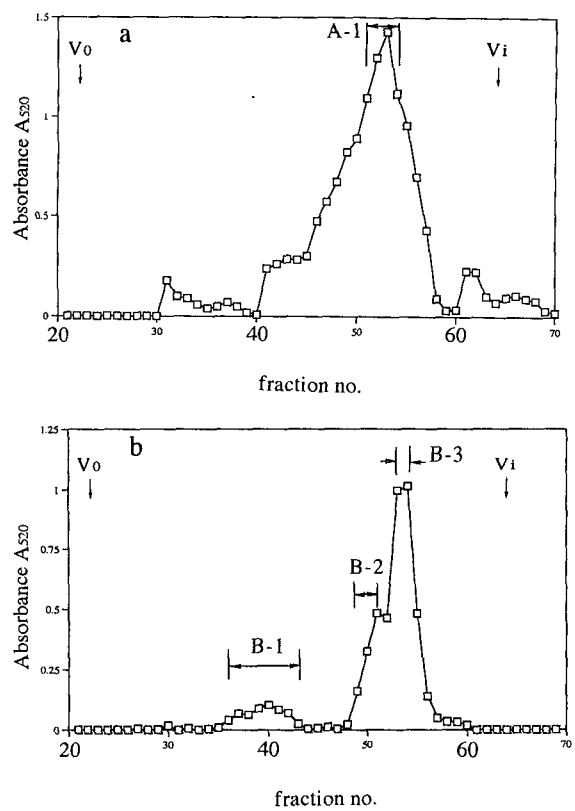


Figure 2. Bio-Gel P-4 gel-permeation chromatography of fraction A (a) and fraction B (b). Acidic sugars were eluted with 200 mM ammonium formate, pH 6.5. Fractions (2 mL) were collected and uronic acid was determined colorimetrically. Fraction nos. 51 to 54 from fraction A, fraction nos. 36 to 43, 49 to 51, and 53 to 54 from fraction B were separately pooled and indicated as A-1, B-1, B-2, and B-3, respectively. The single-headed arrows indicated the void volume (V_o) and the elution volume (V_i) for Glc, respectively.

