

Isolation and Characterisation of an Acetylated Glucomannan from Pine (*Pinus silvestris* L.)

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A mixture of polysaccharides isolated from pine holocellulose has been fractionated under conditions where acetyl groups are stable. A pure glucomannan was obtained with an acetyl content of 5.95 %, corresponding to a DS of 0.24. Periodate oxidation studies on the glucomannan showed that almost all of the acetyl groups are linked to carbon atoms 2 or 3 of the mannose residues. An almost pure glucurono-arabinoxylan isolated from the holocellulose dimethyl sulphoxide extract contained only traces of acetyl groups. It seems likely therefore, that most of the acetyl groups in pine wood are linked to the glucomannan fraction.

Hägglund *et al.*¹ reported that the hemicelluloses extracted from a spruce holocellulose with dimethyl sulfoxide had an acetyl content of 5.0 %. Among other sugars a hydrolysate of the extract contained 23.4 % mannose and 58.6 % xylose. After extraction with dimethyl sulfoxide, the holocellulose was treated with hot water, thereby affording a hemicellulose fraction with an acetyl content of 10.1 %. The hydrolysate of this fraction contained 66.4 % mannose and 14.4 % xylose. These results suggest that the acetyl groups in spruce wood hemicelluloses are linked principally to the glucomannan fraction; this is in contrast to birch wood where they are linked to the xylans^{1,2}.

The main difficulty in the investigation of partially acetylated polysaccharides lies in their isolation and their fractionation into pure components under conditions where the acetyl groups are retained. In the present investigation a partially acetylated glucomannan has been isolated in a pure state by using two new separation methods^{3,4}, and the position of the acetyl groups has been demonstrated by periodate oxidation studies.

A pine chlorite-holocellulose was extracted with dimethyl sulfoxide and then with hot water. The hemicelluloses from the hot water extract (Table 1) were dissolved in water and CTA-Br (cetyl-trimethylammonium bromide) was added³. The precipitate (fraction A, Table 1) was enriched in xylan whereas the supernatant (fraction B) was enriched in glucomannan. However carbohydrate analysis of fraction B showed, that all of the acidic polysaccharides

Table 1. Carbohydrate and acetyl analyses.

	Galactose %	Glucose %	Mannose %	Arabinose %	Xylose %	Acetyl %
Pine wood, extracted with acetone	3.0	65.6	18.9	2.8	9.7	0.85
Holocellulose (yield 70.1 %)	2.0	73.0	14.6	1.5	8.9	1.06
Water extract (after dimethyl sulfoxide extraction)	9.7	15.8	49.0	7.9	17.5	3.31
fraction A	20.3	5.2	14.2	16.0	44.3	1.40
fraction B	8.4	16.5	60.8	2.8	11.5	4.51
fraction B 1	4.8	18.0	77.2	—	—	5.95
Pulp after dimethyl sulfoxide and water extraction	1.8	75.3	13.5	1.0	8.4	0.91

(xylans, galactans) were not removed with CTA-Br. Further fractionation of fraction B was accomplished on a DEAE-cellulose (diethylaminoethyl-cellulose) column in the phosphate form⁴. The first two fractions from the column, eluted with water and 0.05 M monosodium phosphate (adjusted to pH 5.5) respectively, had a similar composition and were combined to give fraction B 1 (Table 1), completely free from glucuronoarabinoxylans. Furthermore the acidic galactans⁵, obviously present in fraction B, had been removed. Fraction B 1 therefore represents a pure glucomannan containing a small number of galactose residues most probably linked terminally to mannose (and possibly glucose) residues through carbon atom 6^{6,7}. The later fractions from the DEAE-cellulose column (eluted with 0.5 M monosodium phosphate (pH 5.5), followed by 0.1 M sodium hydroxide) contained increasing amounts of arabinose and xylose residues.

