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THE ACETYLATION AT O-3 OF GALACTURONIC ACID IN THE RHAMNOSE-RICH PORTION OF PECTINS*

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ABSTRACT

Treatment of cell walls isolated from carrot, cotton, tobacco, and tomato with anhydrous hydrogen fluoride (HF) at −23° generated a high yield of disaccharides of galacturonic acid and rhamnose from the backbone of the rhamnose-rich region of the pectins. ^{1}H-N.m.r. analysis showed ~30 percent of the disaccharides to be acetylated at a unique location. Two-dimensional ^{1}H–^{1}H homonuclear-correlation n.m.r. spectroscopy indicated this to be O-3 of galacturonic acid. During reaction in the HF, the disaccharides were quantitatively converted into the 3-*O*-acetylated and nonacetylated α-D-galactopyranosyluronic acid β-L-rhamnopyranose 1,2′:2,1′-dianhydride.

INTRODUCTION

Pectins are major, and somewhat ill-defined, components of all primary cell-walls of dicots. They are characterized by being rich in galacturonic acid, but they also contain rhamnose, arabinose, galactose, xylose, perhaps glucose, methyl esters, acetic esters, and a combination of methylated sugars.

The arrangement of sugars in pectins seems to exist in three recognizable regions. The most frequently studied is a homogalacturonan which is partially methyl-esterified, but also contains some rhamnose and a low proportion of other neutral sugars. Another region contains a high proportion of neutral sugars and has been called rhamnogalacturonan I (RG-I) by McNeil *et al.*[1], while a third small region containing methylated sugars and some other unusual sugars has been called[2] rhamnogalacturonan II. The rhamnogalacturonan I portion of the pectin from sycamore culture cell-walls has been enzymically solubilized, purified, and studied in detail[1,3,4]. This polysaccharide fragment contains a rhamnogalacturonan backbone composed of approximately equal amounts of linear 2-linked and branched 2,4-linked α-L-rhamnosyl residues, strictly interspaced with 4-linked α-D-

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galactosyluronic residues[4]. Cell walls other than those of suspension-cultured sycamore cells also contain molecules very similar to RG-I, but these polysaccharides have not been studied in detail[5].

From results obtained with bacterial polysaccharides containing rhamnose and uronic acids[6–8], we predicted that the disaccharide galactosyluronic acid rhamnose should be easily obtained by HF solvolysis of cell walls at −23° if they contained significant proportions of the RG-I region. We also expected that any side chains attached to O-4 of the rhamnosyl residues would be removed. Our predictions have been verified, but we found an unexpected acetylation of the disaccharide. Also, an artifactual cyclic glycosylation was induced by the HF solvolysis.

EXPERIMENTAL

Source of cell walls. — Primary cell-walls were obtained from suspension-cultured plant-cells and from whole plant-tissues. Suspension cells from two cotton (*Gossypium hirsutum* L.) lines, Acala 44 and Im 216, which are susceptible and resistant, respectively, to the bacterial pathogen *Xanthomonas campestris* pv. *malvacearum*, the causal agent for cotton blight, were grown as described earlier[9]. The suspension cells were grown in SH medium, and the two-week-old suspension cells were used for wall preparation. Cell walls were prepared as described by York *et al.*[10], except that, instead of a pressure bomb, a polytron (Brinkmann Instruments, Inc., Westbury, NY) was used to break the cells. The cells were collected on a coarse sintered-glass funnel, and then washed with 100mM potassium phosphate (pH 7) five times, followed by 500mM potassium phosphate buffer, pH 7 (four times). The cells were suspended in 1 vol. of 500mM phosphate buffer (pH 7), and broken by using the polytron for 10 min at high speed. Cells were examined under the microscope for complete rupture. The suspension of broken cells was then centrifuged at 2,000*g* for 10 min. The supernatant liquor was decanted, and the pellet was washed twice by suspending it in 5 vol. of the 500mM phosphate buffer, and then centrifuging at 2,000*g* for 10 min. The washing was repeated five times with distilled water, and the washed cell-walls were suspended by vigorous stirring in 5 vol. of 1:1 chloroform–methanol, and placed in a coarse sintered-glass funnel. The organic solvent was removed by applying gentle suction to the funnel, and the cell walls were suspended in 5 vol. of acetone. Cell walls were repeatedly washed with acetone, and then air-dried. The cell walls were tested for the presence of starch, and starch was removed by treating the walls with alpha amylase (Bacillus type II-A) as described by York *et al.*[10].

