Preliminary communication

Hemicelluloses from the stem tissues of the aquatic moss *Fontinalis antipyretica*

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(Received October 26th, 1970; accepted for publication in revised form, January 14th, 1971)

There is very little knowledge of the polysaccharides in mosses (*Bryophytes*), although cellulose may be present in hair-cap moss[1]. We now report the isolation of two hemicellulosic-type polysaccharides, a galactoglucomannan and an arabinogalactan, from the wiry and wood-like stems of the freshwater, aquatic moss *Fontinalis antipyretica*. It has been stated frequently that lignin is absent from mosses[2], but other reports indicate its presence, or the presence of something chemically similar to lignin[3]. Only after stem tissues of *F. antipyretica* had been treated with acid-chlorite[4] was it possible to extract significant quantities of polysaccharides by treatment with alkali. The terms holocellulose, α-cellulose, and hemicellulose used for polysaccharide material derived from lignified tissues are here extended to cover the materials similarly derived from *F. antipyretica*.

Submerged *F. antipyretica* was harvested from the bed of the River Dee (Aberdeenshire). Enzymes in the tissues were inactivated by boiling with ethanol, and the stem tissues were treated with acid-chlorite[4]. The resultant holocellulose was treated twice with methyl sulphoxide at 20° and then with water at 60°. The first methyl sulphoxide extract, on acidification, gave material having OMe, 0.8% and OAc, 5.2%, and the second extract gave hemicellulosic material yielding galactose, glucose, mannose, arabinose, and xylose (in the molar proportions of 13:10:40:6:4) on acid hydrolysis. The aqueous extract on acidification did not yield a precipitate. Further treatment of the residual holocellulose with 5% aqueous potassium hydroxide yielded hemicellulosic material having residues of the sugars mentioned above. Acidic sugars were not detected in hydrolysates of any of the hemicellulosic material studied.

Direct extraction of the holocellulose with aqueous potassium hydroxide (5, 10, and 20%), followed by acidification with acetic acid and precipitation with ethanol, also yielded hemicellulosic materials (20% of holocellulose) which were combined as they were similar. A water-soluble galactoglucomannan ($[\alpha]_D^{18}$ −11°; homogeneous on paper electrophoresis in 0.1 M sodium borate) was isolated from a solution of this material in 5% potassium hydroxide by a series of graded precipitations with barium hydroxide[5]. Acidic hydrolysis gave galactose, glucose, mannose, and xylose in the molar proportions of 10:10:37:trace.

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The galactoglucomannan reduced 1.08 moles of periodate and released 0.107 mole of formic acid per mole of hexose residue; no formaldehyde was released. Smith degradation gave erythritol and glycerol (147:10; g.l.c. of acetates) and traces of mannose and glucose. On partial hydrolysis with acid, the galactoglucomannan yielded mannobiose, mannotriose, mannotetraose, and 4-*O*-β-D-glucopyranosyl-D-mannose, but no cellobiose. Methylation (Haworth, Kuhn[6]) of the galactoglucomannan gave material having a D.P. of 32–37 (ebulliometry in butanone) which, on methanolysis and hydrolysis, yielded 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 2,3,4,6-tetra-*O*-methyl-D-mannopyranose, 2,3,6-tri-*O*-methyl-D-glucopyranose, 2,3,6-tri-*O*-methyl-D-mannopyranose, 2,3-di-*O*-methyl-D-mannose, and 2,3-di-*O*-methyl-D-glucose (identified by conventional procedures) in the molar proportions of 100:25:96:400:55:10. Partial hydrolysis of the galactoglucomannan with acid removed the galactose residues without releasing significant amounts of glucose or of mannose.

It is chemotaxonomically important that the galactoglucomannan from *F. antipyretica* is structurally similar to galactoglucomannans from many softwoods[7,8]. It is water-soluble, has a low specific rotation (−11°) and proportions of galactose:glucose: mannose of 10:10:37, and contains (*a*) β-(1→4)-linked D-mannopyranose and D-glucopyranose residues and (*b*) non-reducing, terminal D-galactopyranose residues attached to position 6 of mannose, and to a lesser extent of glucose, residues in the main chain. The absence of formaldehyde on periodate oxidation showed that there are no terminal galactofuranose residues. It is interesting that the galactose residues are all terminal and are removed by mild acidic treatment. There is no direct evidence of the anomeric nature of the (1→6) linkages, but in certain softwood galactoglucomannans[9-11] it is α-D. A low proportion of non-reducing, terminal D-mannopyranose residues is also present as in other[12-15] galactoglucomannans or glucomannans. Accepting that the D.P. of the moss hemicellulose is *ca.* 34, there are 2–5 non-reducing, terminal side-residues; possibly all of these are D-galactopyranose residues and the non-reducing, terminal D-mannopyranose residues *may* be exclusively at the end of the main glucomannan chain; there is no evidence of non-reducing, terminal D-glucose residues. The presence of contiguous D-mannopyranose residues is shown by the isolation of the D-mannose oligosaccharides, but there is no evidence that D-glucose residues are contiguous The periodate-oxidation and Smith-degradation studies and the methylation analysis show that there is one non-reducing, pyranosidic, terminal residue for every 6–12 other residues. The water-soluble polysaccharide isolated after swelling the holocellulose in methyl sulphoxide was acetylated, and it is reasonable to assume that the galactoglucomannan is acetylated in its natural state.

