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Phenyl*iso*cyanate Derivatives of Carbohydrates

II. Location of the *O*-Acetyl Groups in Birch Xylan

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The distribution of the *O*-acetyl groups in glucuronoxylan from birch wood was re-investigated using phenylcarbamoyl groups as protective substituents. The isolated polysaccharide contained 13.2 % of *O*-acetyl. 58.1 % of the D-xylose residues carried no *O*-acetyl groups, 11.8 % were substituted in the 2-position, 24.0 % in the 3-position and 6.1 % in both the 2- and 3-positions.

While investigating the properties of dimethyl sulphoxide (DMSO) as extractive agent in the isolation of wood hemicelluloses, Hägglund *et al.*[1] isolated a partly acetylated glucuronoxylan from birch wood holocellulose. Subsequently, a good yield of the polysaccharide was obtained by extraction of birch wood holocellulose[2] (prepared by chlorination of the wood followed by extraction with ethanolic ethanolamine) with DMSO. Methylation studies conducted on the partly acetylated glucuronoxylan indicated that the acyl groups (identified as *O*-acetyl groups) were mainly attached at the 3-positions in the original polysaccharide. The procedure employed, however, was inadequate for an unambiguous location of the *O*-acetyl groups in as much as approximately 30 % of these groups were hydrolysed during the methylation of the polysaccharide.

The previous paper in this series[3] investigated the possibility to umambiguously locate *O*-acetyl groups in partly acetylated carbohydrates. This procedure has now been applied to the *O*-acetylated birch wood glucuronoxylan.

Glucuronoxylan, containing 13.2 % of *O*-acetyl, was obtained by extracting birch wood holocellulose (prepared by the chlorite method) with DMSO. Treatment of a solution of the polysaccharide in hot, anhydrous dimethylformamide (DMFA) with an excess of phenyl*iso*cyanate transformed the free hydroxyl groups to phenylcarbamoyl groups.

The polysaccharide derivative thus obtained was insoluble in all the common solvents except anhydrous DMSO and anhydrous (or slightly moist) DMFA. Deacetylation of the material was attempted by dissolving it in DMFA and adding a small amount of aqueous sulphuric acid. The hydrolysing effi-

ciency of that medium gradually decreased however, due to the hydrolysis of the solvent into formic acid and dimethyl amine. The procedure was repeated once. Subsequent methylation showed that about 40 % of the *O*-acetyl groups were removed by this treatment.

Further deacetylation was effected by dissolving the partially deacetylated and methylated glucuronoxylan phenylcarbamoyl derivative in moist tetrahydrofuran containing sulphuric acid. *O*-methyl groups were substituted for all *O*-acetyl groups by alternate deacetylations and methylations.

The methylations were conducted in DMFA[4] with iodomethane and silver oxide. The solubility properties of the product required a modified isolation procedure, by which it was separated from the silver salts by precipitation of the polysaccharide in ethanolic potassium cyanide. Although this procedure gave very good yields of product, its use is limited to materials that are insoluble in ethanol.

The *O*-methylglucuronoxylan phenylcarbamoyl derivative was treated with lithium aluminium hydride in tetrahydrofuran solution to give a good yield of the corresponding partly *O*-methylated glucoxylan. This was hydrolysed and the hydrolysate fractionated on a carbon-Celite column. The mixed fractions were fractionated further by paper chromatography and paper electrophoresis in borate buffer of pH 9.4. Fractionation by chromatography on filter paper impregnated with borate buffer of pH 10[5] was tried but some alkaline degradation of the 3-*O*-methyl-D-xylose occurred during some part of the process. The amount of sugar in each fraction was estimated by hypoiodite oxidation [6]. The results of the fractionation are summarised in Table 1.

The results given in Table 1 show that the 2- and 3-positions in the original polysaccharide are substituted by *O*-acetyl groups in the ratio 1: 1.7. This result agrees fairly well with that previously obtained[2] which indicated that the birch xylan was substituted by *O*-acetyl groups mainly at the 3-positions.

The amount of 4-*O*-methyl-D-glucose found is much less than is required by the molar ratio of the D-xylopyranose and 4-*O*-methyl-D-glucuronic acid residues (91.5 : 8.5) in the original polysaccharide. This is explained by the reaction of phenyl*iso*cyanate with the carboxylic groups of the uronic residues giving *N*-phenylamide groups.[7] The main part of the amide bonds is apparently not hydrolysed during the deacetylations and the corresponding residues are converted into 4-*O*-methyl-6-deoxy-6-*N*-phenylamino-D-glucose residues.[8] The latter substance was not encountered in the hydrolysate. The 4-*O*-methyl-D-glucose found might correspond to uronic acid *N*-phenyl amide residues that were hydrolysed during the deacetylations. The carboxyl groups

Table 1. Methyl ethers from the hydrolysed, partly methylated glucoxylan.