Preparation of cell walls from leaves. — Fully expanded leaves from Acala 44 cotton and tobacco (Burley white) plants were used for cell-wall preparation. Leaves were cleaned, and the mid-veins were removed. The leaves were cut into small pieces, and homogenized thoroughly in 1 vol. of 100mM phosphate buffer (pH 7) in a food processor. The mixture was centrifuged at 2,000*g* for 10 min, and the supernatant liquor was discarded. The rest of the procedure was as already described for suspension-culture cells.

Preparation of cell walls from carrot roots. — Carrots purchased locally were washed, cut into small pieces, blended thoroughly with 100mM phosphate buffer (pH 7), and centrifuged at 2,000*g* for 10 min. The supernatant liquor was removed, the pellet was washed with buffer, and cell walls were prepared as described for the suspension-culture cells.

Determination of sugar composition. — Dry cell-walls or other samples (20–50 μg) were placed in Teflon-lined, screw-capped glass vials containing 10 nmol of inositol as internal standard. Methanolysis and derivatization were performed by a modification of the method of Chaplin[11]. Methanolic HCl (100 μL of 1.5M) and 25 μL of methyl acetate were added to the samples, and sealed vials were kept in a heating block for 16 h at 80°. After cooling, a few drops of *tert*-butanol were added to each vial, and the samples were evaporated under a stream of nitrogen. Trimethylsilylating reagent was prepared by mixing 1 part of Tri-Sil Concentrate (Pierce Chemical Co.) and 3 parts of dry pyridine. Twenty-five μL of the reagent was added to the dried samples, and these were kept for 15 min at room temperature. The derivatized samples were evaporated just to dryness under a stream of argon, and dissolved in 100 μL of iso-octane. A 1-μL aliquot was injected onto a fused-silica capillary column (30 m × 0.25 mm i.d., Durabond-1 liquid phase, J & W Scientific, Inc., Rancho Cordova, CA) installed in a Tracor 560 gas–liquid chromatograph equipped with an on-column injector and helium carrier-gas. The sample was injected at 105°, and the temperature was immediately raised to 140° and held for 4 min before being raised at $1°.min^{-1}$ to 200°. Peak integration and data analysis were performed by using an Apple IIe microcomputer. A Varian 3300 gas chromatograph fitted with the same DB-1 column, and a Varian 4290 integrator, were also used for the sugar analysis.

HF solvolysis of cell walls. — Cell walls were treated with anhydrous liquid HF as described by Mort[12]. In a typical experiment, 250 mg of dry cell-walls were taken in a Teflon reaction vessel and treated with 10 mL of anhydrous liquid HF for 30 min at −23°. The temperature was maintained by an immersion cooler and a temperature regulator (FTS Systems, Inc., NY) using 95% ethanol as the cooling bath. The reaction was stopped by adding cold ether (cooled by adding dry ice to the ether) to the reaction mixture. The quenched reaction mixture was kept for 15 min at room temperature, and then filtered using a Teflon filter. The HF–ether in the filtrate was evaporated under vacuum into a liquid-nitrogen trap. In some cases, the HF–ether mixture was neutralized with $CaCO_3$, and the sugars were extracted from the resulting powder with water.

Gel-filtration chromatography. — The solubilized fractions from the HF reaction were separated on a column (62 × 2.2 cm) of Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA), and eluted with 0.05M sodium acetate buffer (pH 5.2), 2.5-mL fractions being collected. Sugars in the fractions were detected by the phenol–sulfuric acid test[13]. Sodium ions were removed from pooled fractions by passage through a small column of AG-50W X-8 (H^+) cation-exchange resin (Bio-Rad Laboratories), and the samples were freeze-dried.

