The Constitution of an Arabinoxylan from Scots Pine

*(Pinus silvestris L.)*

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Pine *(Pinus silvestris L.)* chlorite holocellulose after extraction first with dimethyl sulphoxide and water gives an arabinoxylan on extraction with 14 % potassium hydroxide. From the results of methylation studies it is concluded that this araboxylan has a backbone of 1,4-linked β-D-xylopyranose residues and that every fifth to sixth xylose residue is substituted in the 2-position with 4-0-methyl-D-glucopyranosyluronic acid residues, while every seventh to eighth xylose residue is substituted in the 3-position with L-arabofuranose residues.

The isolation of an araboxylan from Scots pine *(Pinus silvestris L.)* has been described in a previous communication from this Institute. Pine chlorite holocellulose was extracted successively with dimethyl sulphoxide, hot water, 14 % aqueous potassium hydroxide, 14 % aqueous potassium hydroxide containing 3 % boric acid and finally with 24 % aqueous potassium hydroxide containing 3 % boric acid. The hemicellulose extracted with 14 % potassium hydroxide on fractionation first with Fehling’s solution and then with aqueous barium hydroxide yielded an araboxylan substantially free from other polysaccharides.

The present paper deals with the results of methylation studies on the araboxylan. The molar ratio arabinose: xylose: 4-0-methylgluconic acid was 1 : 7.7 : 1.5, and the DPn estimated by osmometry in aqueous solution was 133.

The araboxylan was methylated with dimethyl sulphate in sodium hydroxide solution and then treated five times with methyl iodide and silver oxide in dimethyl formamide. The product (methoxyl 38.1 %, DPn 115) was subjected to acid hydrolysis and the acidic and neutral fragments were separated on an anion exchange resin. The acidic portion containing the methylated aldobiouronic acid together with a smaller amount of the methylated uronic acid, after esterification and glycosidation, was reduced with lithium aluminium hydride and then hydrolysed.

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The monomeric sugars in the two hydrolysates were fractionated on carbon-Celite columns and estimated quantitatively. The 2-O- and 3-O-methyl-D-xyloses in the neutral fraction of the hydrolysate from the methylated polysaccharide were incompletely separated on the column; their relative proportion was determined by quantitative paper electrophoresis in borate buffer and a preparative fractionation was done on a carbon-Celite column.

**Table 1.** Methyl ethers (mole %) obtained from methylated araboxylan from pine wood.

<table>
<thead>
<tr>
<th>Component</th>
<th>Mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>0.4</td>
</tr>
<tr>
<td>2-O-Methylxylose</td>
<td>9.9</td>
</tr>
<tr>
<td>3-O-Methylxylose</td>
<td>13.7</td>
</tr>
<tr>
<td>&quot;Dimethylarabinose&quot;</td>
<td>0.9</td>
</tr>
<tr>
<td>2,3-Di-O-methylxylose</td>
<td>49.8</td>
</tr>
<tr>
<td>2,3,4-Tri-O-methylxylose</td>
<td>1.3</td>
</tr>
<tr>
<td>2,3,5-Tri-O-methylarabinose</td>
<td>9.7</td>
</tr>
<tr>
<td>2,3,4-Tri-O-methylglucose</td>
<td>14.3</td>
</tr>
</tbody>
</table>

**Table 2.** Fractionation of neutral monosaccharides from a hydrolysate of methylated araboxylan. Gradients: 0—12 % aqueous ethanol (3 l), 12—35 % aqueous ethanol (3 l), 35 % aqueous ethanol-35 % aqueous n-propanol. Fractions collected: 25 ml.

