ACTA CHEMICA SCANDINAVICA 14 (1960) 742–748

Position of the *O*-Acetyl Groups in Birch Xylan *

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A xylan containing 11.8 % uronic acid and 16.9 % *O*-acetyl has been isolated from birch holocellulose by extraction with dimethyl sulphoxide. Methylation studies of the acetyl xylan itself and of a deacetylated sample have shown that the *O*-acetyl groups are attached to the xylose residues mainly in the 3-position and only to a lesser extent in the 2-position.

Birch wood xylan, the predominating hemicellulose in birch wood, has been shown to consist of a chain of 1,4-linked β-D-xylose residues, with every tenth to fifteenth residue substituted in the 2-position with a 4-*O*-methyl-α-D-glucopyranosyluronic acid residue [1-3]. Acetic and formic acids have been isolated from several woods [4] but Timell in an examination of birch wood found only acetic acid [5]. The isolation from birch wood of a partly acetylated xylan [6] localised at least part of the acetic acid in the wood.

In the present investigation the acetylated xylan was isolated by extraction with dimethyl sulphoxide (DMSO) from birch wood holocellulose which had been prepared by chlorination and treatment with ethanolic ethanolamine from a mixture of *Betula verrucosa* Ehrh. and *B. pubescens* Ehrh. The holocellulose was subsequently extracted with hot water and 12 % potassium hydroxide. The yield and composition of the different fractions are given in Table 1. The DMSO extract represented about 50 % of the xylan in the wood and xylose formed about 96 % of the aldose residues of the extract. The present results are thus substiantially different from those obtained with birch chlorite holocellulose [6] from which only about 15 % of the acetylated xylan could be extracted by this method. By osmometry according to Pals and Hermans [7] the $\overline{DP}_n$ of the acetylated xylan was estimated as 200 ± 10.

The positions of the *O*-acetyl groups in the acetylated xylan were investigated by two series of methylations. In the first series the acetylated xylan was fully methylated by a slightly modified version [8] of the method described by Kuhn *et al.*[9] The product (*O*-acetyl, 10.1 %; methoxyl, 28.1 %) was reduced with lithium aluminium hydride to yield a partially methylated glucoxylan

* Preliminary results of this investigation have been published in *Chem. and Ind., 1958*, 1727; the results were presented at the 136th meeting of the American Chemical Society, Atlantic City, New Jersey, September 1959. Abstract 3 E.

Table 1. Yield and composition of extracts from birch holocellulose (%).

	Yield	Lignin	CH_3CO	Uronic acid	OCH_3 %	Aldose residues				
						Galactose	Glucose	Mannose	Arabinose	Xylose
Birch holocellulose	—	7.2				0.9	61.0	2.9	1.1	33.9
DMSO extract	15.0	2	16.9	11.8	1.77	0.8	3.1	—	—	96.2
Hot water extract	0.55	5	4.0	42.7	2.58	19.1	18.2	3.5	8.0	49.6
12 % KOH extract	15.6	3	—	14.6	1.57	1.1	3.2	1.6	1.6	92.0
Residue			—			0.9	88.9	3.5	0.7	6.0

(AMX; $\overline{DP}_n$, 60). In the second series the xylan was methylated with simultaneous deacetylation and then after reduction was converted to a fully methylated glucoxylan (MX; $\overline{DP}_n$, 43) by further methylation. The low $\overline{DP}_n$ values for the two methylated xylans indicate that considerable depolymerisation occurred during the methylations.

The two methylated polysaccharides were hydrolysed and the monomeric sugars obtained were separated on a carbon-Celite column and estimated quantatively. 2-*O*- and 3-*O*-methyl-D-xylose were incompletely separated on the column and were separated for quantitative estimation by paper electrophoresis in borate buffer;[10] fractionation on a preparative scale was done on a carbon-Celite column by gradient elution in the presence of borate buffer [11]. The results of the fractionations are summarised in Table 2.