Ion-exchange chromatography. — Ion-exchange chromatography was performed on a column (5 × 1 cm) of QAE-Sephadex anion-exchanger (Sigma Chemical Co.) with 25mM sodium acetate buffer (pH 5.2), and negatively charged sugars were eluted by using 0.05M sodium acetate buffer, pH 5.2.

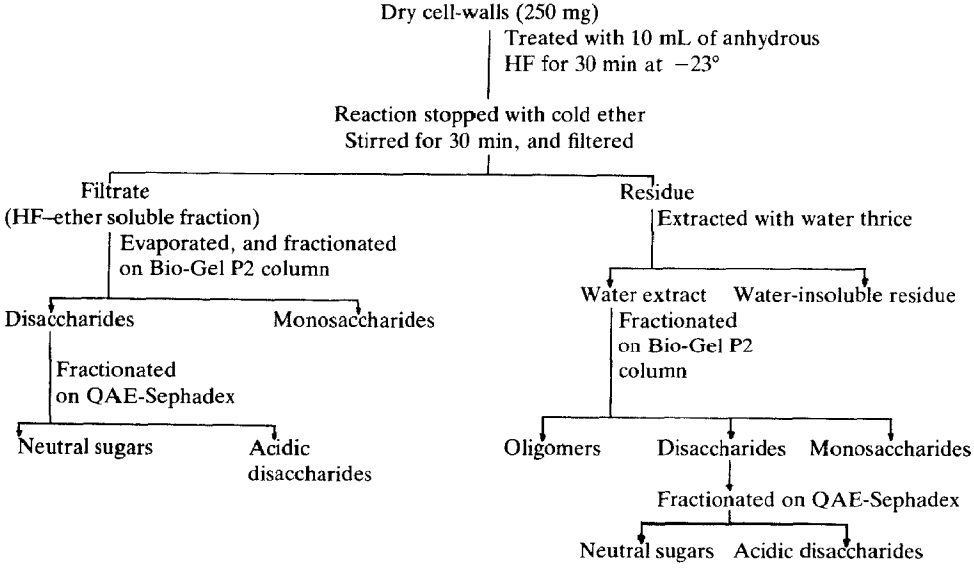
N.m.r. spectroscopy. — ^{1}H-N.m.r. (299.99 MHz) and ^{13}C-n.m.r. (57.22 MHz) spectra were recorded with a Varian (Palo Alto, CA) XL-300 n.m.r. spectrometer at both 25° and 70°. The samples (4–8 mg) were dissolved in 98% D_2O (Sigma Chemical Co.) and freeze-dried. They were again dissolved in 100% D_2O and freeze-dried to exchange the hydrogen with deuterium, and then they were dissolved in 600 μL of 100% D_2O. Sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate (TSP) was used as the internal standard (0.00 p.p.m.).

2D-N.m.r. spectroscopy. — 2D-N.m.r. spectroscopy was performed as described by Gray[14]. The ^{1}H–^{1}H, homonuclear-correlated spectra, and ^{13}C–^{1}H heteronuclear spectra were recorded with a Varian XL-300 n.m.r. spectrometer at 25°.

Methylation analysis was performed by the modified Hakomori method as described earlier[15].

RESULTS AND DISCUSSION

Cell walls from suspension cultures of Acala 44 cotton cells were treated with HF, and fractionated as outlined in Scheme 1. About 27% of the weight of the walls was soluble in the HF–ether mixture that resulted from quenching the reaction. This solubility is characteristic of mono- and di-saccharides[6,8]. Another 21% could be leached from the residue with water.



Scheme 1. HF solvolysis and fractionation of cell walls at −23°.