Sugars	Mole %
Xylose	57.5
2-Methylxylose	11.7
3-Methylxylose	23.8
2,3-Dimethylxylose	6.0
4-Methylglucose	1.0

liberated would be esterified during the methylations and subsequently converted to hydroxyl groups by treatment with lithium aluminium hydride. Hence, it was not possible to obtain meaningful values for the nitrogen and methoxyl contents in the corresponding polysaccharide derivatives. The values given in the experimental part are calculated assuming either that the *N*-phenyl amide groups were unaffected during the deacetylations or that they were removed completely.

The values given in Table 1 correspond to an *O*-methyl glucoxylan containing 10.0 % methoxyl (methoxyl content of the unhydrolysed polysaccharide as analysed, 10.2 %). These values are lower than those (11.7 — 12.3 %) calculated on basis of the methylated glucuronoxylan phenylcarbamate. The low methoxyl content might be explained by the fact that fractionation of the polysaccharide occurred during the isolation.

EXPERIMENTAL

All melting points were corrected. All evaporations were conducted under reduced pressure.

Chromatography. Papers: Whatman No. 1 and 3 MM and Schleicher & Schüll 602 hP. These papers were also used impregnated with 0.1 M sodium borate buffer of pH 10[8]. Spray reagent: Anisidine hydrochloride.

Isolation of O-acetylated birch xylan. Acetone-extracted wood meal (1—2 mm) from a freshly cut log of *Betula verrucosa* Erh. was delignified by the chlorite method[9] at 60° and pH 4.7 to give a holocellulose containing less than 1 % of lignin.

The holocellulose (230 g) was extracted three times at room temperature with 2 l portions of dimethyl sulphoxide (DMSO). Three volumes of ethanol and a small amount of glacial acetic acid were added to the extracts and the precipitated hemicellulose was recovered on a glass filter. The third extract gave only a small amount of precipitate.

The hemicellulose was dissolved in water and insolubles were removed by filtration. The filtrate was acidified with glacial acetic acid and added to ethanol. The precipitated hemicellulose was recovered by filtration, thoroughly washed with ethanol and ether and subsequently dried. The product thus obtained for use in the present investigation was a white powder. Yield 14 g, $[\alpha]_D^{20}$ −54° (*c*, 1.0 in water). Anal. Uronic acid (as glucuronic acid) 10.5 %; *O*-acetyl (as CH_3CO) 13.2 %.

The residual holocellulose was impregnated with 5 % ethanolic monoethanolamine, dried, and extracted three times with DMSO.

Product yield following the isolation and purification procedure described above was 13 g of hemicellulose, $[\alpha]_D^{20}$ −56° (*c*, 1.0 in water). Anal. Uronic acid 13.9 %; *O*-acetyl 9.1 %.

Chromatographic analysis of the two samples after hydrolysis indicated that D-xylose was the only aldose present.

Treatment of birch O-acetyl xylan with phenylisocyanate. After drying in vacuum over phosphorus pentoxide, the polysaccharide (8.0 g) was dissolved in anhydrous *N,N*-dimethyl formamide (DMFA, 200 ml). Phenyl*iso*cyanate (20 ml) was added and the solution kept at 100° for 3 h. It was then poured into ethanol to produce a voluminous precipitate which was collected on a glass filter, washed with ethanol and ether and dried. The treatment with phenyl*iso*cyanate was repeated once and after a final purification by reprecipitation, the product was obtained as a coarse, yellowish powder (15.2 g), $[\alpha]_D^{20}$ −92° (*c*, 1.0 in DMFA), soluble in DMFA and in anhydrous DMSO. However, the addition of only small amounts of water to the DMSO solution of polysaccharide caused an immediate precipitation. (Found: N 6.41 %; CH_3CO 5.83 %. Calc.: N 6.53 %; CH_3CO 6.01 %.)

Deacetylation and methylation of the birch O-acetyl xylan phenylcarbamate. Deacetylation of the above product (14.4 g) was first accomplished by treating it at room tempera-

ture with a mixture of DMFA (250 ml) and 33 % aqueous sulphuric acid (15 ml) for 20 h. After isolation by precipitation in ethanol the treatment was repeated once.

The product was methylated with iodomethane (20 ml) and silver oxide (20 g) in DMFA (100 ml), the reaction time in all methylations being 35—45 h. After trial experiments which involved some loss of material (yield after the first methylation 9.0 g, OCH_3, 2.30 %), the following procedure was adopted for the isolation of the methylated product: The solids in the methylation mixture were centrifuged and washed with DMFA. The combined centrifugates were poured into ethanol (about 1.5 l) containing about 10 g of potassium cyanide in a small amount of water. The solution was allowed to stand for about 15 min with occasional stirring, after which the white, flocculent precipitate was collected on a glass filter, washed with ethanol and ether, and then dried. The yields produced by this procedure were 95—98 %.

The product was dissolved in DMFA (100 ml) and subjected to three additional methylations using half of the amounts of the reagents noted above. These treatments failed to raise the methoxyl content above 2.60 %, indicating that only about 40 % of the *O*-acetyl groups were removed at the deacetylation stage.