The presence of 2-*O*-methyl-D-xylose in the hydrolysed MX cannot be unequivocally accounted for. It is known from studies on cellulose [10,12] that hydroxyl groups in different positions show considerable differences in reactivity on methylation. It is also known from investigations at this Institute (unpublished results) that 2,3-di-*O*-methyl-D-xylose is demethylated to a small extent under the conditions employed for the hydrolysis of AMX and MX, but without

Table 2. Methyl ethers (in mole %) obtained from methylated xylans from birch wood.

Methyl ether	Acetylated methylated xylan (28.1 % OMe)	Deacetylated methylated xylan (37.4 % OMe)
Xylose	5.4	0.1
2-*O*-Methylxylose	17.5	2.5
3-*O*-Methylxylose	9.1	6.3
2,3-Di-*O*-methylxylose	58.1	78.3
2,3,4-Tri-*O*-methylxylose	—	5.2
2,3,4-Tri-*O*-methylglucose	9.8	4.0
2,3,4,6-Tetra-*O*-methylglucose	—	3.7

any preferential demethylation at either the 2- or the 3-position. Thus the presence in the hydrolysate from MX of 2-*O*-methyl-D-xylose which is often found in hydrolysates from methylated xylans (*cf. e.g.* Ref.[2]) might be explained either by incomplete methylation or by demethylation during hydrolysis. Another possibility is the occurrence of some branching points in the original polysaccharide, which might be correlated with the proportion of 2,3,4-tri-*O*-methyl-D-xylose (5.2 mole %) which is higher than that required by a straight chain of about 40 xylose residues (2.3 mole %). This matter however requires further investigation. Aspinall and Das Gupta have recently obtained evidence for a branched xylose chain in a similar xylan from jute.[13] The 2-*O*-methyl-D-xylose in the hydrolysed MX could also be accounted for by the presence of 3-*O*-(4-*O*-methyl-D-glucuronopyranosyl)-D-xylose residues which have been found in *Pinus radiata* D. Don [14] and various grasses.[15] However, a paper chromatographic investigation of a partial hydrolysate did not reveal the presence of this aldobiouronic acid.

The virtual absence of 2,3,4-tri-*O*-methyl-D-xylose from hydrolysed AMX indicates that most of the terminal non-reducing xylose residues carry *O*-acetyl groups.

The results show that the *O*-acetyl groups in the methylated acetylated xylan are attached to the xylose residues mainly at the 3-position and only to a lesser degree at the 2-position. However, the 30 % of the *O*-acetyl groups that is replaced by methoxyl groups is not accounted for. No indications were found of *O*-acetyl groups on the uronic acid residues. As the conditions during methylation [16] and possibly also in the isolation of the polysaccharide favour the migration of acetyl groups it is not possible to extrapolate the results obtained back to the native polysaccharide. In naturally occurring 1-*O*-acetyl-D-mannitol [17] and 6-*O*-acetyl-D-glucose [18] the *O*-acyl groups occupy the most readily acetylated position. No conclusion can be drawn from the present investigation as to whether this is also the case for the acetylated xylan.

EXPERIMENTAL

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

Chromatography. Papers: Whatman No. 1 and 3MM. Solvents: A. Ethyl acetate-acetic acid-water, 3:1:3. B. Butanol-ethanol-water 10:3:5. C. *Iso*propyl ether-light petroleum (40–60°), 1:1 (on DMSO-impregnated paper [19]).

Paper electrophoresis. Papers: Whatman No. 1, Schleicher and Schüll 602 hP. Buffers: 0.1 M borate buffer at pH 10, 0.1 M acetate buffer at pH 4. Spray reagents: Aniline hydrogen phthalate, anisidine hydrochloride.

Preparation of birch wood holocellulose. Acetone-extracted birch wood (a technical mixture of *Betula verrucosa* Ehrh. and *B. pubescens* Ehrh.) of particle size 1–2 mm (462 g) was treated with chlorine in ice-water and extracted with 3 % ethanolic ethanolamine according to Timell and Jahn [20], yielding birch holocellulose (373 g). Yields and analytical data for the holocellulose and the extracts described below are given in Table 1.