TABLE I

MOLE % OF SUGARS IN DIFFERENT FRACTIONS AFTER HF SOLVOLYSIS AT −23° (REFER TO SCHEME 1)

Sugar	*HF–ether-soluble fraction*	*Disaccharide isolated from HF–ether-soluble fraction*	*Water extract*	*Disaccharide isolated from water extract*
Ara	22.2	5.5	4.8	4.4
Rha	11.1	39.3	6.8	34.9
Fuc	3.4			
Xyl	31.9	8.3	6.4	5.2
GlcA	trace		1.3	8.4
4-*O*-Me-GlcA	present	present		
GalA	8.1	40.4	29.9	35.7
Man	0.5		0.8	
Gal	11.7	4.9	6.8	9.7
Glc	11.1	1.6	43.2	1.7

The HF–ether-soluble sugars were crudely separated into mono- and disaccharides by chromatography on Bio Gel P-2, and then the disaccharide fraction was separated into neutral and acidic fractions on a QAE-Sephadex column. Water-soluble sugars extracted from the residue were fractionated by Bio-Gel P-2 and QAE-Sephadex chromatography in the same way. Both the HF–ether-soluble fraction and the water extract yielded acidic disaccharides. The sugar composition of the various fractions is given in Table I.

The ^{1}H-n.m.r. spectrum of the disaccharide from the water extract is shown in Fig. 1a. The spectrum can be readily interpreted as that of a disaccharide of galacturonic acid and rhamnose, having an unusually low chemical-shift of one of the ring protons, indicated by the doublet of doublets at 4.70 p.p.m.

The n.m.r. spectrum of the disaccharide from the HF–ether-soluble fraction was more complex (see Fig. 1b). The singlet at δ 2.18 is indicative of the CH_3 of an acetic ester. From the integration, it appears that ~40% of the disaccharide in this fraction was acetylated. All of the signals are present corresponding to the nonacetylated disaccharide obtained from the water extract. Thus, the HF–ether-soluble fraction is a mixture of acetylated and nonacetylated disaccharides. From the 2D homocorrelation spectrum, the identity of each signal was assigned. For the nonacetylated disaccharide, the signal at 4.7 p.p.m. was found to be from H-3 of galacturonic acid. For the α anomer of free galacturonic acid, H-3 resonates at 3.91 p.p.m., indicating a large perturbation in the electronic environment of H-3 of the galacturonic acid residue in the disaccharide. For the acetylated form of the disaccharide, the resonance for H-3 of galacturonic acid is shifted an additional 1.13 p.p.m. downfield, to 5.83 p.p.m., strongly suggesting that O-3 of the galacturonic acid is the site of the acetylation.

The ^{13}C-n.m.r. spectra of the two samples are quite consistent with the conclusion that C-3 of the galacturonic acid is the unique location of the acetate group.