Fraction B 1 had an acetyl content of 5.95 % corresponding to a DS (degree of substitution) of 0.24. Both B 1 and a deacetylated sample of this fraction were subjected to periodate oxidation. The periodate-oxidised polysaccharides were reduced with sodium borohydride and the polyalcohols so obtained hydrolysed⁸. Glucose, mannose, erythritol and glycerol were identified chromatographically and determined quantitatively (Table 2). No galactose was detected in either of the two samples. In the acetylated sample, a large quantity of mannose survived periodate oxidation whereas only a small amount of glucose was present. The acetyl groups therefore appear to be linked mainly to carbon atoms 2 and 3 of the mannose residues. The small amount of glucose which survived does not necessarily indicate that some glucose residues are also acetylated but may result from incomplete oxidation, or from the presence of a branch point in the glucomannan molecule⁹. Deacetylated fraction B 1 yielded very small amounts of both glucose and mannose possibly due to underoxidation, incomplete deacetylation or a small number of anomalous linkages (units linked 1 → 3 in the main chain, or branch points) in the glucomannan molecule. The glycerol contents of the two B 1 samples (7.4 and 6.8 % for the acetylated and the deacetylated sample, respectively) agree well with the amount of non-reducing end groups in the polysaccharide. Such groups

Table 2. Composition (in mole %) of hydrolysates of fraction B 1 after periodate oxidation and reduction.

	Glucose %	Mannose %	Erythritol %	Glycerol %
B 1 (acetylated)	0.9	21.9	69.9	7.3
B 1 (deacetylated)	1.5	1.7	90.0	6.8

consist principally of galactose residues (4.8 %) and to a minor extent of glucose and mannose units (about 2 % if the polysaccharide with DP 100 has one branch point).

The percentage of surviving mannose residues in the acetylated B 1 sample (21.9 %) indicates a DS of approximately 0.22. The DS which has been calculated from the acetyl analysis of B 1 is 0.24. This close agreement strongly indicates that virtually all of the acetyl groups are linked to carbon atoms 2 or 3 of the mannose residues and that a few, if any, mannose residues carry two acetyl groups, or an acetyl group at carbon atom 6.

The low acetyl content of fraction A (Table 1) suggests that the glucurono-arabinoxylans and other minor hemicellulose components (*e.g.* galactans and arabinogalactans which may be present in this fraction) contain no acetyl groups. In a separate experiment only traces of acetyl were found in an essentially pure glucurono-arabinoxylan. The latter was obtained by CTA-Br fractionation of the dimethyl sulfoxide extract of a pine holocellulose.

Present methods permit the isolation of only a small portion of the glucomannans from softwoods under conditions where the acetyl groups are not cleaved. If all the acetyl groups in pine are assumed to be bound to the glucomannan (which represents about 16 % of the dry weight of the wood) and if the glucomannan has a mean acetyl content of 6 %, the wood should contain 1 % acetyl groups. The pine wood used in this study had an acetyl content of 0.9 % (Table 1), a value lower than those previously reported in the literature¹⁰. The acetyl contents reported in this study were determined by gas-liquid chromatography (described in the experimental section). Although these values were considerably lower than those obtained by conventional acetyl determinations on wood, the method gives correct results with model substances of known composition. However, the variation of the acetyl content in wood from different locations and in stems of different ages, and the changes which might occur under storage, are not well known. It may be that the stem used in this study had a relatively low acetyl content. It is also possible that by other methods which are not specific for acetic acid, acidic degradation products are formed thus giving high values for the acetyl content.

During the course of the present investigation, Koshijima¹¹ reported a study on the acetyl groups in hemicelluloses from *Pinus densiflora*. Without isolating a pure acetylated glucomannan fraction, he obtained good evidence

that at least part of the acetyl groups were linked to glucomannans in the 2 or 3 positions of the mannose residues, in good agreement with the results of the present investigation.

EXPERIMENTAL

The quantitative analysis of sugars by paper chromatography was conducted according to the method of Saeman *et al.*¹³

Acetyl analyses. The method of Spingler and Markert¹³ (somewhat modified) was employed. A sample (20–100 mg) of the polysaccharide was placed in a glass tube, N anhydrous methanolic hydrochloric acid (1 ml) and methanol (1 ml) containing a known amount of methylene chloride (5–10 mg) as internal standard, were added. The tube was sealed and heated for 30 min in a boiling water bath. The reaction product was analysed by gas-liquid chromatography, using an apparatus, recently described by Selleby¹⁴, which was built at this Institute by I. Cedergren. The stationary phase was carbowax 1 500 (Perkin Elmer column K) and the length of the column was 3 m. Helium was used as the carrier gas with a flow rate of 25 ml/min at a temperature of 65°. Samples of about 40 μ l were injected into the column. The procedure was calibrated using penta-O-acetyl-D-galactose as standard. The areas of the methyl acetate and methylene chloride peaks were cut out and compared by weighing.

