Carbohydrate Research, 42 (1975) 297–304

STUDIES OF THE DISTRIBUTION OF THE 4-*O*-METHYL-D-GLUCURONIC ACID RESIDUES IN BIRCH XYLAN

KARL-GUNNAR ROSELL AND SIGFRID SVENSSON*

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm (Sweden)

(Received December 18th, 1974; accepted for publication, January 6th, 1975)

ABSTRACT

The distribution of the 4-*O*-methyl-D-glucuronic acid residues in birch xylan has been studied. Elimination of the 4-*O*-methyl-D-glucuronic acid residues of methylated birch-xylan was followed by specific cleavage of the xylan backbone at the originally branched D-xylose residues, using a technique involving sequential oxidation, β-elimination, and mild hydrolysis with acid. The molecular weight distribution of the resulting methylated oligosaccharides indicates that the 4-*O*-methyl-D-glucuronic acid residues are irregularly distributed in birch xylan.

INTRODUCTION

Previous studies[1] have shown that birch xylan consists of chains of (1→4)-linked β-D-xylopyranose residues, some of which carry a 4-*O*-methyl-α-D-glucopyranosyluronic acid residue at position 2. Some of the D-xylopyranose residues are substituted with *O*-acetyl groups[2,3]. Studies of partial hydrolysis with acid have indicated that the 4-*O*-methyl-D-glucuronic acid residues are randomly distributed along the xylan backbone[4]. In the present study, the distribution of the 4-*O*-methyl-D-glucuronic acid residues has been determined by using specific degradation techniques.

RESULTS AND DISCUSSION

Birch xylan was isolated from birch wood (*Betula verrucosa*) by extraction with alkali[5]. The crude preparation of xylan was further purified by gel-permeation chromatography to remove traces of contaminating products. The purified xylan had $\bar{M}_w$ 31,300 and $\bar{M}_n$ 26,300, giving a $\bar{M}_w/\bar{M}_n$ value of 1.19. The $\bar{M}_n$ value corresponds to $\bar{P}_n$ 175, in accordance with previous data[6]. Sugar analysis showed that the xylan contained 92 mole % of D-xylose and 8 mole % of 4-*O*-methyl-D-glucuronic acid, in agreement with previous results[6].

*Present address: Department of Clinical Chemistry, University Hospital, S-221 85 Lund, Sweden.

TABLE I

METHYLATION ANALYSES OF NATIVE AND DEGRADED BIRCH-XYLAN

Polysaccharide material		*2,3-Me-4-Et-Xyl*[a] (T[c] = *0.60*)	*2,3,4-Xyl* (T *0.68*)	*3-Me-2-Et-Xyl* (T *1.38*)	*2,3-Xyl* (T *1.54*)	*2,3,4-Glc*[b] (T *2.50*)	*3-Xyl* (T *2.92*)
				Mole %			
Native	(*A*)		1		93		6
Native, reduced[d]	(*B*)		1		82	8[e]	9
Degraded, ethylated	(*C*)		1	9	90		
Degraded, oxidised, acid hydrolysis	(*D*)		1		99		
Degraded, oxidised, alkaline treatment, ethylated	(*E*)	9	1		90		

[a]2,3-Me-4-Et-Xyl = 2,3-di-*O*-methyl-4-*O*-ethyl-D-xylose, etc. [b]2,3,4-Glc = 2,3,4-tri-*O*-methyl-D-glucose. [c]Retention time of the alditol acetates relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an ECNSS-M column at 170°. [d]Carboxyl-reduced after methylation. [e]Deuterium labelling at position 6.

The xylan was methylated with methylsulphinyl anion/methyl iodide in methyl sulphoxide[7], the methylated xylan was hydrolysed, and the resulting partially methylated sugars were analysed, before and after carboxyl-reduction, as their alditol acetates by g.l.c.–m.s.[8] (Table I, *A* and *B*). These analyses were in accordance with previous data[6].