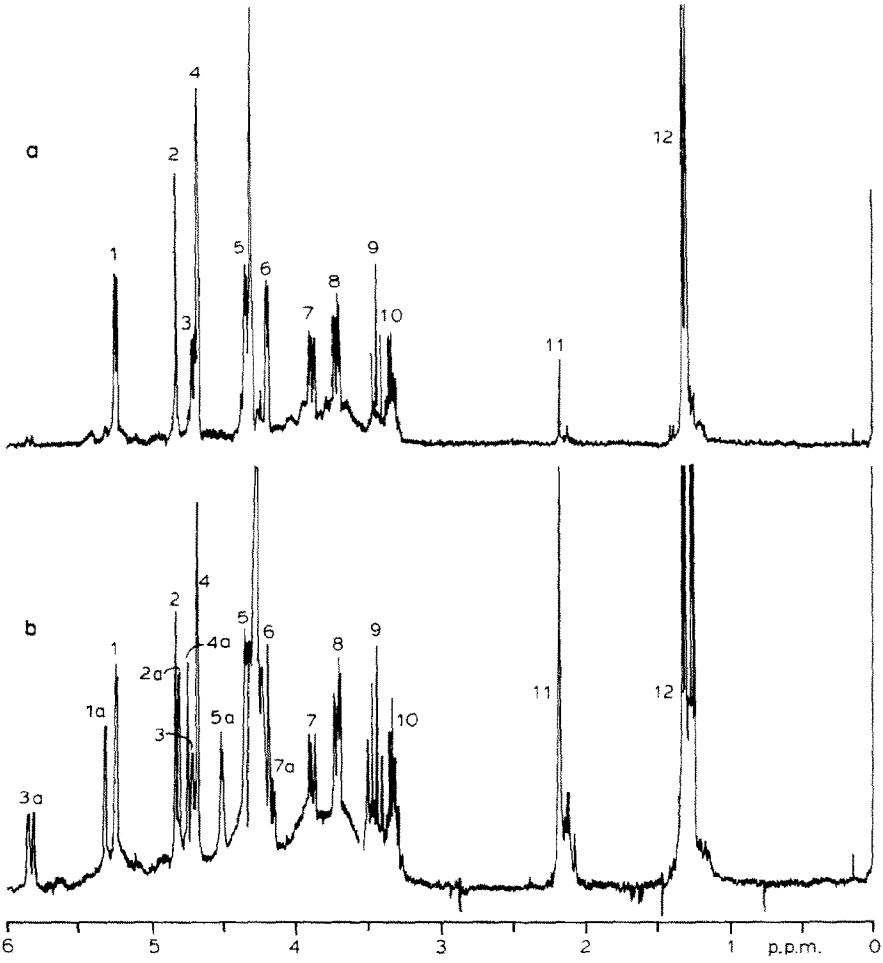


Fig. 1. (a) The ^{1}H-n.m.r. spectrum of the disaccharide *O*-galactosyluronic acid-rhamnose (GalA–Rha) isolated from the water-soluble fraction; (b) the ^{1}H-n.m.r. spectrum of the disaccharide *O*-galactosyluronic acid-rhamnose isolated from the HF–ether-soluble fraction (refer to Scheme 1). Peaks are assigned as follows. Plain numbers refer to signals in the non-acetylated disaccharide and the numbers with an "a" refer to signals in the acetylated disaccharide. (1) H-1 of GalA, (2) H-1 of Rha, (3) H-3 of GalA, (4) H-5 of GalA, (5) H-4 of GalA, (6) H-2 of Rha, (7) H-2 of GalA, (8) H-3 of Rha, (9) H-4 of Rha, (10) H-5 of Rha, (11) CH_3 of acetate, and (12) CH_3 of Rha.

Each signal was identified by using the ^{13}C–^{1}H heterocorrelation spectrum (see Table II). The signal for C-3 of the acetylated galacturonic acid was shifted 3.81 p.p.m. downfield with respect to that of the nonacetylated form. Both the C-2 and C-4 signals were shifted upfield, by 3.23 and 2.55 p.p.m., respectively.

When compared with free α-galacturonic acid (see Table II), most of the ^{13}C and ^{1}H chemical shifts are quite close to what would be expected, except that that of C-2 in our sample is downfield in the ^{13}C spectrum, and H-3 is shifted far

TABLE II

ASSIGNMENTS OF CARBON AND HYDROGEN ATOMS TO THE SIGNALS OBSERVED IN THE ^{1}H- AND ^{13}C-N.M.R. SPECTRA[a] OF THE DISACCHARIDE (NONACETYLATED AND ACETYLATED) AND THE REFERENCE COMPOUNDS, (α-GALACTURONIC ACID AND β-RHAMNOSE)

