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An unusual (4-*O*-methyl-D-glucurono)-D-xylan isolated from the mucilage of seeds of the quince tree (*Cydonia oblonga*)

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ABSTRACT

The major water-soluble polysaccharide in the mucilage of the seeds of the quince tree, *Cydonia oblonga*, is a partially *O*-acetylated (4-*O*-methyl-D-glucurono)-D-xylan having an exceptionally high proportion of glycuronic acid residues. The 4-*O*-methyl-α-D-glucopyranosyluronic and α-D-glucopyranosyluronic acid groups are linked to positions 2 of a (1→4)-β-D-xylan that is somewhat branced through the 2-positions. The *O*-acetyl groups are linked to the β-D-xylopyranosyl residues. The molar proportions of D-Xyl, 4-*O*-Me-D-GlcA, D-GlcA, and *O*-Ac are ~2:0.8:0.2:1.

INTRODUCTION

The mucilage extracted from the seeds of the quince tree, *Cydonia oblonga* Mill. (also referred to as *C. vulgaris*), has had limited commercial importance[1]. Early studies (summarised in ref. 2) demonstrated the mucilage to be a mixture of cellulose and water-soluble polysaccharides. The latter, on acid hydrolysis, yielded L-arabinose, D-xylose, and aldobiouronic acids, the last containing *O*-methyl groups. The cellulose microfibrils in the mucilage, when deposited in a cavity associated with each cell in the epidermal layer on the surface of the seed, are arranged helicoidally[3]. It has been proposed that the mucilagenous assemblage is cholesteric liquid crystalline in character[3,4] and that the soluble component of the mucilage is responsible for the generation of the cholesteric (helicoidal) form[4]. It has been proposed also that changes in the orientation of cellulose from layer to layer in plant cell walls originate in many instances through cholesteric liquid crystallisation[5]. Since the cell-wall architecture is of fundamental significance in determining the properties of plant cells, it is important to know more about the mucilage, and we now report on the structure of the major component of its soluble part.

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RESULTS AND DISCUSSION

A hydrolysate of the mucilage contained arabinose, xylose, mannose, galactose, and glucose in the proportions 8:46:4:10:32. The content of uronic acid, determined by the carbazole method, was 35%. The cellulose microfibrils were separated from the soluble components by centrifugation of a dilute aqueous solution. The acidic polysaccharide in the supernatant solution was then isolated by elution from DEAE-Sepharose with a gradient of aqueous sodium chloride. Only a single acidic component was obtained.

Sugar analysis of the acidic polysaccharide yielded xylose as the only neutral sugar. Methanolysis, carboxyl reduction, and hydrolysis with acid yielded D-xylose, D-glucose, and 4-*O*-methylglucose, in the proportions 1:0.1:0.4. The 4-*O*-methylglucose, analysed as the [1-^{2}H]alditol acetate, was indistinguishable, by g.l.c.–m.s., from an authentic sample. The absolute configuration was not determined but was assumed to be D, as 4-*O*-methyl-D-glucuronic acid is a component of all known acidic (1→4)-β-D-xylans from plants.

Methylation analysis, with carboxyl reduction of the methylated polysaccharide, yielded 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-D-glucose in the molar proportions ~1:3.0:3.3:3.6. The high percentage of 2,3,4-tri-*O*-methyl-D-xylose indicated the xylan backbone to be branched, evidently through the 2-positions.

The ^{1}H-n.m.r. spectrum of the *O*-deacetylated polysaccharide contained, *inter alia*, signals for anomeric protons at δ 5.38 (0.2 H, not resolved), 5.28 (0.8 H, not resolved), 4.67, 4.59, and 4.50. The last three signals each had $J_{1,2}$ 8 Hz, and corresponded, in all, to 2 H. In the ^{1}H-n.m.r. spectrum of *O*-deacetylated jute xylan[6], which is a (4-*O*-methyl-D-glucurono)-D-xylan of the usual type, there were corresponding signals at δ 5.26, 4.65, and 4.49, assigned to H-1 of 4-*O*-methyl-α-D-glucopyranosyluronic acid groups, β-D-xylopyranosyl residues substituted with such groups, and β-D-xylopyranosyl residues, respectively. One additional signal (δ 5.38) in the spectrum of the quince xylan was most probably due to H-1 of the α-D-glucopyranosyluronic acid groups. The remaining, weak signal at δ 4.59 may have been due to H-1 of terminal β-D-xylopyranosyl groups or to β-D-xylopyranosyl residues which, as well as one or both of their neighbours, carried a uronic acid substituent.