A sample of the hemicellulosic material remaining after the isolation of the galactoglucomannan was subjected to repeated fractionation using cetylpyridinium hydroxide[16,17] and cetyltrimethylammonium hydroxide[17], and a small amount of a water-soluble arabinogalactan was finally obtained. The arabinogalactan, $[\alpha]_D^{19}$ +18°, travelled as a single zone on paper electrophoretograms in 0.1 M sodium borate and in 0.1 M cetyltrimethylammonium borate. Arabinose and galactose were present in a hydrolysate in the molar ratio of 7:2, and there was a trace of xylose. The polysaccharide reduced 1.13 moles of periodate and released 0.45 mole of formic acid per mole of monosaccharide residue. After borohydride reduction of the resulting oxopolysaccharide, the product was hydrolysed and again reduced; glycerol and galactitol were formed (molar ratio, 152:100; g.l.c. of acetates). A partial, acid hydrolysate of the arabinogalactan contained 6-*O*-β-D-galactopyranosyl-D-galactose (methylation analysis) and probably 3-*O*-β-D-galactopyranosyl-D-galactose (paper chromatography). The methylated (Haworth, Kuhn[18]) arabinogalactan, on methanolysis and hydrolysis, yielded 2,3,4-tri-*O*-methyl-L-arabinopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 2,3,4-tri-*O*-methyl-D-galactopyranose, and 2,4-di-*O*-methyl-D-galactopyranose in the molar proportions of 21:10:30:18 (based on charcoal–Celite chromatography). Methanolysis of the methylated arabinogalactan gave (g.l.c.) the methyl glycosides of 2,3,4-tri-*O*-methyl-L-arabinopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 2,3,4- and 2,4,6-tri-*O*-methyl-D-galactopyranoses, and 2,4-di-*O*-methyl-D-galactopyranose in the molar proportions of 203:100:307:290. Treatment of the arabinogalactan with 10mM hydrochloric acid (3 h, 100°) liberated arabinose and a trace of galactose; the hemicellulose degraded by acid then retained only a trace amount of arabinose residues. This galactan was methylated (Haworth, Kuhn[18]), and methanolysis then gave the glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 2,3,4-tri-*O*-methyl-D-galactopyranose, 2,4,6-tri-*O*-methyl-D-galactopyranose, and 2,4-di-*O*-methyl-D-galactopyranose in the molar proportions of 100:90:75:90. A trace of material was found that corresponded in its retention times on g.l.c. to methyl 2,3,4-tri-*O*-methyl-L-arabinopyranoside. The acid-treated arabinogalactan reduced 1.04 moles of periodate per mole of hexose residue. The D.P. (hypoiodite oxidation) of the arabinogalactan was *ca.* 60, and the D.P. of the methylated arabinogalactan was *ca.* 107 (ebulliometry in butanone).

The arabinogalactan has non-reducing, terminal D-galactopyranose and L-arabinopyranose residues in the ratio of 2:1. It is concluded to have a highly branched galactan core with β-(1→3)- and β-(1→6)-linked D-galactopyranosidic residues. A number of non-reducing, terminal L-arabinopyranosidic and D-galactopyranosidic residues are attached to the core, and the methylation and periodate-oxidation studies indicate that *ca.* 1 in 3 of these residues is (1→3)-linked; the remainder are (1→6)-linked. The difference in the D.P. values of the arabinogalactan (*ca.* 60) and of the methylated derivative (*ca.* 107) indicates that there has been a selective loss of hemicellulose of low D.P. during the methylation procedures, as is usual. The structure of the arabinogalactan from *F. antipyretica* is similar to that of others found in softwoods and in members of the *Larix* genus.

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