Atoms	*α-Galacturonic acid*		*Galacturonic acid in nonacetylated disaccharide*		*Galacturonic acid in acetylated disaccharide*		*β-Rhamnose*		*β-Rhamnose in nonacetylated disaccharide*		*β-Rhamnose in acetylated disaccharide*	
	^1H[b] (p.p.m.)	^{13}C (p.p.m.)	^1H (p.p.m.)	^{13}C (p.p.m.)	^1H (p.p.m.)	^{13}C (p.p.m.)	^1H[c] (p.p.m.)	^{13}C[d] (p.p.m.)	^1H (p.p.m.)	^{13}C (p.p.m.)	^1H (p.p.m.)	^{13}C (p.p.m.)
	$^3J_{H,H}$ (Hz)		$^3J_{H,H}$ (Hz)		$^3J_{H,H}$ (Hz)		$^3J_{H,H}$ (Hz)		$^3J_{H,H}$ (Hz)		$^3J_{H,H}$ (Hz)	
C-1, H-1	5.30	95.26	5.24	97.60	5.31	97.49	4.86	96.35	4.83	94.29	4.80	94.02
	$J_{1,2}$ 3.8		$J_{1,2}$ 3.6		$J_{1,2}$ 3.6		$J_{1,2}$ 1.1		$J_{1,2}$ <2		$J_{1,2}$ <2	
C-2, H-2	3.81	70.73	3.88	73.37	4.16	70.14	3.94	74.17	4.20	78.71	4.22	78.57
	$J_{2,3}$ 10.0		$J_{2,3}$ 10.4		$J_{2,3}$ 10.7		$J_{2,3}$ 3.5		$J_{2,3}$ 3.6		$J_{2,3}$ 3.6	
C-3, H-3	3.91	71.58	4.70	71.45	5.83	75.26	3.60	75.59	3.71	73.85	3.71	73.68
	$J_{3,4}$ 3.5		$J_{3,4}$ 3.2		$J_{3,4}$ 3.2		$J_{3,4}$ 9.5		$J_{3,4}$ 9.5		$J_{3,4}$ 9.5	
C-4, H-4	4.26	72.58	4.33	73.29	4.51	70.75	3.36	74.66	3.47	74.49	3.44	74.31
	$J_{4,5}$ 1.5		$J_{4,5}$ <2		$J_{4,5}$ <2		$J_{4,5}$ 9.6		$J_{4,5}$ 9.5		$J_{4,5}$ 9.5	
C-5, H-5	4.41	75.24	4.68	75.82	4.75	75.36	3.40	74.88	3.35	74.95	3.35	75.11
							$J_{5,6}$ 6.2		$J_{5,6}$ 6.0		$J_{5,6}$ 6.0	
C-6, H-6		174.99		175.51		175.09	1.29	19.59	1.31	19.92	1.29	19.76

CH_3 of acetate: ^{1}H 2.18, ^{13}C 23.46; >C=O of acetate: ^{13}C 176.37

[a]The ^{1}H- and ^{13}C-n.m.r. spectra were recorded for compounds in D_2O solution, using TSP as the internal standard. [b]Ref. 16. [c]Ref. 17. [d]Ref. 18.

downfield in the ^1H spectrum. Another peculiarity indicated by both the ^{13}C and ^1H spectra is that rhamnose occurs in only one anomeric form.

Were the rhamnose at the reducing end of the disaccharides, an equilibrium mixture of anomers would be expected. Both the ^1H and ^{13}C spectra indicated that the rhamnose is in the β configuration. The H-1 atom of rhamnose resonates at 4.83 p.p.m., which is indicative of β-rhamnose[17]. In the ^{13}C spectrum, the signal for C-5 of rhamnose is at almost 75 p.p.m. (as it is for all β-rhamnosyl residues), not at ~71 p.p.m., as it would be for α-rhamnosyl residues[18]. In the fully ^1H-coupled, ^{13}C spectrum, the H-1, C-1 coupling of galacturonic acid is 169.7 and 170.9 Hz for the acetylated and nonacetylated forms, respectively, which indicates that the galacturonic acid residue is the α anomer. However, the H-1, C-1 coupling of the rhamnose residue was 166 and 167 Hz in the nonacetylated and acetylated cases, respectively. These couplings are suggestive of the α configuration or a form intermediate[18] between α and β.

Using space-filling models, it is quite possible to build a dianhydride in which galacturonic acid is linked α-(1→2) to rhamnose, with rhamnose linked β-(1→2) back to the galacturonic acid. This is not possible with the rhamnose as the α anomer. During the solvolysis of polysaccharides in HF, the initial products are glycosyl fluorides. These are somewhat reactive, especially in liquid HF. If high concentrations of sugar fluorides are generated in HF, they tend to polymerize[19,20]. The polymerization can usually be avoided by working at low concentrations. However, in the case of gellan gum[7], we observed what we believe to have been oligomerization of the repeat unit of the gum during reaction in HF even at low concentrations. In the case of galacturonic acid → rhamnosyl fluoride, the fluorine on C-1 of the rhamnose is held adjacent to the O-2 atom of galacturonic acid, and could easily react to form the β-(1→2) cyclic glycoside. Formation of the glycoside at C-2 of galacturonic acid could explain the downfield shift of the C-2 signal in the ^{13}C spectrum, compared to that in free galacturonic acid. In the dianhydride thus formed, H-3 of galacturonic acid is held in very close proximity to O-2 and O-5 of rhamnose, which could explain its downfield position in the ^1H spectrum[21]. Comparison of the coupling constants of the sugar residues to those of free α-galacturonic acid and rhamnose (see Table II) shows that there is not much distortion from the expected $^4C_1$ and $^1C_4$ conformations, respectively.