Extraction of the holocellulose. The holocellulose (300 g) was extracted twice with redistilled dimethyl sulphoxide (3 l) at room temperature. After filtration the combined solutions were acidified with acetic acid and the hemicellulose was precipitated by adding two volumes of ethanol and removed by centrifugation. It was obtained as a powder after reprecipitation from aqueous solution with ethanol, washing with ethanol and ether and drying in a vacuum. The hemicellulose had $\overline{DP}_n$ 200 ± 10 and $[\alpha]_D^{20}$ −69° (*c*, 1.0 in water).

Table 3. Distillation at constant volume of the volatile acid from birch wood xylan and of some known acids.

Distillate ml	Formic acid %	Acetic acid %	Propionic acid %	Acid from xylan
25	17	27	46	28
50	31	46	70	46
75	41	60	82	59
100	50	69	89	68
125	56	76	93	75
150	62	81		80
175	66	85		84
200	70	88		87
225	74	90		90

The residual holocellulose was extracted twice with boiling water for 2 h and then twice with 12 % aqueous potassium hydroxide under nitrogen overnight at room temperature. The extracts were worked up as described above. The lignin in the holocellulose was estimated as Klason lignin, that in the hemicellulose fractions by measuring the absorption at 280 mμ [21]. Uronic acid contents were determined according to Johansson *et al.* [22], and quantitative paper chromatography on aldoses in the hemicellulose hydrolysates was done according to Saeman *et al* [23].

Identification of the O-*acetyl groups.* The acetic acid was identified by Dyers method [24]. The xylan (0.25 g) was heated in N sodium hydroxide at 100° for 15 min. The solution was acidified and the volatile acid was removed by distillation. After neutralisation with sodium hydroxide and concentration the solution containing the acetate was passed through Amberlite IR-120 (H^+), made up to 50 ml and distilled at constant volume. The distillate was collected in 25 ml fractions which were titrated with 0.01 N sodium hydroxide. Solutions with comparable concentrations of authentic formic, acetic and propionic acid were distilled in a similar manner. The results are summarised in Table 3 which shows that only acetic acid was obtained from the xylan.

Methylation of acetylated birch wood xylan. DMSO-extracted xylan (4 g) was dissolved in dry dimethyl formamide (150 ml). Methyl iodide (10 ml) and silver oxide (9 g) were added in small portions over 5 h and the solution was stirred vigorously and cooled externally. The reaction was allowed to proceed for a further 20 h and the product was then isolated as previously described [8]. The product was methylated in the same way a further three times. Yield 1.55 g, OCH_3 28.1 %, CH_3CO 10.1 %. (The calculated methoxyl content for a fully substituted xylan with 10.1 % *O*-acetyl is 28.4 %). This material is referred to as AMX.

Methylation with deacetylation of birch xylan. The xylan (4 g) was first methylated with simultaneous deacetylation using methyl sulphate in aqueous sodium hydroxide and then methylated three times with methyl iodide and silver oxide in dimethyl formamide. Yield 3.32 g, OCH_3 37.4 % (theoretical value 38.3 %). This material is referred to as MX.

Reduction of AMX and MX. AMX (1.5 g) was reduced with lithium aluminium hydride (2 g) in boiling, anhydrous tetrahydrofuran (100 ml) for 4 h. After addition of water to destroy excess reducing agent and neutralisation with sulphuric acid, the reaction mixture was filtered and concentrated to dryness. The product was extracted with chloroform and precipitated in light petroleum from benzene solution. Yield 1.4 g. MX (1.3 g) was reduced similarly to yield 1.2 g of product. This was then methylated twice with methyl iodide and silver oxide in the usual manner. Yield 1.1 g, OCH_3 37.9 %.