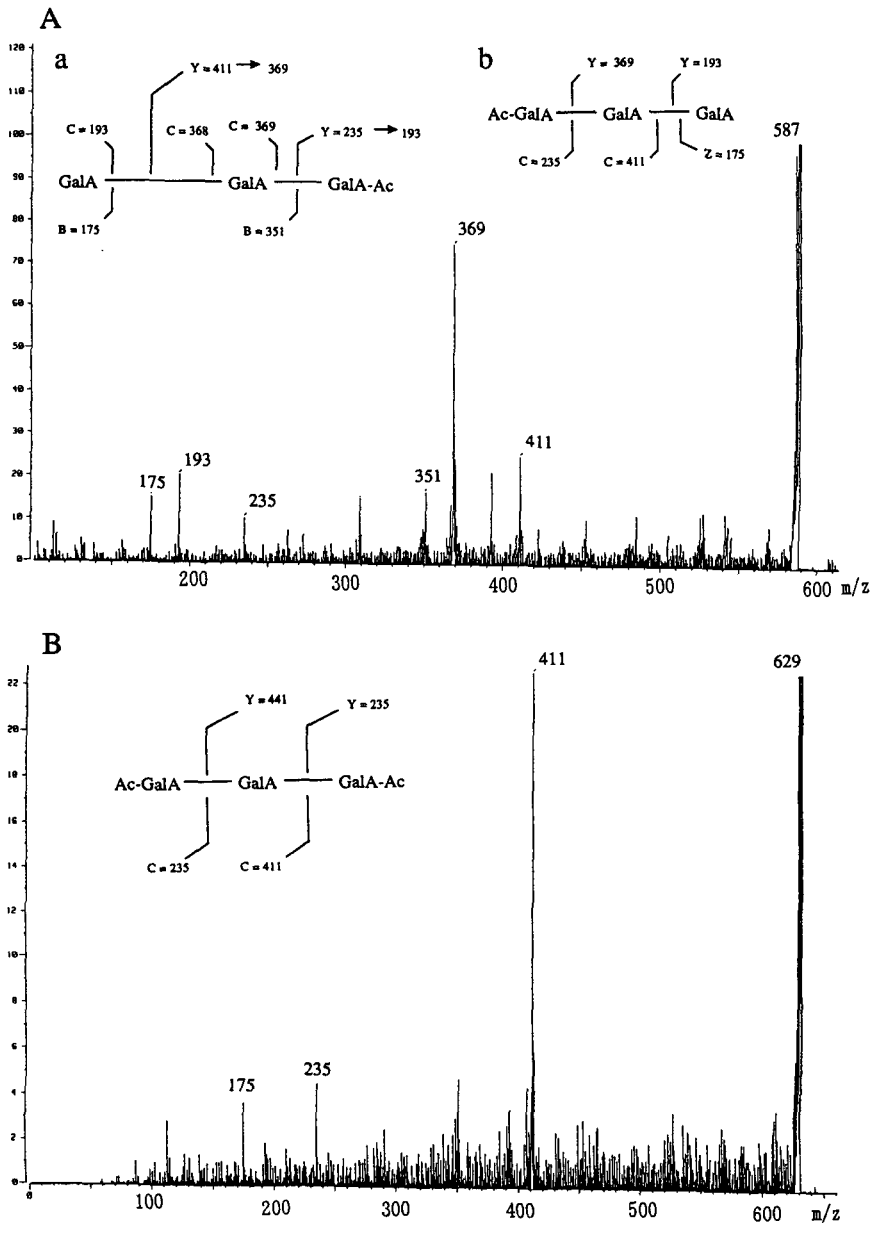


Figure 3. Negative-ion mode linked-scan FAB-MS spectrum of a daughter ion at *m/z* 587 of monoacetylated trigalacturonide (fraction B-3) (A) and at *m/z* 629 of diacetylated trigalacturonide (fraction A-1) (B). Two alternative fragmentation patterns (a and b in A) are shown. NMR analysis showed pattern "a" was correct.

5.14, respectively, at the site of esterification (Fig. 4A). Taken together, these results established that *O*-3 of the reducing galacturonic acid is the site of acetylation (Fig. 5).

Characterization of Diacetylated Trigalacturonides Present in Fraction A-1

Fraction A-1 contained only D-galacturonic acid residues. Glycosyl-linkage analysis of the aldehyde and carboxyl-reduced oligomer from fraction A-1 gave the derivatives expected for 6,6'-dideuterio substituted for 4-linked galactitol (27 mol%), 4-linked Gal (38 mol%), and terminal Gal (36 mol%). The negative-ion mode FAB-MS spectrum of fraction A-1 contained a high-abundance ion at *m/z* 629 $(M-H)^-$ and a low-abundance ion at *m/z* 587 $(M-H)^-$. The ratio of ions at *m/z* 629 and 587 was about 6:1. The negative-ion mode linked-scan spectrum of the quasimolecular ion at *m/z* 629 gave a series of intense daughter ions at *m/z* 411 and 235 ("Y" or "C") (Fig. 3B), and the spectrum of the quasimolecular ion at *m/z* 587 gave the same ions as observed in fraction B-3 (Fig. 3A). These results indicated that fraction A-1 contained at least two compounds, a monoacetylated trigalacturonide (molecular weight 588) and a diacetylated trigalacturonide (molecular weight 630), and that acetylation occurred both at the reducing and nonreducing ends of the diacetylated trigalacturonide.

The ^{1}H-NMR spectrum of fraction A-1 contained two signals at δ 2.08 and 2.10, with a peak ratio of 1:3, which are characteristic of the methyl protons of the *O*-acetyl groups. The spectrum also contained a broad signal at δ 5.53 (H-4 terminal nonreducing galacturonic acid) and doublets of doublets at δ 5.14 (H-3 reducing α-galacturonic acid), 5.02