In the ^{13}C-n.m.r. spectrum of jute xylan[6], signals for the anomeric carbons of unsubstituted and substituted β-D-xylopyranosyl residues and 4-*O*-methyl-α-D-glucopyranosyluronic acid groups appeared at δ 102.2, 101.9, and 98.1, respectively. The signal for C-2 of substituted β-D-xylopyranosyl residues appeared at δ 83.0. There is good agreement between these signals and those given by the quince xylan, at δ 102.62, 102.21, 98.45, and 83.10.

The ^{1}H-n.m.r. spectrum of the native quince xylan contained a strong signal for *O*-acetyl groups at δ 2.19, corresponding to ~0.5 group per xylosyl residue. The polysaccharide gave poorly resolved n.m.r. spectra, most probably because of the non-regular distribution of the uronic acid and *O*-acetyl groups, so that the latter could

not be located. On treatment of the polysaccharide with periodate, however, all of the uronic acid groups were oxidised, as demonstrated by sugar analysis of the product obtained by borohydride reduction, methanolysis, and carboxyl reduction. Xylose, but no glucose or 4-*O*-methylglucose, was obtained, thus demonstrating the *O*-acetyl groups to be located on xylose residues only. *O*-Acetyl groups can migrate between the 2- and 3-positions in a 4-substituted xylopyranosyl residue and, most probably, are present in both of these positions.

Thus, it is concluded that the major water-soluble component of quince-seed mucilage is a (4-*O*-methyl-D-glucurono)-D-xylan of a structural type that is common in Nature, but with an exceptionally high content of uronic acid groups (1 per 2 xylose residues). The polysaccharide comprises a (1→4)-β-D-xylan backbone, with 4-*O*-methyl-α-D-glucopyranosyluronic acid groups and, to a lesser extent, α-D-glucopyranosyluronic acid groups, linked to the 2-positions in the β-D-xylopyranosyl residues. The xylan backbone carries *O*-acetyl groups, which is a common feature in this group of polysaccharides, and is somewhat branched through the 2-positions.

EXPERIMENTAL

General methods. — These were the same as in the investigation of the *Klebsiella* K 21b capsular polysaccharide[7].

Isolation of the (4-O-methyl-D-glucurono)-D-xylan. — A mixture of quince-seed mucilage (1.0 g) and water (100 mL) was centrifuged and the clear supernatant solution freeze-dried. Part of the product (100 mg) was fractionated on a column (100 × 2 cm) of DEAE-Sepharose, equilibrated with 0.02M acetate buffer (pH 4.5) containing 0.02% of sodium azide. The column was eluted with the buffer and then with a gradient of sodium chloride (0→M). The fractionation was monitored using the phenol–sulfuric acid reagent. A single acidic component was obtained. Separate fractions that contained the neutral and acidic polysaccharides were dialysed and freeze-dried to give 20 and 55 mg of material, respectively.

O-*Deacetylation.* — A solution of the xylan in 0.1M sodium hydroxide was kept for 6 h at room temperature, then neutralised, dialysed against distilled water, and freeze-dried.

Periodate oxidation. — A solution of the xylan (5 mg) in 0.04M sodium metaperiodate (1 mL) was kept for 4 h in the dark. Ethylene glycol (one drop) was added followed by sodium borohydride (10 mg) and, after 1 h, the solution was treated with Dowex 50 (H^+) resin, filtered, and concentrated. The residue was dried in a vacuum over phosphorus pentaoxide. Methanolic M hydrogen chloride (5 mL), prepared from anhydrous methanol and acetyl chloride, was added, and, after boiling under reflux for 10 h when all of the material had dissolved, silver carbonate was added, and the solution centrifuged. Lithium borohydride (20 mg) was added to the supernatant solution, which was then boiled under reflux for 6 h. After conventional work-up, the product was subjected to sugar analysis, when xylose was the only sugar found.

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