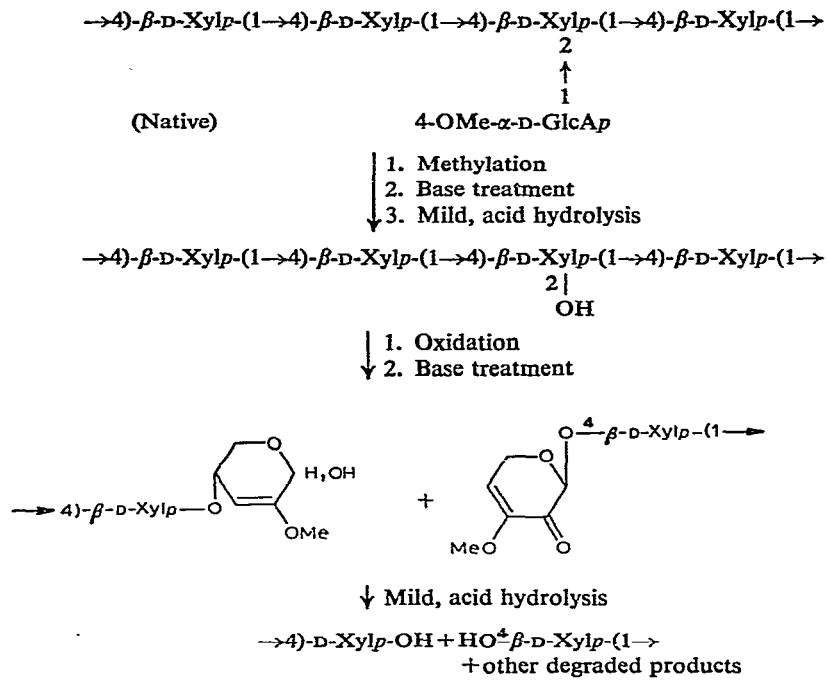
The methylated xylan was treated with base under anhydrous conditions, and subsequently with weak acid in order to remove the methylated uronic acid residues[9]. Part of the degraded material was ethylated and hydrolysed, and the resulting alkylated sugars were analysed, as alditol acetates, by g.l.c.–m.s.[8] (Table I, *C*). This analysis shows that essentially all of the uronic acid residues had been eliminated, leaving a hydroxyl group at position 2 of each of the originally branched D-xylose residues. The $\bar{P}_n$ of the degraded material, as indicated by the percentage of 2,3,4-tri-*O*-methyl-D-xylose in an acid hydrolysate, was essentially the same as that for methylated 4-*O*-methyl-D-glucuronoxylan.

The methylated, degraded xylan was oxidized with the methyl sulphoxide–chlorine complex in dichloromethane[10]. The oxidized product showed i.r. absorption for carbonyl but not for hydroxyl groups. Part of the oxidized material was hydrolysed and the resulting methylated sugars were analysed, as their alditol acetates, by g.l.c.–m.s.[8] (Table I, *D*). The absence of 3-*O*-methyl-D-xylose demonstrated that the oxidation had gone to completion.

The methylated, degraded, and oxidized xylan was treated with base, which results in elimination of the substituent at position 4 of each of the oxidized D-xylose residues[11], *i.e.*, cleavage of the xylan backbone at the original branch-points. The elimination reaction produces a new reducing-terminal, which is further degraded under the basic conditions[12,13]. Treatment of the base-degraded material with mild acid results in further degradation of the α,β-unsaturated keto-sugars with concomitant release of the ring-substituents[12,14]. These reactions are depicted in Scheme 1; in this scheme, all hydroxyl groups are methylated unless otherwise indicated.

Part of the degraded material was ethylated and hydrolysed. Analysis of the resulting alkylated sugars, as alditol acetates, by g.l.c.–m.s.[8] (Table I, *E*) showed that 9 mole % of new non-reducing terminals (4-*O*-ethyl-2,3-di-*O*-methyl-D-xylose) had been formed. This result demonstrates that, in the original birch-xylan, the main portion of the D-xylose residues substituted with 4-*O*-methyl-D-glucuronic residues are separated by at least two non-substituted D-xylose residues.

The degraded material was also analysed, after methylation, by high-pressure liquid chromatography on a Microporasil column, calibrated with the aid of methylated xylose oligosaccharides. The oligosaccharides[15] were methylated with methylsulphinyl anion–methyl iodide in methyl sulphoxide[7]. The elution volumes were determined for oligosaccharides up to $n = 8$ (Fig. 1). From these elution volumes and assuming that higher oligosaccharides of a homologous series will have a retention volume dependent only on length, the expected elution volumes for higher oligosaccharides were calculated. Based on these calculated volumes, it was concluded that



Scheme 1.