Table I. *^1H NMR data for trigalacturonides (pH 7.2)*

Oligo-galacturonide	Residue	^1H Chemical Shift (δ)[a] (Coupling Constants)[b]					
		H-1	H-2	H-3	H-4	H-5	CH_3
Trigalacturonide							
Reducing	GalAα	5.27 (3.8)	3.80 (10.5)	3.96 (3.0)	4.39 (1.1)	4.37	–
Reducing	GalAβ	4.57 (7.3)	3.47 (10.2)	3.72 (3.2)	4.34 (1.1)	4.02	–
Internal	GalpA	5.07 (3.8)	3.74 (10.4)	α3.97 (3.2)[c] β3.99 (3.3)	4.38 (1.1)	4.72	–
Terminal	GalpA	5.01 (3.8)	3.69 (10.5)	3.88 (3.3)	4.23 (1.1)	4.71	–
Monoacetyl trigalacturonide (fraction B-3)							
Reducing	GalAα (3Ac)	5.30 (3.8)	4.00 (10.6)	5.14 (3.1)	4.46 (1.1)	4.42	2.05
Reducing	GalAβ (3Ac)	4.64 (7.8)	3.64 (10.2)	4.91 (3.3)	4.41 (1.1)	4.07	2.05
Internal	GalpA	4.96 (3.7)	3.63 (10.8)	3.96 (3.2)	4.33 (1.1)	4.50	–
Terminal	GalpA	4.97 (3.9)	3.68 (10.5)	3.82 (3.3)	4.18 (1.1)	4.69	–
Diacetyl trigalacturonide (fraction A-1)							
Reducing	GalAα (3Ac)	5.30 (3.8)	4.00 (10.4)	5.14 (3.0)	4.47 (1.1)	4.44	2.08
Reducing	GalAβ (3Ac)	4.64 (7.3)	3.64 (10.2)	4.91 (3.1)	4.42 (1.1)	4.06	2.08
Internal	GalpA	4.98 (3.7)	3.68 (10.2)	3.97 (3.0)	4.34 (1.1)	4.50	–
Terminal	GalpA (3Ac)	5.02 (3.7)	3.66 (10.4)	5.02 (3.0)	4.34 (1.1)	4.76	2.08
	GalpA (4Ac)	5.02 (3.7)	3.64 (10.4)	4.04 (3.0)	5.53 (1.1)	4.85	2.10

[a] In D_2O at 25°C. [b] Observed first-order splittings in Hz. [c] The chemical shift of H-3 of the internal GalA residue is significantly affected by the anomeric configuration (α and β) of the reducing GalA residue.

(H-3 nonreducing galacturonic acid), and 4.91 (H-3 reducing β-galacturonic acid) linked to the *O*-acetylated carbons, respectively. The H-1 and H-3 protons of the 3-*O*-acetylated, nonreducing residue were overlapped (Table I). The HMBC analysis of fraction A-1 confirmed the linkage positions of the acetyl groups (Fig. 4B). Acetyl carbonyl-carbon signals at δ 173.08 and 173.26 were correlated with the proton signals at the site of esterification, i.e. H-4 (δ 5.53), H-3 (δ 5.02), Hα-3 (δ 5.14), and Hβ-3 (δ 4.91), respectively. From these results, we conclude that *O*-4 or *O*-3 of the nonreducing end and *O*-3 of the reducing end are the sites of acetylation (Fig. 5).

Characterization of Acetylated RG Oligomers in Fractions B-1 and B-2

Fractions B-1 and B-2 were analyzed by HPAEC. They contained RG-I-derived heptamer and petamer that co-

Table II. *Assignments of signals in the ^{13}C NMR spectra of trigalacturonides*

Oligogalacturonide	Residue	Chemical Shift (δ)[a]							
		C-1	C-2	C-3	C-4	C-5	C-6	CH_3	CO
Trigalacturonide									
Reducing	GalAα	92.21	67.64	67.91	78.39	69.31	172.09	–	–
Reducing	GalAβ	96.19	70.91	71.37	77.59	72.78	171.17	–	–
Internal	GalpA	99.80	67.71	67.90	78.07	70.08	172.09	–	–
Terminal	GalpA	99.90	67.71	68.58	68.82	70.79	172.71	–	–
Monoacetyl trigalacturonide (fraction B-3)									
Reducing	GalAα (3Ac)	92.00	66.02	71.47	76.82	70.65	174.27	20.09[b]	173.28
Reducing	GalAβ (3Ac)	95.75	68.19	74.25	75.98	74.25	173.49	20.15[b]	173.28
Internal	GalpA	98.83 99.87	67.81	68.52 68.56	78.00	71.47	174.97	–	–
Terminal	GalpA	99.00	68.19	69.41	70.65	72.14	175.86	–	–
Diacetyl trigalacturonide (fraction A-1)									
Reducing	GalAα (3Ac)	92.42	66.43	71.85	77.23	68.78	173.78	20.14[c]	173.08[d]
Reducing	GalAβ (3Ac)	96.18	69.80	74.63	76.39	na[e]	173.48	20.31[c]	173.26[d]
Internal	GalpA	100.26 100.22	68.15	68.85	78.39	73.19	174.81	–	–
Terminal	GalpA (3Ac)	99.45	68.34	73.34	68.85	na[e]	174.24	20.14[c]	173.08[d]
Terminal	GalpA (4Ac)	99.45	68.34	68.73	73.34	70.62	174.24	20.31[c]	173.26[d]

