Birch Wood Constituents

I. Carbohydrates of Low Molecular Weight

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Myo-inositol, glucose, fructose, sucrose, raffinose, stachyose and verbascose have been isolated from birch wood (Betula verrucosa). The presence of two higher oligosaccharides, probably belonging to the same series as verbascose, was also indicated.

During recent years hardwood has attracted considerable interest as pulp wood. In the Scandinavian countries the birches (Betula verrucosa and B. pubescens) are the only hardwoods sufficiently abundant to be of general importance. A chemical investigation of the constituents in birch wood is therefore warranted and has been started at this institute. The present communication reports studies on the low molecular weight carbohydrates in the wood of birch (B. verrucosa). The two species (B. verrucosa and B. pubescens) are closely related and major differences in their chemical composition would not be expected.

A tree was felled in January 1958, and a log cut from it was chopped and milled. The product was treated with acetone as rapidly as possible to inactivate the enzyme systems and thereby to minimise post-mortem changes. The milled wood was then treated with various solvents and the extracts dealt with as described in the experimental part. The carbohydrate fraction was subfractionated by chromatography on carbon-Celite columns and the following substances were isolated (they are listed in the order in which they emerged from one of the columns).

\[
\begin{align*}
&\text{Myo-inositol} & 0.02 \\
&\text{Fructose} & 0.56 \\
&\text{Glucose} & 0.62 \\
&\text{Sucrose} & 0.04 \\
&\text{Raffinose} & 0.03 \\
&\text{Stachyose} & 0.02 \\
\end{align*}
\]

The yields reported are approximate and should be regarded as representative only of the tree in question at the time when it was felled.

Glucose and fructose were not isolated but were identified by their paper chromatographic and electrophoretic behaviour. The other sugars, with the exception of verbascose, were isolated in the crystalline states. Verbascose was obtained as an amorphous powder and was chromatographically and electrophoretically indistinguishable from an authentic sample, and had a specific rotation, +146°, which agreed well with the value recorded in the literature.

The three highest oligosaccharides all possessed the structure: \( O-\alpha-D\)-galactopyranosyl-(1→6)-[O-\alpha-D\)-galactopyranosyl-(1→6)]_3-\beta-D\)-glucopyranosyl-(1→2)-\beta-D\)-fructofuranoside. (In verbascose \( n = 5 \).) The chemistry of such oligosaccharides has recently been summarized by French and by Wickström. The two next higher members of the series \( (n = 6 \) and \( n = 7 \)) were chromatographically indicated to be present in a fraction eluted from the column after the verbascose.

All of the oligosaccharides possessed a fructofuranosidic linkage, known to be very easily hydrolysed either by enzymes or by acids. Small amounts of melibiose, mannotriose and verbascotetraose were chromatographically indicated to be present and may have arisen from post-mortem reactions. Their presence does not necessarily imply that they were present in significant amounts in the living tree.

In addition to the carbohydrates described above, a fraction containing a mixture of phenolic substances was obtained, and is now under investigation.

**Experimental**

Paper chromatography was carried out on Whatman No. 1 filter paper using the following solvent systems (v/v): A. Ethyl acetate-acetic acid-water (3:1:3, upper layer); B. n-Butanol-ethanol-water (10:3:5); C. Ethyl acetate-pyridine-water (2:1:2, upper layer).

The chromatograms were developed with one of the following spray reagents: silver nitrate in acetone and ethanolic sodium hydroxide, or \( p \)-anisidine hydrochloride in butanol or resorcinol in hydrochloric acid.

Electrophoresis was carried out on Whatman No. 3 MM filter paper in borate buffer solution (pH 10).

A birch, about 40 years old, was felled and from the trunk a log (100 × 20 cm) was sawn, 1 1/2–2 1/2 m from the root end. After one day in the cold, the bark of the log was stripped off. The wood was cut into chips, milled and the wood meal (>30 mesh) covered with acetone and kept at 40°. After 20 h the acetone extract was decanted off and taken to dryness. The residue was dissolved in water (200 ml) and extracted with ether (3 × 100 ml) and the ether layer was taken to dryness. The aqueous layer contained a yellow-brown bulky precipitate, which was centrifuged off. The clear solution was decanted and the precipitate in the centrifuge bottle was washed with water. The aqueous solutions were combined and concentrated to a small volume under reduced pressure at 35°. This solution contained compounds with chromatographic characteristics similar to those of phenols. It was subjected to countercurrent extraction between water and ethyl-methyl-ketone. The extraction was performed in 6 steps with a ratio of water to ethyl-methyl-ketone of 1:3 (v/v). The first two aqueous phases (80 ml) contained most of the carbohydrates. After concentration to dryness it yielded a syrup (20 g), which on chromatograms gave no spots on spraying the papers with diazotised sulphuric acid.

