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Preliminary communication

Isolation and characterization of a homogalacturonan in the primary walls of *Rosa* cells cultures *in vitro*

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A characteristic feature of primary cell walls is their high content of galacturonic acid in the pectic polysaccharides. In galactosyluronic-containing polymers, rhamnosyl residues have been demonstrated[1,2] as integral components of the polysaccharide chain, as shown by the isolation through partial hydrolysis of rhamnose-containing oligoglycuronic acids. Arabinosyl and galactosyl residues are also covalently attached to the rhamnogalacturonan[3]. A more-complex heterogalacturonan has been recently isolated and characterized[3], in which 10 different monosaccharides have been shown to be glycosidically linked, including such rare sugars as apiose and 2-methyl ethers of xylose and fucose. However, the existence of a pure homogalacturonan, as found in apple pectin[4], in sunflower seeds[5], and in the bark of Amabilis fir[6], has been mentioned only recently as a constituent of the primary cell wall of *Acer pseudoplatanus*[7].

This communication describes the isolation of a homogalacturonan from the wall of suspension-cultured cells of *Rosa glauca*, and its chemical characterization.

Sequential extraction with EDTA and ammonium oxalate solutions of the cell walls from 14-day-old cultures gave a first fraction of galacturonic acid-containing polysaccharides corresponding to 21% of the total glycuronans initially present in the cell walls[8]. Subsequent extractions with sodium hydroxide solutions of increasing concentration (1.25, 2.5, and 4.3 M) left a residual material that still contained ~60% of the initial glycuronan[8]. A similar result was achieved when the alkaline extraction was limited to a single stepwith 2.5 M sodium hydroxide, and an insoluble residue R (55% of the wall) in which ~50% of the initial glycuronan present was accounted for, was obtained. Total hydrolysis of residue R revealed that glucose accounted for 60% of the neutral sugars, and thus cellulose was the predominant polysaccharide constituent. It seems at this stage that the glycuronans are tightly associated with cellulose, as further extraction with sodium hydroxide did not solubilize a significant amount of pectic material. When hydrolytic conditions (M sulfuric acid, 120°) known to hydrolyse only hemicellulose material[9] were applied to R, an insoluble residue was obtained in which glucose constituted 95% of the neutral sugars. Washing this residue with 0.15 M sodium hydroxide solubilized a fraction that was reprecipitated at the lower pH, and constituted 9% of R (5% of the wall).

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Analysis of this material showed it to contain only galacturonic acid, with no accompanying, neutral-sugar residues. Carboxyl reduction[10] followed by hydrolysis afforded only galactose and 3% of residual galacturonic acid. Methylation analysis of the galactan obtained by carboxylreduction of the galacturonan gave 2,3,6-tri-*O*-methyl galactose as the only methylated derivative, showing that the galactosyluronic residues were 1→4 linked. As no peak corresponding to 2,3,4,6-tetra-*O*-methylgalactose could be identified by g.l.c.–m.s. of the hydrolysate of the permethylated galactan, it may be deduced that the galacturonan is linear and had a degree of polymerization at least >100. The highly positive value of the specific optical rotation, $[\alpha]_D^{20}$ +125°(*c* 1.6, water) suggests that the galactosyluronic units have the α-D configuration. This supposition was further substantiated by n.m.r. spectroscopy. The proton spectrum of the galacturonan showed a signal at δ 4.35 p.p.m. ($J_{1,2}$ = 3.5 Hz) characteristic of α linkages[11]. The ^{13}C-n.m.r. spectrum of the sodium salt in D_2O at 85° showed six well-resolved signals, which were attributed as follows, with reference to the CH_3 signal of acetone (δ 31.07 p.p.m.) : C-1 (100.25), C-2 (69.25), C-3 (69.75), C-4 (79.05), C-5 (71.89), and C-6 (174.50), and the α configuration of the glycosidic linkages was further demonstrated by the $^1J_{C\text{-}1,H\text{-}1}$ coupling constant (172 Hz) as measured by the gated-decoupling technique.

The fact that M sulfuric acid had been used to release the galacturonan could readily cause preferential removal of side chains containing neutral residues. We therefore tried milder conditions to isolate the polymer, namely: 0.25 M sulfuric acid which does not cleave aldobiouronic acids and also affords neutral oligosaccharides[12]. As before, a glycuronan fraction (4.7% of the wall) was obtained when the residue from hydrolysis was washed with 0.15 M sodium hydroxide. The polymer contained no neutral sugars, and the structure established by methylation analysis, n.m.r. spectroscopy, and specific rotation was identical to that of the aforementioned homogalacturonan. Additional evidence of the association of the homogalacturonan with cellulose through acid-labile linkages was obtained by a new extraction scheme. This procedure is based on the use of *N*-methylmorphiline *N*-oxide (MMNO), a new solvent for cellulose that proved efficient for structural studies on polysaccharides[13]. The entire cell walls were solubilized in MMNO–Me_2SO, and fractionation was achieved by precipitation with water. The precipitate (48% of the wall) had a composition very similar to that of residue R, mostly in its cellulose and glycuronan content. Treatment of the precipitate with 0.25 M sulfuric acid and washing with 0.15 M sodium hydroxide yielded a homogalacturonan (5.7% of the wall) that was characterized as before and was identical with the preceding material. The isolation with MMNO of a residue similar to R suggests that two groups of polysaccharide complexes exist in the primary walls of *Rosa glauca* suspension cells: one that remains in solution when water is added to the solution of the walls in MMNO–Me_2SO, and a second that is insoluble. From our study on the use of this solvent system with different model polysaccharides[13], we can assume that no covalent linkage is broken during the solubilization step, and we think, therefore, that the two groups of polysaccharides constituting the original cell wall are not covalently linked to each other. On the other hand, the fact that acidic hydrolysis was necessary for isolation of the galacturonan suggests that this polymer is linked through acid-labile bonds. Investigations in progress concerning that point, involving selective hydrolysis with oxalic acid, suggest that arabinofuranosyl residues may be involved in the attachment of the homogalacturonan to the fibrillar[14] cellulose of the primary wall.

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