[a] In D_2O at 25°C. [b-d] These assignments may be interchanged. [e] Not assigned.

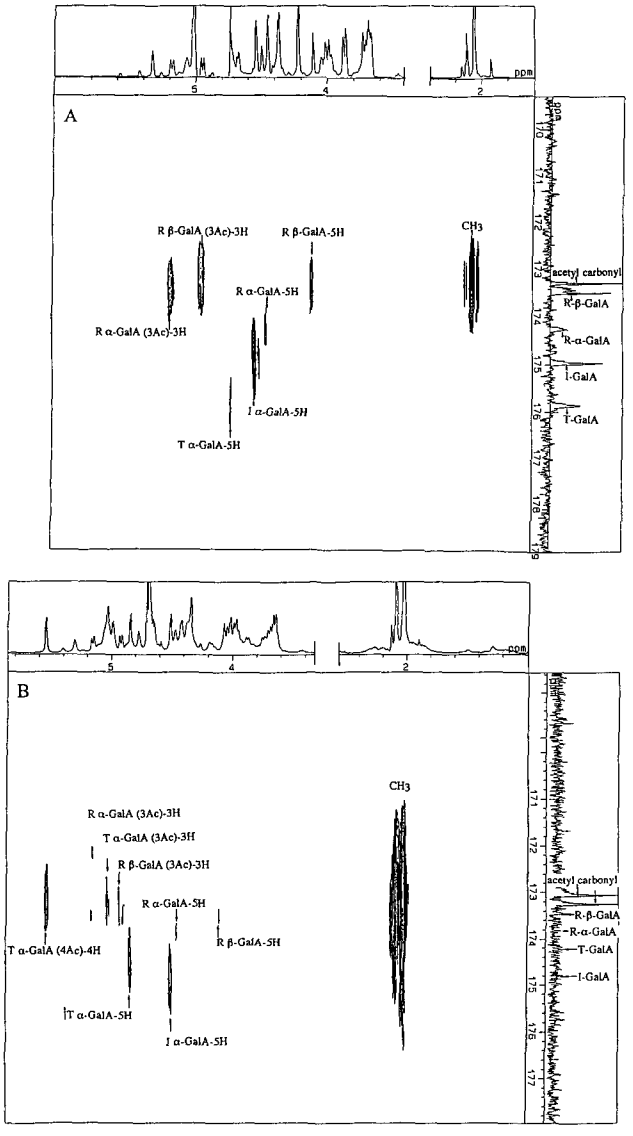
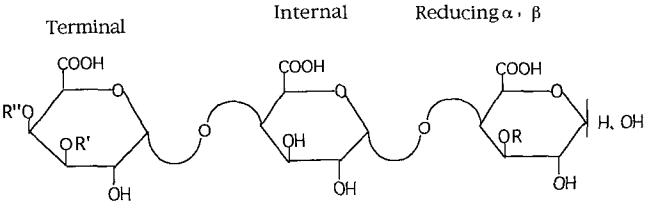


Figure 4. Partial HMBC spectra of mono *O*-acetylated trigalacturonide (fraction B-3) (A) and di-*O*-acetylated trigalacturonide (fraction A-1) (B) showing 3-bond connectivities from *O*-acetyl carbonyl carbon to protons on *O*-acetylated carbons, 2-bond connectivities from *O*-acetyl methyl carbons to *O*-acetyl methyl protons, and 2-bond connectivities from C-6 carbonyl carbons to H-5 of GalA residues.