The airdried wood-meal (2 520 g) from the acetone treatment was continuously extracted in a Soxhlet-type apparatus for 3 days with ether and thereafter once again dried in

*Acta Chem. Scand. 12 (1958) No. 7*
the air, and the residue extracted in a similar way with methanol for 4 days. The extract was withdrawn and replaced with fresh methanol every day to minimise the hydrolysis of the extracted carbohydrates. The methanolic extracts were combined and taken to dryness under reduced pressure and the residue after dissolution in water (150 ml), was extracted with chloroform (3 × 50 ml). The chloroform solution contained only traces of material and was discarded. The aqueous phase was centrifuged and the then clear solution on concentration gave a yellow syrup (16 g) which was chromatographically free of phenolic compounds.

**Separation of carbohydrates on carbon-Celite columns**

The carbohydrate fractions from the acetone and the methanolic extracts were combined (36 g) and, after dissolution in water (150 ml) were added to the top of a carbon-Celite (1:1) column (65 × 7.0 cm). The column was irrigated with the following solvents using the gradient-elution technique:

<table>
<thead>
<tr>
<th>Water</th>
<th>20 l</th>
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<tbody>
<tr>
<td>Aqueous ethanol</td>
<td>1 %</td>
</tr>
<tr>
<td>1 → 8 %</td>
<td>20 l</td>
</tr>
<tr>
<td>8 → 25 %</td>
<td>20 l</td>
</tr>
<tr>
<td>25 → 35 %</td>
<td>12 l</td>
</tr>
</tbody>
</table>

Fractions (225 ml) were collected automatically and examined on paper chromatograms. Those fractions which were similar were combined and taken to dryness.

*Fraction No. 1* (270 mg) contained myo-inositol, which crystallised when the solvent was removed. By crystallisation from aqueous ethanol pure myo-inositol was obtained. It had m. p. and mixed m. p. 230–233°.

*Fraction No. 2* (9.21 g) contained most of the glucose and fructose and a small amount of myo-inositol.

*Fraction No. 3* (3.35 g) contained glucose and fructose only.

*Fraction No. 4* (14.1 g) contained sucrose and traces of melibiose. Crystallisations from aqueous ethanol yielded pure sucrose, $[\alpha]_D^{15} +66.6^\circ$ (c, 2.0 in water) and m. p. and mixed m. p. 191–192°.

*Fraction No. 5* (187 mg) contained sucrose, raffinose and traces of manno-inositol.

*Fraction No. 6* (1.43 g) contained raffinose, stachyose and traces of sucrose. This fraction was sub-fractionated on a smaller carbon-Celite (2:1) column (40 × 3.8 cm). The syrup was dissolved in water (50 ml) and added to the top of the column. The column was irrigated with aqueous ethanol the concentration of which was linearly increased from 7 to 15% (6 l). Fractions (50 ml) were collected and treated as above. Two compounds, later identified as raffinose and stachyose, were obtained in the crystalline state. The former (320 mg) crystallised as the pentahydrate from aqueous ethanol and had $[\alpha]_D^{15} +104^\circ$ (c, 1.8 in water). On heating it lost its crystalline properties at 75.5–80°, due to dehydration. The shape of the material was unaffected but examination in polarised light revealed that the material was no longer crystalline; the solid residue collapsed at ca. 135°. Authentic raffinose pentahydrate behaved in the same way and no depression was observed on admixture of the two samples. A saturated solution of the other major component in aqueous ethanol on nucelation with crystalline stachyose deposited crystals (240 mg) having $[\alpha]_D^{15} +132^\circ$ (c, 1.9 in water) and m. p. and mixed m. p. 153–155°.

*Fraction No. 7* (758 mg) contained verbascose, stachyose and raffinose.

*Fraction No. 8* (880 mg) contained mainly verbascose and stachyose. This fraction was sub-fractionated on a small carbon-Celite (1:1) column (22 × 3.2 cm). The column was irrigated with aqueous ethanol (9→15%; 4 l) and fractions (25 ml) were collected and treated as previously described. In this way a compound (217 mg) was obtained which was chromatographically and electrophoretically indistinguishable from verbascose. It was a white, anhydrous, powder which had $[\alpha]_D^{15} +146^\circ$ (c, 2.1 in water). The product

was at first believed to be crystalline, but an investigation under the polarizing microscope revealed it to be amorphous. The substance has previously been obtained as a crystalline pentahydrate.\(^1\)

This separation also yielded chromatographically pure verbascotetraose (314 mg) which may possibly have been formed from verbascose by hydrolysis by traces of acid on the column. The substance failed to crystallise and was precipitated from an aqueous solution with absolute ethanol. It yielded a white amorphous powder which had \([\alpha]_D^{19} +161^\circ\) (c, 2.0 in water).

Fraction No. 9 (160 mg) contained verbascose and traces of two compounds with lower \(R_F\)-values which gave the same colour reactions as did verbascose.

The authors are indebted to Docent A. Wickström, Farmasøytisk Institutt, Oslo, for gifts of authentic stachyose and verbascose.

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Received May 29, 1958.