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INVESTIGATION OF STRUCTURAL FEATURES OF THE PECTIC POLYSACCHARIDES OF ONION BY ^{13}C-N.M.R. SPECTROSCOPY

PETER RYDEN, IAN J. COLQUHOUN, AND ROBERT R. SELVENDRAN*
AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA (Great Britain)

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ABSTRACT

The Na_2CO_3-soluble pectic polysaccharides of mature onions were separated by anion-exchange chromatography, and the structural features of two of the pectic polysaccharides were examined by methylation analysis and ^{13}C-n.m.r. spectroscopy. The oligosaccharide side-chains containing β-D-galactose were (1→4)-linked and were shown to have an average, d.p. of 7–8 by methylation analysis and 8–9 by n.m.r. spectroscopy. The α-L-arabinose side-chains were (1→5)-linked and had an average d.p. of 3, as shown by methylation analysis, but it was not possible to obtain a value from the n.m.r. data.

INTRODUCTION

The isolation and partial characterisation by methylation analysis of onion cell-wall polysaccharides has been described[1]. No unambiguous data on the average length of the neutral side-chains of the pectic polysaccharides were obtained. We now report the application of methylation analysis together with ^{13}C-n.m.r. spectroscopy which provided more information on the structure of these side chains. The 100-MHz ^{13}C-n.m.r. spectra contained minor signals for the anomeric carbons of the terminal residues of the side chains, which enabled estimation of their lengths. The method was first described by Gidley[2] for the measurement of the degree of branching of degraded starches.

RESULTS AND DISCUSSION

Fractionation of the Na_2CO_3-soluble material by anion-exchange chromatography gave 5 pectic fractions. This fractionation differs from that reported[1], in that significant amounts of more acidic fractions were found. The carbohydrate compositions of the fractions are shown in Table I, and the ^{13}C- and ^{1}H-n.m.r. spectra were obtained for fractions N1B and N1C. Fig. 1 shows the ^{13}C-n.m.r.

*Author for correspondence.

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TABLE I

CARBOHYDRATE COMPOSITION OF Na_2CO_3-SOLUBLE FRACTIONS

Fraction		*Yield (%)*	*Sugar composition (mol%)*[a]					*Total sugars (μg/mg)*
			Rha	*Ara*	*Gal*	*Glc*	*Uronic acid*	
Buffer	N1A	6.9	1	5	88	1	5	893
NaCl 0.125M	N1B	9.1	3	5	70	6	16	906
0.25M	N1C	37.0	3	6	36	4	51	852
0.5M	N1D	18.0	2	3	20	2	73	999
1M	N1E	2.9	5	6	61	6	23	599

[a]"Anhydro sugar" values after Saeman hydrolysis.

TABLE II

ASSIGNMENTS OF ^{13}C-N.M.R. SPECTRA[a] OF FRACTION N1B

Sugar residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
(1→5)-α-L-Arabinofuranose	108.4	81.7	77.7	83.2	67.8	
Terminal β-D-galactopyranose	104.4	71.7	73.7	69.6	75.9	61.8
(1→4)-β-D-Galactopyranose	105.2	72.8	74.2	78.5	75.4	61.7

[a]In D_2O at 55° (internal acetone).

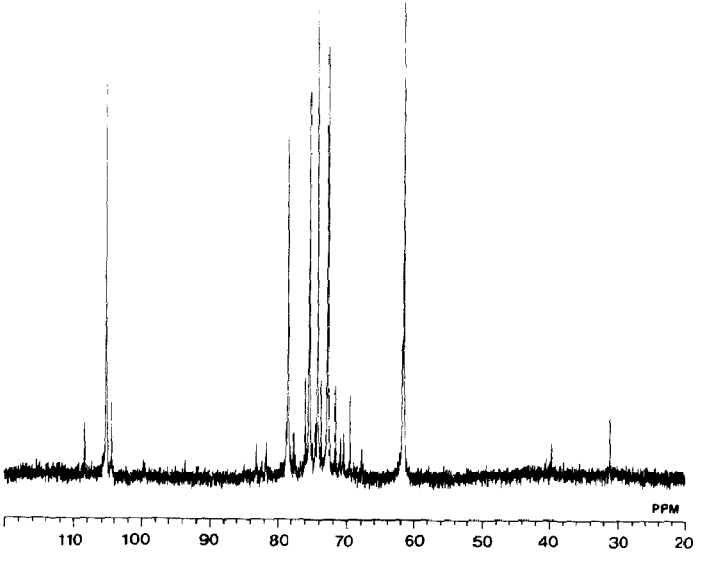

Fig. 1. The ^{13}C-n.m.r. spectrum of onion pectin N1B.