When the disaccharide was methylated, followed by reduction of the galacturonic acid residue to galactose, hydrolysis, and conversion into alditol acetates, 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylgalactitol was obtained, not the 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol that would be expected from the noncyclized disaccharide. All of the rhamnose was converted into 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol.

The generation of a dianhydride from a pectin-like polysaccharide from Wobaku bark had been observed; Fujiwara and Arai[22] found that, after mild methanolysis and acetylation of the disaccharide fraction obtained following partial acid hydrolysis of the polysaccharide, a crystalline product could be obtained which

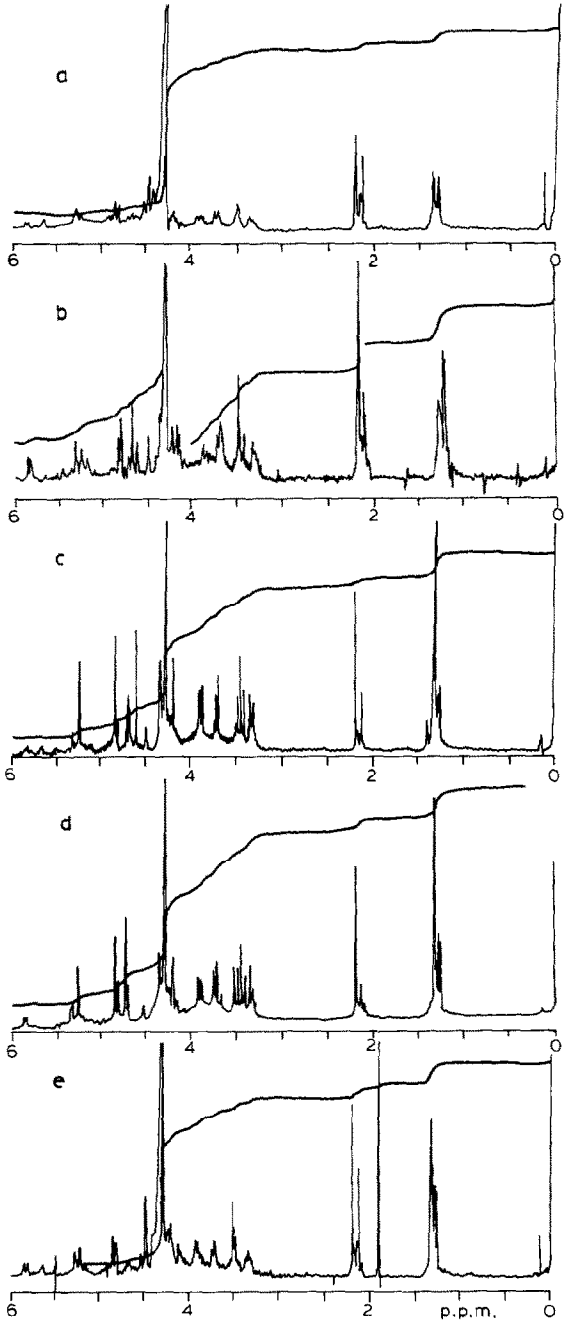


Fig. 2. The ^{1}H-n.m.r. spectrum of the disaccharide *O*-galactosyluronic acid-rhamnose isolated from the HF–ether-soluble fraction (refer to Scheme 1) of different plant species. (a) Im 216 Cotton suspension cell-walls, (b) Acala 44 Cotton-leaf cell-walls, (c) tomato suspension cell-walls, (d) tobacco-leaf cell-walls, (e) carrot-root cell-walls. Samples (a), (c), and (e) were produced by using $CaCO_3$ to neutralize the acid in the HF–ether mixture, and they exhibit signals for two sites of acetylation.