eluted with authentic RG-I heptamer and pentamer from bamboo shoot cell walls (Ishii, 1995a). Glycosyl composition analysis of TMS-derivatized sugars showed that fraction B-2 consisted of rhamnose and galacturonic acid in the L and D absolute configurations, respectively. Methylation analysis of the aldehyde and carboxyl-reduced oligomer from fraction B-2 revealed 6,6′-dideuterio 4-linked galactitol (14 mol%), 4-linked Gal (22 mol%), and terminal Gal (20 mol%), along with two substituted rhamnosyl residues (41 mol%). These results suggest that galacturonic acid was present at both reducing and nonreducing terminals and that both 2-linked rhamnose and 4-linked galacturonic acid residues were internal. The ^{1}H NMR spectrum of fraction B-2 contained signals at δ 2.13, 2.19, and 2.22, which are characteristic of the methyl protons of the *O*-acetyl groups. The negative-ion mode FAB-MS spectrum showed intense ions at *m/z* 837 $(M-H)^-$, 879 $(M-H)^-$, 921 $(M-H)^-$, and 963 $(M-H)^-$.

The positive-ion mode spectrum of fraction B-2 that contained four abundant ions at *m/z* 861, 903, 945, and 987 corresponds to $(M+Na)^+$ of the nonacetylated, mono-, di-, and tri-*O*-acetylated RG-I pentamers, respectively. Fraction B-2 was analyzed by negative-ion and linked-scan mode FAB-MS to elucidate the glycosyl sequence and the location of the acetyl groups in the RG-I heptamer, as described previously (Ishii, 1995a). For example, the quasimolecular ion at *m/z* 921 produced fragment ions at *m/z* 703, 557, 381, and 235 (these ions correspond to either the "Y" or "C" ions.). The formation of the Y or C ion with m/z 703, 557, 381, and 235 suggested that acetylation occurred both at the reducing and nonreducing ends, and that rhamnosyl residues were not acetylated. The deduced structures of RG-I oligomers are summarized in Table III. The ^{1}H-NMR spectrum contained signals at δ 5.31 and 5.34, which are assigned to H-2 protons that are linked to *O*-acetylated carbons in galacturonic acid residues (Lerouge et al., 1993; Ishii, 1995a). These results confirm that acetylation occurs at *O*-2 of galacturonic acid residues and that rhamnose residues are not acetylated (Komalavilas and Mort 1989; Lerouge et al., 1993; Ishii, 1995a).

Fraction B-1 was analyzed by FAB-MS, ^{1}H-NMR spectroscopy, glycosyl-composition, and glycosyl-linkage analyses in the same way as fraction B-2. The fraction contained more than three different structures of *O*-acetylated RG-I heptasaccharides with four, five, or six acetyl residues of α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A (Table III). Signals at δ 5.30 and 5.53 in the ^{1}H-NMR spectrum were assigned to the H-2 and H-3 protons that were linked to the *O*-acetylated carbons, respectively (Lerouge et al., 1993; Ishii, 1995a). In heptamers, acetylation occurred not only at *O*-2 but also at *O*-3 of galacturonic acid residues. Similar acetylated RG-I oligomers were isolated from suspension-cultured sycamore RG-I (Lerouge et al., 1993)



Trigalacturonide	R = R' = R'' = H
Monoacetylatedtrigalacturonide	R = CH_3CO, R' = R'' = H
Diacetylatedtrigalacturonide	R = R' = CH_3CO, R'' = H
	R = R'' = CH_3CO, R' = H

Figure 5. Proposed structures of the mono- and di-*O*-acetylated trigalacturonides.

Table III. *Structure of RG oligomers obtained from Driselase hydrolyzates of potato cell walls*

Fraction	Compound	Molecular Wt	Structure
B-1	Tetraacetyl RG-7	1328	α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-αRhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-GalpA | | | | Ac Ac Ac Ac
	Pentaacetyl RG-7	1370	α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-αRhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-GalpA | | | | Ac Ac_{1or2}[a] Ac_{1or2}[a] Ac_{1or2}[a]
	Hexaacetyl RG-7	1412	α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-GalpA | | | | Ac Ac_{1or2}[a] Ac_{1or2}[a] Ac_{1or2}[a]
B-2	Nonacetyl RG-5	838	α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA
	Monoacetyl RG-5	880	α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA | | Ac^a Ac^a
	Diacetyl RG-5	922	α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA | | | Ac Ac^a Ac^a
	Triacetyl RG-5	964	α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA-(1→2)-α-Rhap-(1→4)-α-GalpA | | | Ac Ac Ac

[a] Acetyl groups cannot be assigned to specific GalA residues.

and the Driselase hydrolyzates of the bamboo shoot cell-walls (Ishii, 1995a).