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Component</th>
<th>Millimoles</th>
<th>Mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td>42—50</td>
<td>Xylose</td>
<td>0.0212</td>
<td>0.5</td>
</tr>
<tr>
<td>70—75</td>
<td>3-O-Methylxylose</td>
<td>0.0622</td>
<td>1.5</td>
</tr>
<tr>
<td>76—116</td>
<td>2-O- and 3-O-Methylxylose</td>
<td>0.855</td>
<td>16.0</td>
</tr>
<tr>
<td>117—132</td>
<td>&quot;Dimethylarabinose&quot;</td>
<td>0.0596</td>
<td>1.5</td>
</tr>
<tr>
<td>133—220</td>
<td>2,3-Di-O-methylxylose</td>
<td>2.70</td>
<td>66.0</td>
</tr>
<tr>
<td>250—274</td>
<td>2,3,5-Tri-O-methylarabinose</td>
<td>0.524</td>
<td>12.8</td>
</tr>
<tr>
<td>275—298</td>
<td>2,3,4-Tri-O-methylglucose</td>
<td>0.0685</td>
<td>1.7</td>
</tr>
</tbody>
</table>

The ratio 2-O- : 3-O-methylxylose in fraction 76—116 was 4.5:1.

**Table 3.** Fractionation of the hydrolysate from the acidic part of the methylated araboxylan hydrolysate. Gradient: 0—50 % aqueous ethanol (5 l). Eluted with 50 % aqueous ethanol. Fractions collected: 25 ml.

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>Component</th>
<th>Millimole</th>
<th>Mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td>24—80</td>
<td>3-O-Methylxylose</td>
<td>0.434</td>
<td>42.0</td>
</tr>
<tr>
<td>Remainder</td>
<td>2,3,4-Tri-O-methylglucose</td>
<td>0.600</td>
<td>58.0</td>
</tr>
</tbody>
</table>

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by gradient elution with borate buffer. The results of the fractionations are summarised in Table 1. In correlating the proportions obtained in the two fractionations (see Tables 2 and 3) to get the values given in Table 1, the ratio of the total amount of monomers in each of the two fractionations was adjusted to give the same uronic acid content as that present in the original araboxylan.

The hydrolysates obtained from the acidic fragments of the methylated polysaccharide hydrolysate contained only 3-O-methyl-D-xylene and 2,3,4-tri-O-methyl-D-glucose. The aldobionionic acid from the pine wood thus clearly is 2-O-(4-O-methyl-D-glucuronopyranosyl)-D-xylene which is the usual aldobionionic acid fragment found in wood xylans. There were no indications of 3-O-(4-O-methyl-D-glucuronopyranosyl)-D-xylene residues the presence of which has been reported in hydrolysates from Pinus radiata D. Don and various grasses. The aldobionionic acid was isolated from a partial hydrolysate of the araboxylan and was converted to the crystalline methyl-2-O-(methyl 2,3-di-O-acetyl-4-O-methyl-a-D-glucuronopyranosyl)-3,4-di-O-acetyl-D-xylpyranoside according to Timell. Some 3-O-methyl-D-xylene was found in the hydrolysate from the neutral portion of the methylated polysaccharide. This probably arises from partial hydrolysis of the methylated aldobionionic acid fragment, as the yield agreed well with the excess of 2,3,4-tri-O-methyl-D-glucose from the acid portion. The L-arabofuranose residues therefore appear to be attached exclusively to the 3-positions of the anhydroxylose residues. This is substantiated by the relative proportions of 2-O-methyl-D-xylene and 2,3,5-tri-O-methyl-L-arabinose. The results give no indication whether the L-arabofuranose residues are linked directly to the anhydroxylose back-bone or are the terminal residues of side-chains of β-1,4-linked xylose units. From the results of previous investigations, however, it seems likely that the L-arabofuranose residues are linked directly to the anhydroxylose back-bone. The amount of the fraction containing 2,3,4-tri-O-methyl-D-xylene (1.3 mole %) appears somewhat higher than that required by a DPn of 115 (0.9 mole %). This fraction did, however, contain a smaller amount of 2,3,5-O-tri-methyl-arabinose, and there is therefore little indication of any branching in the β-1,4-linked anhydroxylose back-bone.

Previous studies of the hemicelluloses of coniferous wood have indicated the presence of a xylan composed only of 4-O-methyl-glucuronic acid and xylose. This arabinose-free xylan has been extracted directly with alkali from finely ground wood and the hemicelluloses have been fractionated to yield the xylans. Fractionation of alkaline holocellulose extracts has also yielded arabinose-free xylans. The yields have usually been of the order of 1 % or less of the wood. Xylans containing arabinose residues have been isolated from Norway spruce, Western hemlock European larch and Sitka spruce; the constitutions of those from Western hemlock and European larch have been investigated and found similar to that of the araboxylan examined in the present work. Structural investigation of the xylans present in Southern pine pulps has indicated a similar structure for the native araboxylan in this case also. The xylan in the present investigation represents 80 % of the total xylan in the wood which amounted to 9—10 % of the dry extractive-free wood. In view of the results of previous investigations, the
presence in the pine wood of an arabinose-free xylan cannot be excluded, but it seems however probable that if present it constitutes only a minor part of the xylan in the wood.

