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Note

^{13}C-N.m.r. spectrum of a D-galactose-rich polysaccharide from tomato fruit

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The action of tomato galacturonanase on tomato cell-walls isolated from green fruit releases galacturonans of low molecular weight, and also a D-galactose-rich polysaccharide having a high, apparent molecular weight[1,2]. This polysaccharide contains 58% of D-galactose, 14.5% of arabinose, 3.5% of rhamnose, 22% of galacturonic acid, and 2% of several minor components. An enzyme has been isolated[2] from ripe tomatoes that hydrolyzes most of the D-galactose in this polysaccharide. To help establish the substrate specificity of the D-galactanase, a study was undertaken to determine the D-galactose linkages in the polysaccharide.

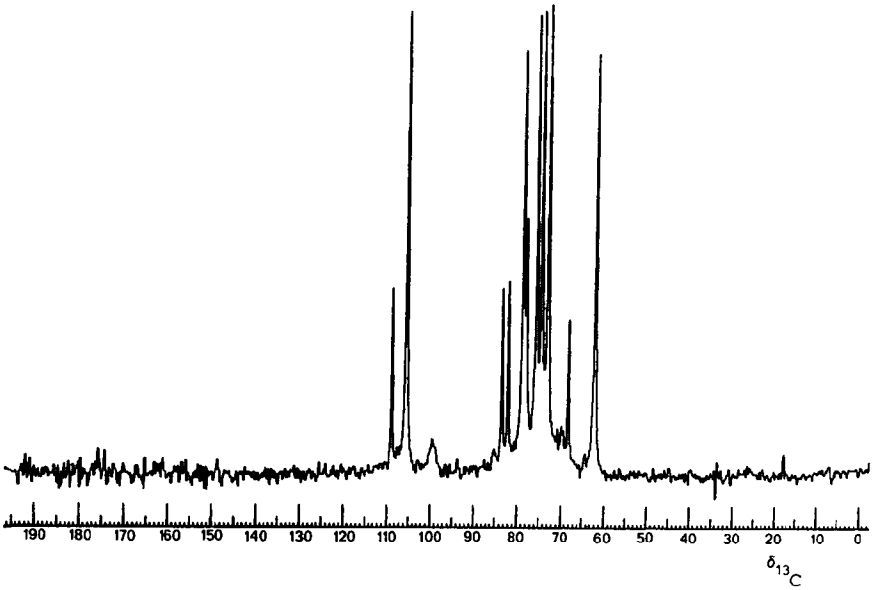

Fig. 1. ^{13}C-N.m.r. spectrum of the D-galactose-rich, tomato polysaccharide, recorded relative to external tetramethylsilane

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We report here the results of ^{13}C-n.m.r.-spectral studies that indicate the structure not only of the D-galactan but also of the arabinan of the polysaccharide.

Fig. 1 shows the ^{13}C-n.m.r. spectrum of the tomato polysaccharide. Peaks for C-1 and C-4 of the D-galactosyl residues in the polysaccharide were observed at 105.6 and 78.9 p.p.m., respectively. The corresponding peaks occur at 99.5 and 69.3 p.p.m. for methyl α-D-galactopyranoside, and at 103.9 and 68.8 p.p.m. for methyl β-D-galactopyranoside[3]. The downfield shift of ~10 p.p.m. for C-4 of the D-galactosyl residues in the polysaccharide is particularly characteristic of a (1→4) linkage. The data are also consistent with a β-galactosidic linkage, based on an expected, small downfield shift for C-1 of a D-galactopyranosyl D-galactopyranoside compared with the methyl derivative.

Peaks for C-1 and C-5 of the arabinose in the polysaccharide were respectively observed at 108.9 and 68.3 p.p.m. (see Fig. 1). Ritchie *et al.*[4] reported peaks for C-1 and C-5 in the spectrum of methyl α-L-arabinofuranoside at 109.2 and 62.2 p.p.m., respectively, and in that of methyl β-L-arabinofuranoside at 103.1 and 62.4 p.p.m., respectively. Thus, the shifts observed for the arabinose in the polysaccharide indicate α-(1→5) linkages. This is consistent with almost identical signals (after correction for the difference in the reference systems) reported by Joseleau *et al.*[5] for this linkage in two L-arabinans isolated from *Rosa glauca* stems.

Integration of the anomeric-carbon resonances at 108.9 and 105.6 p.p.m. indicated a 4:1 ratio of galactose to arabinose in the tomato polysaccharide. This is in agreement with the relative levels of these sugars as determined[1] by g.l.c.