Hydrolysis of reduced AMX and MX. Reduced AMX (1 g) was treated with formic acid at 100° for 8 h and then with 0.5 N hydrochloric acid at 100° overnight. The solution was passed through Amberlite IR 4B (free base) and concentrated to a syrup. Yield 0.85 g.

Reduced MX (0.80 g) was hydrolysed in a mixture of 20 ml acetic acid and 40 ml 0.5 N sulphuric acid at 100° overnight. The hydrolysate was treated with barium hydroxide, filtered and concentrated to a small volume. After extraction with ethanol and concentration the mixture of monomeric sugars was obtained as a syrup (0.81 g).

Table 4. Fractionation of hydrolysed AMX. *Gradients:* 0 → 12 % aqueous ethanol (3 l), 12 → 35 % aqueous ethanol (3 l). 20-ml fractions were collected.

Tube No.	Component	Millimole	Mole %
21—38	Xylose	0.198	5.4
41—44	3-*O*-Methylxylose	0.112	3.0
45—88	2-*O*- + 3-*O*-Methylxylose	0.870	23.6
108—166	2,3-Di-*O*-methylxylose	2.14	58.1
Residue	2,3,4-Tri-*O*-methylglucose	0.360	9.8

Ratio: 2-*O*-: 3-*O*-methylxylose in fraction 45—88 was 2.9:1.

Fractionation of hydrolysed AMX and MX. The hydrolysates were fractionated on a carbon-Celite column (4 × 46 cm) by gradient elution with aqueous ethanol [25]. The column was subsequently washed with 50 % acetone. The amount of reducing sugar in each of the combined fractions was determined by hypoiodite oxidation [26]. The fractions containing both 2-*O*- and 3-*O*-methyl-D-xylose were analysed for each component by quantitative paper electrophoresis in borate [10] while fractionation on a preparative scale was done on a carbon-Celite column (2.8 × 30 cm) by gradient elution (2 l 0—6 % aqueous ethanol) in the presence of borate (0.1 M, pH 10); 3-*O*-methyl-D-xylose was eluted first. The results of the fractionations are summarised in Tables 4 and 5.

Characterisation of components

D-*Xylose.* Table 4: Crystallised from ethanol-water, m. p. and mixed m. p. 143—145°. Table 5: Characterised by paper chromatography.

2-O-*Methyl*-D-*xylose.* Table 4: Crystallised from acetone, m. p. 131—133°. Table 5: The ether was indistinguishable by paper electrophoresis from an authentic specimen, kindly supplied by Dr. T. E. Timell, Montreal.

Table 5. Fractionation of hydrolysed MX. *Gradients:* 0 → 12 % aqueous ethanol (3 l), 12 → 30 % aqueous ethanol (2 l), 30 % aqueous ethanol → 30 % aqueous acetone (3 l), 30 % → 50 % aqueous acetone (2 l). 25 ml fractions were collected.

Tube No.	Component	Millimole	Mole %
24— 42	Xylose	0.031	0.1
54— 71	3-*O*-Methylxylose	0.203	4.9
72—120	2-*O*- + 3-*O*-Methylxylose	0.163	3.9
134—224	2,3-Di-*O*-methylxylose	3.25	78.1
230—254	2,3,4-Tri-*O*-methylglucose	0.166	4.0
255—284	2,3,4-Tri-*O*-methylxylose	0.216	5.2
Residue	2,3,4,6-Tetra-*O*-methylglucose	0.154	3.7

Ratio: 2-*O*- : 3-*O*-methylxylose in fraction 72—120 was 1.8:1.

3-O-Methyl-D-xylose. Tables 4 and 5: Crystallised from acetone and methyl ethyl ketone, m. p. and mixed m. p. 96—98°. The ether was indistinguishable from authentic material on paper electrophoresis.