spectrum of N1B, and the assignments, based on those of published spectra of tomato pectin[3] and *Vicia faba* arabinan[4], are shown in Table II. The spectrum contains well resolved signals for arabinose and galactose with narrow line-widths, but no signals for uronic acid or rhamnose. This finding demonstrated the expected structural arrangement of mobile galactose- and arabinose-containing side-chains and a backbone of uronic acid with reduced segmental motion. The signals for mid-chain and terminal residues were resolved in the ^{13}C-n.m.r. spectra for galactose, but not for arabinose. The amount of terminal arabinose was too small to be detected. The ^{1}H-n.m.r. spectra at 400 MHz were complicated by signals from the backbone, and the minor signals for the terminal galactose were not resolved.

The fractions N1B and N1C were each methylated once, and the products were reduced with $LiAl^2H_4$ and analysed conventionally by conversion into the partially methylated alditol acetates. The reduced galacturonic acid residues were labelled with deuterium, but the recoveries were not quantitative, as observed previously[1]. As the total amounts of 2,3-Me_2-Gal, 3,6-Me_2-Gal, and 2,6-Me_2-Gal residues from the branched residues of N1B and N1C without carboxyl-reduction were 7% and 8%, respectively, in excess of the terminal residues, fractions N1B and N1C were methylated twice. It was essential that, during methylation, the samples were completely soluble in methyl sulfoxide. Concentrations of pectin >3 mg/mL formed gels, and the samples were not methylated efficiently. Carboxyl-reduction during methylation was not important since the main objective was to determine the structures of the side chains. After the second methylation (Table III), the proportion of Me_2-Gal residues was negligible.

The average lengths of the side chains, inferred from the relative amounts of terminal to mid-chain residues, were 3.4 and 2.9 for the "arabinan" and 7.5 and 7.7 for the "(1→4)-galactan" in N1B and N1C, respectively. The chain lengths calculated by integration of the ^{13}C resonances for anomeric carbons at 104.4 and

TABLE III

PARTIALLY METHYLATED ALDITOL ACETATES FROM METHYLATED SAMPLES OF Na_2CO_3-SOLUBLE FRACTIONS

Alditol acetates	*Relative mol %*[a]		*Linkage*
	N1B	*N1C*	
3,4-Me_2-Rha[b]	4.8	4.5	→2)-Rha*p*-(1→
3-Me-Rha	3.3	3.2	→2,4)-Rha*p*-(1→
2,3,5-Me_3-Ara	5.6	3.1	Ara*f*-(1→
2,3-Me_2-Ara	13.3	5.9	→5)Ara*f*-(1→
2,3,4,6-Me_4-Gal	9.4	10.3	Gal*p*-(1→
2,4,6-Me_3-Gal	2.5	4.1	→3)-Gal*p*-(1→
2,3,6-Me_3-Gal	61.0	68.8	→4)-Gal*p*-(1→

[a]Values corrected using the molar response factors of Sweet *et al.*[5]. [b]3,4-Me_2-Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol, *etc.*

105.2 p.p.m. indicated average "galactan" chain-lengths of 8.5 and 9.2 for N1B and N1C, respectively. Accurate quantification requires[6] equal n.O.e. and a repetition time of acquisition $>5\ T_1$. Although no T_1 measurements were made, the T_1 values were expected to be closer for the C-1 signals than for other pairs of signals and the repetition time of 2 s is adequate.