Isolation and fractionation of the polysaccharides. Pine wood meal (0.5–2.0 mm) was extracted with acetone and the residue delignified by the chlorite method at 60° and pH 4.7. The holocellulose was obtained in a yield of 70.1%. A sample (450 g) was treated for one hour in a Valley beater and twice extracted overnight with dimethyl sulfoxide (2 \times 10 l). The residue was filtered, washed thoroughly with cold water and extracted twice with water (2 \times 10 l) at 100° for 30 min. The aqueous extracts were combined and concentrated to about 2 l, adjusted to pH 4 with hydrochloric acid and the polysaccharides, precipitated with 8 l of ethanol, were centrifuged and washed several times with ethanol and acetone (yield 14.5 g). A sample (13 g) was dissolved in water (200 ml), a small insoluble fraction centrifuged and a 5% aqueous solution of CTA-Br added until no further precipitate was formed. After centrifugation the polysaccharides were recovered by dissolving the precipitate in very dilute hydrochloric acid and precipitating with ethanol (yield 1.7 g, fraction A, Table 1). The supernatant was acidified with a small amount of hydrochloric acid and the polysaccharides precipitated with ethanol, washed and dried (yield 7.8 g, fraction B, Table 1).

A sample of fraction B (3 g) was dissolved in water (20 ml) and added to the top of a diethylaminoethyl-cellulose column (4 \times 40 cm), which had been saturated with 0.5 M monosodiumphosphate (adjusted to pH 5.5) and washed with water. The polysaccharides were then eluted successively with water (2 l), 0.05 M monosodiumphosphate (2 l), 0.5 M monosodiumphosphate (2 l), and 0.1 N sodium hydroxide (2 l). The fractions were recovered by freeze drying, the second, third and fourth after deionization (Amberlite IR 120 and IR 4B, mixed bed). The hydrolysates of the water and 0.05 M monosodiumphosphate eluates had similar compositions and therefore were combined (fraction B 1, yield 770 mg). The fractions eluted with 0.5 M monosodiumphosphate (yield 960 mg) and 0.1 N sodium hydroxide (yield 350 mg) contained glucurono-arabinoxylan in addition to glucomannan as indicated by the composition of their hydrolysates.

Periodate oxidation. A sample of B 1 (40 mg) was dissolved in water (10 ml), 0.1 M sodium metaperiodate (10 ml) added and the solution kept in the dark at room temperature. Another sample was deacetylated by treating overnight with 10% sodium hydroxide. The solution was deionized and the polysaccharide precipitated with ethanol, washed, dried and then treated in a manner similar to the first sample. The consumption of periodate, determined spectrophotometrically¹⁵, reached an almost constant value after 300 h. This amounted to 0.85 and 1.10 moles per anhydrohexose unit for the acetylated and deacetylated samples, respectively. After precipitation of the periodate and the iodate with barium carbonate, an aliquot of both samples (10 ml) was reduced with sodium borohydride (30 mg). Sulfuric acid was added to a concentration of 4% and the samples were hydrolysed by heating at 120° for one hour. After neutralisation with barium hydroxide, the solutions were passed through a cation exchange resin (IR 120) and the residual boric acid was removed by codistillation with methanol. An aliquot (containing approxi-

mately 0.4 mg) of each of the samples was applied to chromatographic paper (Schleicher and Schüll No. 2043 a) and the chromatograms irrigated with ethyl acetate-pyridine-water (8:2:1). Glucose, mannose, erythritol and glycerol, localised with the aid of guide strips, were well separated and were eluted from the paper. The reducing sugars were determined quantitatively according to the method of Saeman *et al.*¹² and erythritol and glycerol according to the procedure of Lambert and Neish¹⁶. The determinations were performed in triplicate, the results being in good agreement.

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