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Note

A ^{13}C-n.m.r. study of sugar-beet pectin

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Sugar-beet pectin is obtained from sugar-beet pulp, the residue remaining following extraction of sugar from sugar beet. This polysaccharide has a relatively low molecular weight (≤80,000) and contains galacturonic acid (80%), arabinose (10%), galactose (6%), rhamnose (2%), and traces of xylose and mannose. Of the acid groups, ~40% are methylated and 16% of the residues are acetylated[1]. The structure is unknown, but is said to be complex[2,3]. We now report the results of a ^{13}C-n.m.r. study of sugar-beet pectin.

Fig. 1 shows the ^{13}C-n.m.r. spectrum of sugar-beet pectin. The resonances at 100.6, 79.2, and 174.0 p.p.m. are identical to those obtained for a commercial (1→4)-α-galacturonan (Sigma). Pressey and Himmelbach[4] detected a weak, broad signal at ~100 p.p.m. for glycosyluronic residues in a polysaccharide extracted from tomato fruit, and inferred that they occurred in the backbone and were held too rigidly to be seen relative to the other constituent sugars. Our observation of narrow resonances for these residues implies that they arise from some mobile portion of the (1→4)-α-galacturonan, which is probably not representative of backbone material. If all glycosyluronic residues in sugar-beet pectin were observed, their resonances would be about eight times more intense than any others. This is not the case, and therefore we conclude that the signals for (1→4)-α-galacturonan probably arise from side chains or from mobile chain-ends. However, by analogy with other pectins of plant origin[5], it seems reasonable that the backbone glycosyluronic residues should be linked similarly. Indeed, apart from signals representing methyl carbon atoms, the spectrum obtained for solid sugar-beet pectin using cross-polarisation magic-angle spinning methods[6] was dominated by features identical to those observed in the spectrum of solid (1→4)-α-galacturonan, indicating that much of sugar-beet pectin comprises that structure.

The intense signal at 53.4 p.p.m. is assigned to COO*Me* of esterified glycosyluronic residues, and the resonances at 171.1 p.p.m., and at 21.0 and 20.6 p.p.m., arise from O-*C*OCH$_3$ and O-CO*C*H$_3$, respectively. These values are consistent with those reported[7,8] for *O*-acetyl groups in bacterial polysaccharides. A

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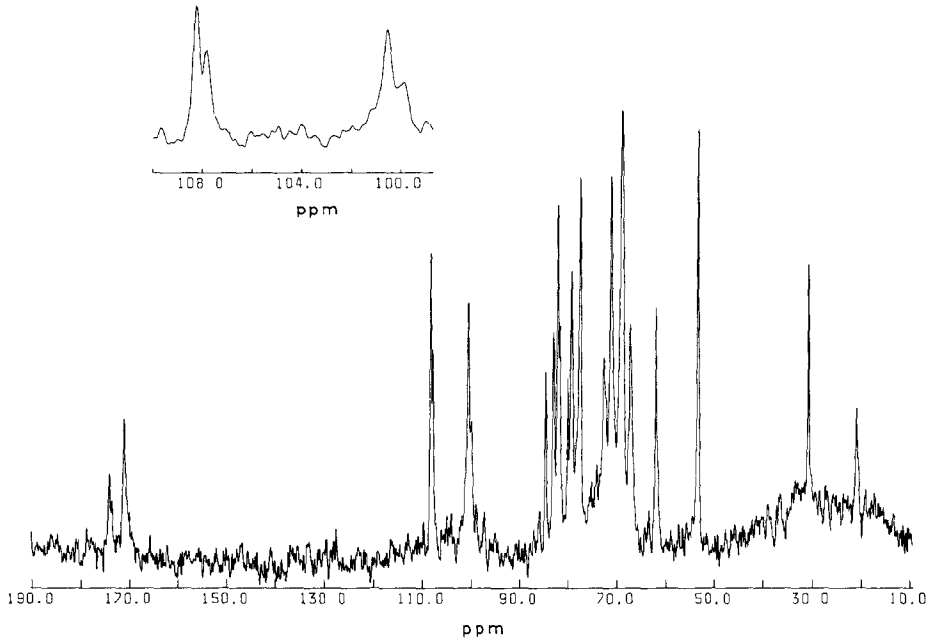