Complete deacetylation was effected by dissolving the material in tetrahydrofuran (280 ml) containing aqueous 33 % sulphuric acid (20 ml), the reaction being conducted at room temperature. This process was repeated twice, all treatments being followed by two methylation steps. The total time for these acid deacetylation treatments was about 5 days. Following the last methylation the product was dissolved in tetrahydrofuran, filtered, precipitated in ethyl ether, finally being obtained as a white powder (5.2 g), $[\alpha]_D^{20}$ −7° (*c*, 1.0 in DMFA). (Found: OCH_3 5.22 %; N 6.50 %. Calc.: OCH_3 5.0 — 5.8 %; N 6.1 — 6.4 %.)

Added in proof. It has later been shown that a material with better solubility properties is obtained by conversion of the phenylcarbamoyl into *N*-methyl-phenylcarbamoyl groups. Therefore, the best procedure for deacetylation of the *O*-acetyl glucuronoxylan phenylcarbamate involves an initial *N*-methylation of the phenylcarbamoyl groups with iodomethane and silver oxide. The product obtained is then deacetylated with sulphuric acid in moist tetrahydrofuran.

Reduction of the methylated xylan phenylcarbamoyl derivative. The above product (4.80 g) was placed in a 500 ml flask fitted with a condenser and dissolved in anhydrous tetrahydrofuran (200 ml). The addition of lithium aluminium hydride (300 mg) caused the solution to gelatinise. The gel dispersed by gentle warming on a steam bath. More lithium aluminium hydride (1.2 g) was added and the reaction mixture agitated with a magnetic stirrer; the gel dissolved gradually. The mixture was gently agitated overnight at room temperature and thereafter refluxed for 1 h to ensure complete reaction. After adding water the reaction mixture was neutralised with dilute phosphoric acid, this acid being used in order to convert the hydroxides into salts with low solubility in water. The reaction mixture was filtered, the solids washed with cold water and the combined filtrates were concentrated and added to ethanol. The precipitate thus formed was collected on a glass filter, washed with ethanol and ether and dried. Inorganic salts were removed by dissolving the product in cold water, filtering and precipitating it in ethanol: the process was repeated. It was finally obtained as a white powder, $[\alpha]_D^{20}$ −86° (*c*, 1.0 in water). Yield 1.64 g = 84 %. (Found: OCH_3 10.2 %; Calc.: OCH_3 11.7 — 12.3 %.)

Table 2. Fractionation of hydrolysed *O*-methyl glucoxylan.

Fract. No.	mmoles (total)	Sugars	% of fraction	mmoles
23—38	4.42	Xylose		
39—96	3.05	Xylose	4.8	0.15
		2-Methylxylose	30.5	0.93
		3-Methylxylose	62.0	1.89
		4-Methylglucose	2.7	0.08
Residue	0.48	2,3-Dimethylxylose		

Chromatographic analysis on paper impregnated with borate buffer showed that the hydrolysed sample contained D-xylose, 2-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and 4-*O*-methyl-D-glucose.

Hydrolysis and fractionation of the O-methyl glucoxylan. The polysaccharide (1.3 g) was hydrolysed in 0.5 N sulphuric acid at 100° overnight and then neutralised with barium carbonate. The salts were filtered and the hydrolysate concentrated to a small volume and added to the top of a carbon-Celite column (3.5 by 43 cm) and the components were eluted with 5 1 2 → 20 % aqueous ethanol, 30 ml fractions being collected. The column was finally washed with 50 % aqueous ethanol. Mixed fractions were resolved on thick filter paper impregnated with borate buffer. The fractionations are summarised in Table 2.

By resolving a sample of fracs. 39—96 on ordinary filter paper an estimate of the relative amounts of D-xylose, 4-*O*-methyl-D-glucose and 2-*O*-methyl-D-xylose + 3-*O*-methyl-D-xylose was possible. In a separate experiment the monomethylxyloses were isolated; after resolving the fraction by paper electrophoresis in borate buffer of pH 9.4 the relative amounts of 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose were estimated. The data in Table 2 were then calculated from the values obtained.

Characterisation of the components. 4-O-Methyl-D-glucose. This substance was chromatographically indistinguishable from an authentic specimen both on ordinary paper and on paper impregnated with borate buffer.

α-D-Xylose. The sugar had $[\alpha]_D^{20}$ +18° (*c*, 1.0 in water, final value) and m.p. and mixed m.p. 144—146°.

2-O-Methyl-β-D-xylose. The ether had $[\alpha]_D^{20}$ −21 → +34° (*c*, 1.0 in water) and m.p. and mixed m.p. 130—132°.

3-O-Methyl-α-D-xylose. The ether had m.p. and mixed m.p. 95—97°.

2,3-Di-O-methyl-D-xylose. The ether had $[\alpha]_D^{20}$ +23° (*c*, 0.6 in water). It was converted to its aniline derivative, m.p. and mixed m.p. 126—128°.

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