DISCUSSION

Diacetylated trigalacturonides were isolated from the Driselase digest of potato tuber cell walls. Acetyl groups were located at *O*-3 and *O*-4 of the nonreducing galacturosyl residue and at *O*-3 of the reducing galacturonic acid. Our results suggest that *O*-4 of the nonreducing galacturonic acid residues of homogalacturonan in pectin may be *O*-acetylated. However, acetyl groups are known to migrate. For example, acetates on *cis*-diols of neuramic acids have been shown to migrate spontaneously without an enzyme (Kamerling et al., 1987). Thus, the 3-*O*-acetyl group of the nonreducing end in diacetylated trigalacturonide may migrate to position 4 during the preparation and purification of the acetylated trigalacturonides.

Direct detection of the acetylated galacturonic acid residues in the pectin from potato cell walls by NMR spectroscopy was unsuccessful. The pectin was extracted with 500 mM imidazole-HCl buffer from de-starched potato, according to the method of Mort et al. (1991), and analyzed by ^{1}H-NMR spectroscopy. The yield of pectin was 5% (w/w) based on the de-starched potato. The signals are broad and the acetyl content of the pectin is low. It was not possible to detect signals at δ of approximately 5.5, which would be characteristic for H-4 signal of 4-*O*-acetylated galacturonic acid (T. Ishii, unpublished data). This is partly due to the fact that a high degree of acetylation is present in the modified hairy region of potato (Schols and Voragen, 1994) and that the pectin extracted with 500 mM imidazole-HCl buffer would not represent the modified hairy region. Further evidence is required to establish that the nonreducing residue is naturally *O*-acetylated at position 4.

Two acetylated RG-I oligomers were isolated from potato tube cell walls. Acetylation mainly occurred at *O*-2 with some at *O*-3 of the galacturonic acid residues. No acetylation was observed at the rhamnosyl residues of RG-I. *O*-Acetyl groups are located at *O*-3, and at *O*-2 or *O*-3 of the galacturonic acid residues in cotton RG-I (Komalavilas and Mort, 1989) and sycamore RG-I (Lerouge et al., 1993), respectively. The *O*-acetylated RG-I-derived pentamers and heptamers were isolated from bamboo (monocots) shoot cell walls (Ishii, 1995a).

The acetylated RG-I oligomers obtained from the Driselase digest had galacturonic acids as nonreducing and reducing ends. This indicates that Driselase, a hydrolytic enzyme mixture, contains an α-L-rhamnosidase in addition to *endo*-rhamnogalacturonase. An et al. (1994) isolated RG-I oligomers (degree of polymerization 2–11) from sycamore cell walls with recombinant *Aspergillus acculeatus* enzymes. The predominant RG-I oligomers had nonreducing galacturonic acid. Schols et al. (1994) isolated RG-I oligomers from the hairy regions of apple pectin with a pure rhamnogalacturonase treatment. The nonreducing ends of the oligomers were all rhamnosyl residue.

Driselase digest of the potato cell walls gave a series of acetylated trigalacturonides and RG-I oligomers. A small amount of nonacetylated trigalacturonide was present in the hydrolyzates. The presence of the acetyl groups may affect the interaction of homogalacturonan and RG-I with hydrolytic enzymes such as EPG and *endo*-rhamnogalacturonase.

Liners et al. (1994) reported that the *O*-acetylation of cell wall pectin correlates with the cell size of suspension-cultured sugar beet cells. We found that nonembryogenic carrot callus is smaller in cell size and has a lower acetyl content (3.5%) in the cell wall, compared with that of embryogenic callus (6.0%) (T. Ishii, unpublished results).

This suggests that the physical properties of the pectin of the walls of plant cells can be affected by the presence of acetyl substituents. The potential of *O*-acetyl substituents to affect the ability of pectin to modulate or control cell elongation calls for further study.