2,3-Di-O-methyl-D-xylose. Table 4: $[\alpha]_D^{20}$ +23° (*c*, 1.5 in water). The anilide had m. p. 124—126°. Table 5: $[\alpha]_D^{20}$ +23.5° (*c*, 1.5 in water). The anilide had m. p. 126—128°. These values are in good agreement with those previously reported [27].

2,3,4-Tri-O-methyl-D-xylose. $[\alpha]_D^{20}$ +18° (*c*, 1.1 in water). The anilide after crystallisation from ligroin had m. p. and mixed m. p. 99—101°. The ether was indistinguishable from authentic material by paper chromatography on DMSO-impregnated paper in solvent C.

2,3,4-Tri-O-methyl-D-glucose. The ether was in both cases indistinguishable from an authentic sample on paper chromatography in solvents A, B and C. Attempts to crystallise the aniline derivative failed; on paper chromatography in solvent C it gave two spots corresponding to the α and β forms, both of which were indistinguishable from the spots given by 2,3,4-tri-*O*-methyl-D-glucopyranosyl-N-phenylamine.

2,3,4,6-Tetra-O-methyl-D-glucose. Demethylation gave glucose only. The ether was indistinguishable from an authentic sample on paper chromatography in solvent A, B and C.

Partial hydrolysis of birch wood xylan. Xylan (5 g) from the 12 % potassium hydroxide extract was dispersed in water (300 ml) and 2 N sulphuric acid (100 ml) was added. The mixture after keeping at 100° for 3 h gave a clear solution. After neutralisation with barium hydroxide and filtration, the solution was concentrated to a small volume. Paper chromatography in the ethyl acetate-pyridine-water, 2:1:2 solvent indicated the presence of at least two acidic components together with xylose and small amounts of other sugars. The barium salts of the acids were precipitated with ethanol. From the supernatant liquid further amounts of barium salts of acids were obtained after concentration and repeated precipitation. The precipitates were washed with ethanol and reprecipitated. The free acids (0.76 g) were obtained after removal of the cations with Amberlite IR-120 (H^+) and concentration to dryness.

Fractionation of the acidic components. The acidic fraction (0.76 g) was adsorbed on a cellulose column, 2.5 × 35 cm, and eluted with ethyl acetate-acetic acid-formic acid-water 19:3:1:4 (3 l). 25 ml fractions were collected. Suitable fractions were combined, evaporated to dryness and examined by paper chromatography in ethyl acetate-acetic acid-water 3:1:1, in ethyl acetate-acetic acid-formic acid-water 19:3:1:4 and by paper electrophoresis in borate and acetate buffer.

Fraction 1 (75 mg) contained mainly 4-*O*-methyl-D-glucuronic acid together with a smaller amount of 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose, both indistinguishable from authentic specimens.

Fraction 2 (250 mg) contained 2-*O*-(4-*O*-methyl-D-glucuronopyranosyl)-D-xylose and a trace of a component of lower R_F-value on paper chromatography. The aldobiouronic acid was indistinguishable from authentic material on chromatography and on electrophoresis.

Fraction 3 (320 mg) consisted of a mixture of higher acids and oligosaccharides and was not examined further. No aldobiouronic acid was observed corresponding to 3-*O*-(4-*O*-methyl-D-glucuronopyranosyl)-D-xylose, which is reported to move faster than 2-*O*-(4-*O*-methyl-D-glucuronopyranosyl)-D-xylose on paper chromatography in ethyl acetate: acetic acid:formic acid-water, 19:3:1:4 [14].

Osmometric DP_n determinations. Osmometric determinations were made on the sodium salt of the acetylated xylan in 0.1 N sodium chloride [7] using "Ultrafein allerfeinst" membranes, supplied by Membranfiltergesellschaft Göttingen. Determinations of the DP_n of AMX and MX were carried out in chloroform and butyl acetate, respectively, using "Ultracellafilter allerfeinst" membranes supplied by the same firm.

One of the authors (PJG) is indebted to *Papirindustriens Forskningsinstitutt*, Oslo, Norway, for a fellowship.

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Received November 3, 1959.