EXPERIMENTAL

All melting points are corrected. Evaporations were done under reduced pressure at a bath temperature below 40°.


Isolation of the araboxylan. The araboxylan was extracted from Scots pine (Pinus silvestris L.) chlorite holocellulose. It was obtained in a substantially pure form by the fractionation techniques previously described. OCH₃ 2.6 %; 4-O-methylglucuronic acid 20.7 %; the ratio galactose:glucose:mannose:arabinose-xylose was trace:trace:11:12.7:87.3.

DE₃ (in aqueous solution), [α]Dᵇ 59° (c, 1.2 in water).

Methylation of the araboxylan. The araboxylan (5 g) was first methylated with methyl sulphate in aqueous sodium hydroxide and then treated five times with methyl iodide and silver oxide in dimethyl formamide. Yield 4.4 g, [α]Dᵇ 50° (c, 1 in chloroform), OCH₃ 38.1 %, (calculated methoxyl content for a fully substituted araboxylan with 20.7 % methyluronic acid is 38.1), DE₃ 115.

Hydrolysis of the methylated araboxylan and separation of acid and neutral components. The methylated araboxylan (1.3 g) was hydrolysed first with 72 % sulphuric acid (10 ml) at room temperature for 0.5 h and then with 8 % sulphuric acid (90 ml) at 100° for 2 h. The hydrolysate was neutralised with barium carbonate, filtered, and passed through Amberlite IR 120 (H⁺ form). The acidic components were absorbed on Dowex 2 (acetate form), 1.5 × 12 cm, and the column was eluted with water (1.5 l) and the eluate concentrated to give the neutral fraction (950 mg). The acids were displaced from the column with 3 N acetic acid (500 ml); the eluate was concentrated to give the acidic fraction (275 mg). Paper chromatograms of the two fractions using solvent B indicated a complete separation of neutral and acidic material; the neutral fraction contained methylxyllose, dimethylxyllose, trimethylarabinose and probably some trimethylxylose; the acidic fraction contained two components, probably methylated uronic acid (minor component) and methylated aldoxuronic acid (major component). Paper electrophoresis in the acetate buffer gave two spots with slightly lower mobilities than those of 4-O-methyl-d-glucuronic acid and 2-O-(4-O-methyl-d-glucuronopyranosyl)-d-xylose, respectively.

Glycosidation, esterification and reduction of the acidic components. The acidic fraction (275 mg) was refluxed with 2.1 % dry methanolic hydrogen chloride for 8 h and the solution was then neutralised with silver carbonate, filtered and concentrated to dryness. The product was reduced with lithium aluminium hydride (1 g) in refluxing ethyl ether (50 ml) for 4 h. Excess hydride was destroyed with anhydrous ethanol and the alcoholsates were decomposed with water. Aluminium hydroxide was removed by centrifuging and the supernatant solution was neutralised with sulphuric acid. Lithium sulphate was removed by filtration of the product in ethanolic solution and the filtrate was concentrated to a syrup (280 mg).

Hydrolysis of the reduced acidic fraction. The reduced acidic components (260 mg) were hydrolysed overnight at 100° with 0.5 N sulphuric acid (20 ml), neutralised with barium hydroxide, filtered and concentrated to dryness. Paper chromatography in solvent B and paper electrophoresis in the borate buffer indicated the presence of 3-O-methyl-xyllose and 2,3,4-tri-O-methyl-glucose.