The one-bond, ^{13}C–^{1}H coupling-constants ($^{1}J_{CH}$) were also determined by gated decoupling. Carbon atom 1 of the galactosyl and arabinosyl units gave $^{1}J_{CH}$ values of 161.1 and 174.6 Hz, respectively. These results are in accord with the values reported for methyl β-D-galactopyranoside[6] and methyl α-L-arabinofuranoside[5].

The results indicate that the tomato polysaccharide contains relatively long, homogeneous blocks of α-(1→5)-linked arabinofuranosyl and β-(1→4)-linked galactopyranosyl residues. Presumably, these neutral polymers occur as side chains on a rhamnogalacturonan backbone, as postulated for the pectic polysaccharide from suspension-cultured, sycamore cells[7]. Ishii[8] recently isolated from potato tubers a galactose-rich polysaccharide that appears to be similar to the tomato polysaccharide.

The small signal at 18.0 p.p.m. indicates the presence of a low level of rhamnose[3] in the polysaccharide. However, the n.m.r. spectrum shows very weak signals for galacturonic acid, a major component of the polysaccharide[1,2]. Except for the broad, anomeric resonance near 100 p.p.m., most of the signals due to glycosyluronic residues, as determined for α-(1→4)-galacturonan (Sigma Chemical Co.), were not readily discernible from the "noise". Part of the problem may be due to short T_1 (spin–lattice) or T_2 (spin–spin) relaxation values for glycosyluronic residues, which would result in signal broadening and diminution, but the very low signals for galacturonic acid suggest an unusual, motional effect. Aggregation of

the polysaccharide under the experimental conditions employed may be involved. Another possible explanation is that the glucosyluronic residues occur in the backbone of the polysaccharide, and that this part of the molecule is too large, or too rigid, or both, to yield detectable signals. In contrast, the strong signals for the arabinan and galactan portions indicate that they are highly flexible, as would be expected for side chains in the molecule.

The n.m.r.-spectral data established that the galactose in the tomato polysaccharide is β-(1→4)-linked, and that the enzyme, isolated[2] from ripe tomatoes, that hydrolyzes the galactan portion of the polysaccharide must be a (1→4)-β-galactanase.

EXPERIMENTAL

Preparation of the polysaccharide. — The polysaccharide was solubilized from cell walls isolated from green tomato fruit, as described previously[1]. The cell walls were treated[2] with a combination of tomato pectinesterase and galacturonanase at pH 3.5 for 1 h at 37°. The suspension was filtered, and the filtrate was boiled for 5 min to inactivate the enzymes. The solubilized fraction was ultrafiltered through a PM-10 membrane (Amicon Corp.) to remove the solutes of low molecular weight. The retained macrosolute was then applied to a column (2.5 × 40 cm) of Bio-Gel A-15 m equilibrated with 0.15M NaCl. The large peak of anthrone-positive material that was eluted near the exclusion limit of the gel represented the galactose-rich polysaccharide. It was further purified on a column of DEAE-Sephadex A-50, which removed a small amount of uronic acid. The polysaccharide was precipitated by adding ethanol (2 vol.) to the solution, successively washed with ethanol and acetone, and dried. The yield was 43 mg from 1 g of cell walls.

^{13}C-N.m.r. spectroscopy. — The tomato polysaccharide (100 mg) was dissolved in D_2O (1 mL) at 70°. The ^{13}C-n.m.r. spectrum was recorded with a JEOL PS/PFT 100 n.m.r. spectrometer interfaced with a Nicolet 1083 computer system. The spectrometer was operated at 25.2 MHz for carbon, utilizing proton-decoupling at 100.0 MHz, and locked on the deuterium signal at 15.4 MHz. In the complete-decoupling experiment, a pulse repetition time of 2.0 s with a 90° pulse angle (20 s in width) and a 5-kHz spectral window were employed, to accumulate 42,000 spectral scans in 8 k of computer core. In the gated-decoupling experiment, the pulse repetition-time was changed to 5.0 s, and 65,000 scans were accumulated. In both experiments, only the first 2 k of the free induction decay (f.i.d.) signal was utilized for the Fourier transform, as essentially all of the signal was contained in this portion of the time-domain spectrum. The remainder of the f.i.d. (6 k) was zero-filled, to improve apparent resolution and s/n value. The spectra were referenced to external tetramethylsilane contained in a 1-mm wide, capillary tube centered in a 10-mm n.m.r. tube that contained the solvent. The spectra were obtained at 70°, to increase the concentration of the solution of polysaccharide.

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