The (1→3)-linked galactose residues found by methylation analysis were not detected by n.m.r. spectroscopy, either because the amount was too small or possibly because they were less mobile in a region close to the backbone[7]. The proportion of terminal galactose residues which should be assigned to either (1→3)- or (1→4)-linked galactan chains could not be determined from the results of methylation analysis, which may account for the discrepancy between the estimates from methylation analysis and ^{13}C-n.m.r. spectroscopy. It is also possible that a small proportion of the side chains were lost during dialysis after the first methylation. Thus, the n.m.r. data showed the major pectic side-chains to be oligosaccharides containing (1→4)-linked β-galactose residues with an average d.p. of 8–9.

The ^{13}C-n.m.r. spectrum of N1B contained five unassigned signals at 78.8, 74.6, 70.9, 70.5, and 39.7 p.p.m. with intensities slightly greater than those for C-2,3,4,5 of arabinose. In comparison with the signals for C-2,3,4,5, the C-1 signal of arabinose had twice the intensity expected and probably overlapped the signal of an anomeric carbon corresponding to the unassigned signals. The chemical shifts suggested a (1→6)-deoxyhexopyranose; the resonance at 39.7 p.p.m. is close to the chemical shift of the resonance for C-3 in 3-deoxy-β-*ribo*-hexopyranose[8]. This unidentified sugar was not detected by chemical analysis, probably because it was acid-labile, which demonstrates the need for mild extraction of pectic fractions and the benefits of non-destructive analysis.

Thus, ^{13}C-n.m.r. spectroscopy at 100 MHz is useful, in conjunction with methylation analysis, for structural analysis of the side chains of pectic substances. The complete assignment of the mid-chain and terminal galactose resonances is made possible by the considerable difference in segmental motion between the side chains and the backbone, which conveniently simplifies the spectrum.

EXPERIMENTAL

General methods. — Neutral sugars were released by a Saeman hydrolysis and analysed[9] as their alditol acetates by g.l.c. Uronic acid was determined colorimetrically by a modification[9,10] of the method of Blumenkrantz and Asboe-Hansen[11]. Polysaccharides were methylated twice by the modified[12] Hakomori method. The samples (8 mg) dissolved in methyl sulfoxide (3 mL) after sonication for 2 days at 30°. The methylated products were recovered after the first methylation by dialysis against water and, after the second methylation, by partition between water and dichloromethane. Partially methylated alditol acetates were separated by g.l.c. on a capillary column (10 m × 0.32 mm) of CP Sil 43CB.

Combined g.l.c.–m.s. was performed on an AEI MS-30 mass spectrometer, using a wide bore (25 m × 0.5 mm) OV-225 capillary column.

Preparation of the polysaccharides. — The polysaccharides were extracted[1] from onion cell-wall material. The cell-wall material was extracted twice with 50mM cyclohexane-*trans*-1,2-diamine tetra-acetate (pH 6.5) at 20° and then with 50mM Na_2CO_3 for 20 h at 1°. The Na_2CO_3(1°)-soluble material was neutralised, dialysed, and freeze-dried. The residue (800 mg) was suspended in distilled water (360 mL) overnight at 1° and for 4 h at 20°, 0.5M phosphate buffer (40 mL, pH 6.5) was added, and the solution was passed through a column (3 × 40 cm) of DEAE-Trisacryl M (phosphate form) at 40 mL/h. The fractions were eluted sequentially with 300 mL of 0.05M buffer and 400 mL of 0.05M buffer containing 0.125, 0.25, 0.5 and M NaCl. Fractions (3/h) were collected, and 0.1-mL portions were assayed by the phenol–sulphuric method[13]. The amounts of the sample recovered in each fraction were as follows: buffer, 55 mg; 0.125M NaCl, 73 mg; 0.25M NaCl, 296 mg; 0.5M NaCl, 144 mg; and M NaCl, 23 mg.

^{13}C-N.m.r. spectroscopy. — Spectra were recorded under conditions of broad-band proton decoupling on a JEOL GX400 spectrometer at an operating frequency of 100.4 MHz. Samples were examined as solutions in D_2O (50 mg in 3 mL of solvent) in 10-mm spinning tubes at 55°. Spectra were obtained using 90° pulses with a pulse repetition time of 2 s, and 27,000 transients were acquired. Acetone was the internal reference and the chemical shift of the acetone methyl group was taken to be 31.07 p.p.m. with respect to that of Me_4Si.

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