Fractionation of the hydrolysates from the neutral and acidic components. The neutral components (950 mg) from the hydrolysate of the methylated araboxylan were fractionated on a carbon-Celite (1:1) column (4 × 46 cm) by gradient elution; the hydrolysate from the acidic components (280 mg) was fractionated on a carbon-Celite column (3.3 × 25 cm) in the same way. The amount of reducing sugar in each of the combined

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fractions was determined by hypodiode oxidation. 2-O- and 3-O-methyl-D-xylose were incompletely separated and the relative proportions of these components in the combined mixed fraction were determined by quantitative paper electrophoresis in borate buffer. A preparative fractionation was done on a carbon-Celite (1:1) column (3.3 × 25 cm) using borate buffer (0.1 M; pH 10) and gradient elution (3.1 0–10 % aqueous ethanol). 3-O-methyl-D-xylose was eluted first. The results of the fractionations are summarised in Tables 2 and 3.

Characterisation of components

D-Xylose. Characterised only by paper chromatography. Indistinguishable from D-xylose in solvents A and B.

2-O-Methyl-D-xylose. Crystallised from acetone, m.p. 130–132°C, undepressed on admixture with authentic material. Indistinguishable from authentic material on paper electrophoresis in borate buffer and on paper chromatography in solvent B.

3-O-Methyl-D-xylose. Crystallised from acetone and methyl ethyl ketone, m.p. 95–97°C, undepressed on admixture with authentic material. Indistinguishable from authentic material on paper electrophoresis in borate and on paper chromatography in solvent B. "Dimethylarabinose" (Table 2). Only examined by paper chromatography. This fraction contained a main component with a mobility slightly lower than that of 2,3-di-O-methylxylose, as well as some methylxylose and 2,3-di-O-methylxylose. Demethylation indicated arabinose and a smaller amount of xylose.

2,3-Di-O-methyl-D-xylose. The anilide had m.p. 133–135°C, undepressed on admixture with authentic material.

2,3,4-Tri-O-methyl-D-xylose. Demethylation indicated xylose and a small amount of arabinose. The methyl sugar and its anilide failed to crystallise. The methyl sugar was indistinguishable from authentic material on paper chromatography in solvent C.

2,3,5-Tri-O-methyl-D-arabinose. Demethylation gave arabinose only. The 2,3,5-tri-O-methyl-L-arabinonamide had m.p. 135–137°C, undepressed on admixture with authentic material.

2,3,4-Tri-O-methyl-D-glucose. The 1,6-diazobenzoate crystallised from ligroin-chloroform (25:1) m.p. 162–164°C, undepressed on admixture with authentic material.

A crystalline derivative of the aldobiouronic acid. The araboxylan (2 g) was hydrolysed with 0.5 N sulphuric acid (160 ml) at 100°C for 3 h and the hydrolysate was neutralised with 0.5 N barium hydroxide, filtered and concentrated to a small volume. Barium ions were removed by passage through Amberlite IR 120 (H+ form) and the uronic acids were absorbed on Dowex 2 (acetate form). Neutral sugars were eluted with water (2 l). The acids with 3 N acetic acid (500 ml) and then with water until the eluate was neutral. Concentration yielded the acid fraction (0.45 g). Paper chromatography in ethyl acetate-formic acid-acetic acid-water 19:1:3:4 and ethyl acetate-pyridine-water 2:1:2 indicated that the acid fraction contained the aldobiouronic acid slightly contaminated with aldorotri- and tetrauronic acids but no spots corresponding to 4-O-methylglucuronic acid were observed. The aldobiouronic acid was indistinguishable from 2-O-(4-O-methyl-D-glucuronopyranosyl)-D-xylopyranose. The crystalline methyl-2-O-(methyl 2,3-di-O-acetyl-4-O-methyl-α-D-glucuronopyranosyl)3,4-di-O-acetyl-D-xylopyranoside prepared according to Timell crystallised from ethyl ether and was recrystallised from ethyl ether-ethanol 1:1, [α]D +103° (c, 0.5 in chloroform), m.p. 202–203°C undepressed on admixture with authentic material kindly supplied by Dr. T. E. Timell, Montreal.

Osmometric DP Determinations. Osmometric DP determinations on the araboxylan were done on the sodium salt in 0.1 N aqueous sodium chloride using "Ultrafein feinst" membranes supplied by Membranfiltergesellschaft, Göttingen. The DPn of the methylated araboxylan was determined in butyl acetate solution using "Ultrazellfilter allerfeinst" membranes supplied by the same firm.
REFERENCES

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