Fig. 1. The ^{13}C-n.m.r. spectrum of sugar-beet pectin.

different number of resonances for methyl and carbonyl carbon atoms in compounds containing more than one *O*-acetyl group is not unusual; for example, α-D-glucose penta-acetate has three and five resonances, respectively[9]. *O*-Acetyl groups are most probably attached to glycosyluronic residues[10], and the dual methyl resonances observed could indicate that both of the available ring positions, namely, 2 and 3, are occupied. An alternative location for the *O*-acetyl groups cannot be dismissed by our data.

Peaks representing C-1 of galactosyl and arabinosyl residues were observed at 107.8 and 108.3 p.p.m., and were present in the ratio 0.6:1, which corresponds closely to the relative amounts of these residues measured by chemical analysis[1]. Although their chemical shifts differ from those of the methyl glycosides of the individual sugars[11,12], they are similar to the values reported[4] for C-1 of (1→4)-linked β-D-galactopyranosyl residues and (1→5)-linked α-L-arabinofuranosyl residues in the polysaccharide of tomato fruit. Supporting the arabinosyl assignment, sugar-beet pectin has resonances at 82.1, 77.5, and 68.9 p.p.m. with an intensity equal to that of the signal at 108.3 p.p.m., and which correspond to the combination of shifts reported[4] for these residues. The signal at 68.9 p.p.m., taken together with that at 108.3 p.p.m., strongly indicates arabinofuranose in a (1→5)-α-linked polymer[13]. Therefore we conclude that arabinose in sugar-beet pectin is present primarily as (1→5)-linked α-L-arabinofuranosyl residues.

The resonances at 84.7, 83.0, and 81.7 p.p.m. are at too high field for the four major sugars present in sugar-beet pectin, and are too intense to be from trace

components. However, the resonance frequencies observed by us for C-1 to C-5 of α/β-D-galacturonic acid closely resemble those for α/β-D-galactopyranose. Methylation of α- or β-D-galactopyranose at position 2 shifts the C-2 signal upfield by 8 p.p.m.[14], whilst methylation at position 3 effects a 10-p.p.m. upfield shift of the signal for C-3. Although no spectral data are available for ring-esterified galacturonic acid, we tentatively assign these peaks to α-D-galactosyluronic acid ring-carbon atoms that are acetylated and/or to α-D-galactosyluronic acid and β-D-galactopyranosyl ring-carbon atoms involved in chain branching.

From the signal-to-noise ratios, a signal at 17.9 p.p.m., indicative of the C-6 of rhamnose[15], would have been expected because rhamnose is present in sugar-beet pectin in amounts comparable to those of the tomato polysaccharide for which such a peak was observed[4]. Therefore, we infer that rhamnosyl residues do not occupy a flexible position in the sugar-beet pectin molecule and are backbone constituents.

The ^{13}C-n.m.r. data establish that sugar-beet pectin is a branched (1→4)-linked α-D-galacturonan containing a small proportion of rhamnose, with side chains composed of (1→5)-linked α-L-arabinofuranosyl residues and β-galactosyl residues, probably (1→4)-linked.

EXPERIMENTAL

A Bruker CXP 300 spectrometer operating at 300.00 MHz for ^{1}H and 75.4 MHz for ^{13}C was used with a sweep width of 20 kHz and the collection of 1K data points. Before Fourier transformation, the data table was zero filled to 8K and an exponential apodization function of equivalent 15-Hz line broadening was used. Data were acquired at 55° under conditions of broad-band decoupling with a recycle time of 2 s and a flip angle of 60°; 30,000 transients were acquired.

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