ACKNOWLEDGMENTS

I would like to thank Drs. W.S. York and M.A. O'Neill (Complex Carbohydrate Research Center, the University of Georgia, Athens) and K. Yamamoto (Hokuren, Sapporo, Japan), for critical reading of the manuscript and for the gift of de-starched potato, respectively, and Mrs. Y. Takeda for technical help and for typing the manuscript. I also would like to thank Dr. W.S. York for performing ^{1}H-NMR analysis of trigalacturonic acid.

Received September 23, 1996; accepted January 5, 1997.
Copyright Clearance Center: 0032–0889/97/113/1265/08.

LITERATURE CITED

An J, Zhang L, O'Neill MA, Albersheim P, Darvill AG (1994) Isolation and structural characterization of endo-rhamnogalacturonase-generated fragment of the backbone of rhamnogalacturonan I. Carbohyr Res **264:** 83–96

Blumenkratz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem **54:** 484–489

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J **3:** 1–30

Domon B, Costello CE (1988) A systematic nomenclature for carbohydrate fragmentation in FAB-MS/MS spectra of glycoconjugates. Glycoconjugate J **5:** 397–409

Gerwig GJ, Kamerling JP, Vliegenthart JFG (1979) Determination of the absolute configuration of monosaccharides in complex carbohydrates by capillary G.L.C. Carbohydr Res **77:** 1–7

Ishii T (1995a) Isolation and characterization of acetylated rhamnogalacturonan oligomers liberated from bamboo shoot cell-walls by Driselase. Mokuzai Gakkaishi **41:** 561–572

Ishii T (1995b) Pectic polysaccharides from bamboo shoot cell-walls. Mokuzai Gakkaishi **41:** 669–676

Ishii T, Thomas J, Darvill A, Albersheim P (1989) Structure of plant cell walls. XXVI. The walls of suspension-cultured sycamore cells contain a family of rhamnogalacturonan-I-like pectic polysaccharides. Plant Physiol **89:** 421–428

Kamerling JP, Schauer R, Shukla AK, Stoll S, Van Halbeek H, Vliegenthart JFG (1987) Migration of *O*-acetyl groups in *N,O*-acetylneuramic acid. Eur J Biochem **162:** 601–607

Komalavilas P, Mort AJ (1989) The acetylation at O–3 of galacturonic acid in the rhamnose-rich portion of pectins. Carbohydr Res **189:** 261–272

Lerouge P, O'Neill MA, Darvill AG, Albersheim P (1993) Structural characterization of endo-glycanase-generated oligoglycosyl side chains of rhamnogalacturonan I. Carbohydr Res **243:** 359–371

Liners F, Gaspar T, Van Cutsem P (1994) Acetyl- and methylesterification of pectins of friable and compact sugar-beet calli: consequences for intercellular adhesion. Planta **192:** 545–556

Lo V-M, Hahn MG, Van Halbeek H (1994) Preparation, purification, and structural characterization of linear oligogalacturonides. A FAB-mass spectrometric and NMR spectroscopic study. Carbohydr Res **255:** 271–284

Mort AJ, Moerschbacher BM, Pierce ML, Maness NO (1991) Problems encountered during the extraction, purification, and chromatography of pectic fragments, and some solutions of them. Carbohydr Res **215:** 219–227

O'Neill M, Albersheim P, Darvill A (1990) Pectic polysaccharides. *In* Dey PM, Harbonrne JB, eds, Methods in Carbohydrate Chemistry, Vol 1. Academic Press, New York, pp 478–512

Schols HA, Voragen AGJ (1994) Occurrence of pectic hairy regions in various plant cell wall materials and their degradability by rhamnogalacturonase. Carbohydr Res **256:** 83–95

Schols HA, Voragen AGJ, Colquhoun IJ (1994) Isolation and characterization of rhamnogalcturonan oligomers, liberated during degradation of pectic hairy regions by rhamnogalacturonase. Carbohydr Res **256:** 97–111

Waeghe TJ, Darvill AG, Albersheim P (1983) Determination, by methylation analysis, of the glycosyl-linkage compositions of microgram quantities of complex carbohydrates. Carbohydr Res **123:** 281–304

York WS, Darvill AG, McNeil M, Stevenson T, Albersheim P (1985) Isolation and characterization of plant cell walls and cell wall components. Methods Enzymol **118:** 3–40