proved to be the peracetylated methyl ester of the same disaccharide dianhydride that we obtained. Because of competing reactions, the yield of the dianhydride was low. The ^{1}H-n.m.r. spectrum of the dianhydride showed the same "anomalously" large downfield-shift for the H-3 signal of the galacturonic acid, but, otherwise, no severe distortion from the chair conformations of the sugars.

In order to find out whether the acetylation of rhamnogalacturonan is a general phenomenon and if there is an RG-I-like region containing a fairly strict repeating disaccharide backbone in many plants, a variety of cell-wall sources was extracted in the same way as were those of the Acala 44 suspension-culture cell-walls. Cell walls were prepared from a cell suspension-culture of cotton from a different cotton line (Im 216), from leaves of Acala 44 cotton plants, from tomato suspension-culture, from tobacco leaves, and from carrot roots. In all cases, a good yield of the disaccharide could be obtained and could be shown by ^{1}H-n.m.r. spectroscopy to have a similar degree of acetylation (see Fig. 2, signal at 2.18 p.p.m.). Many of these samples were prepared by using calcium carbonate to neutralize the HF in ether, rather than by direct evaporation. It may be seen that two positions are acetylated in these cases. The additional signal, at 5.7 p.p.m., has the splittings that would be expected for H-4 of galacturonic acid, but is 1.41 p.p.m. *downfield* from the signal in the nonacetylated case (at 4.33 p.p.m.). Because O-4 of galacturonic acid was involved in a glycosidic linkage in the original polymer, there must have been acyl migration induced (from O-3 to O-4) by the calcium carbonate during the neutralization reaction. There was no detectable acetylation of O-4 if the HF–ether was removed by evaporation. We take the lack of acetate on C-4 of the galacturonic acid residue, in the experiments in which HF–ether was evaporated, to show that acyl migration did *not* take place in those cases. The possibility that O-2 of the galacturonic acid residue was acetylated in the original polymer seems unlikely, because of the quantitative glycosylation of O-2 during the HF treatment which should have been blocked by the acetylation[23]. In previous work[6], we did not observe acyl migration from O-3 to O-2 in an acetylated glucose residue under similar conditions in HF. Also, a 3-hydroxybutanoic ester on O-3 of a galactose residue did not migrate to O-2. Under harsher conditions, *e.g.* for several h at 0 or 18°, fully acetylated sugars with *cis*-hydroxyl groups undergo Walden inversion and, sometimes, ring contraction[24].

Preliminary experiments indicated that fragments of the backbone in which only the reducing-terminal rhamnose has cyclized to form the dianhydride can be obtained by performing the HF solvolysis at −40° instead of −23°. From the ratio of recovery of disaccharide from the HF–ether and water extracts, and the ~40% acetylation of the former, it was estimated that, overall, 30% of the galacturonic acid residues are acetylated.

Sun *et al.*[25] reported that, in pectic preparations they obtained from tobacco leaves, the portion which was richest in the neutral sugars arabinose and galactose was the only one with significant acetylation. In contrast, this fraction contained the fewest methyl-esterified galacturonic acid residues. This fraction probably con-

tained a large proportion of the RG-I-like region. We found no evidence for methyl esterification of any of the disaccharide we isolated. Thus, it is likely that acetylation of pectin is mostly confined to the RG-I region, and methyl esterification to the homogalacturonan region.

CONCLUSION

In the cell walls of all dicots tested, there is a significant proportion of a polysaccharide which, after solvolysis at −23° with HF, gives rise to a mixture of acetylated and nonacetylated disaccharides of galacturonic acid and rhamnose. The only known polymer that would produce these disaccharides is that called RG-I by McNeil *et al.*[1]. Thus, it appears that this region of pectin is acetylated on O-3 of about one in three of the galacturonic acid residues in its backbone.

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