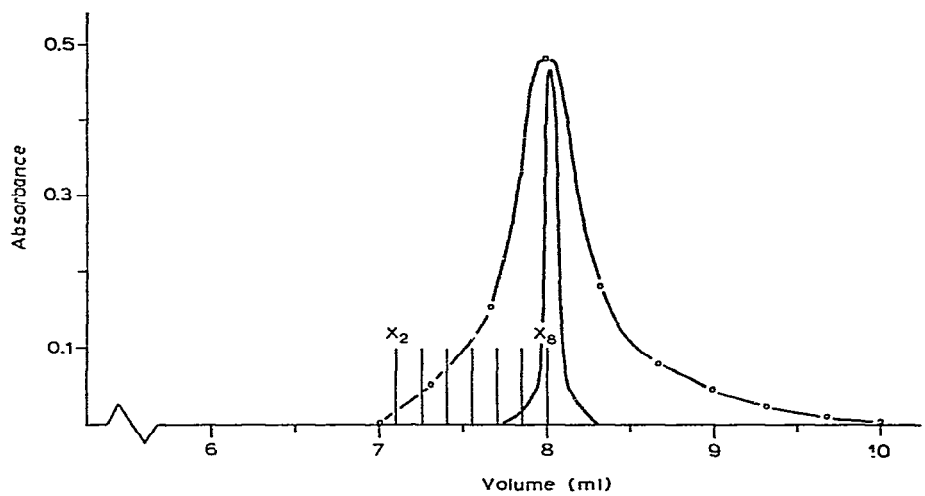


Fig. 1. Separation of methylated oligosaccharides on Microporasil columns. The conditions of the separation are given in the Experimental section; X_2 = permethylated β-D-Xylp-(1→4)-D-Xyl. The vertical bars indicate elution volumes for the reference oligosaccharides, and the elution curve for X_8 is shown.

oligosaccharides up to $n = 18$ were present in the mixture obtained from the oxidation and β-elimination degradative procedure.

These results strongly indicate that the 4-*O*-methyl-D-glucuronic acid residues are irregularly distributed along the D-xylan backbone.

EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at bath temperatures not exceeding 40°. For g.l.c., a Perkin–Elmer 990 instrument, fitted with flame-ionisation detectors, was used. Separations were performed on glass columns (190 × 0.15 cm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 170°, and on ECNSS-M S.C.O.T. columns (15 m × 0.5 mm) at 190°. For quantitative evaluations of the g.l.c., a Hewlett–Packard 3370B integrator was used. For mass spectrometry, the mixture of alditol acetates, dissolved in chloroform, was injected into a Perkin–Elmer 270 combined gas chromatograph–mass spectrometer. The mass spectra were recorded at an ionisation potential of 70 eV, ionisation current of 80 μA, and an ion-source temperature of 80°. High-pressure liquid chromatography was performed with a Waters Solvent delivery system 6000 constant-flow pump, and the column effluent was monitored with a Waters R401 differential refractometer. Separations were performed on two Waters Microporasil columns (30 × 0.5 cm) connected in series, using acetonitrile as eluant. Optical rotations were recorded using a 10-cm micro-cell with a Perkin–Elmer 141 instrument, i.r. spectra were recorded with a Perkin–Elmer 257 instrument, and absorbances were measured with a Beckman DB spectrophotometer.

Preparation of the polysaccharide material. — Acetone-extracted birch wood (*Betula verrucosa*) of particle size 0.5–2 mm was extracted with 24% (w/w) aqueous potassium hydroxide under nitrogen, and worked up as described by Glaudemans and Timell[5]. The yield of the hemicellulose was 26%. The material (2.5 g) was dissolved in water (50 ml) and applied to a column (60 × 8 cm) of Sephadex G-75, which was irrigated with water. The separation was monitored by optical rotation. A single peak was obtained, the leading and trailing edges of which were rejected, and the product [1.9 g, $[\alpha]_{578}^{22}$ −80° (*c* 0.5, water)] was recovered by dialysis followed by lyophilization.

Sugar analysis. — The polysaccharide material (5 mg) and D-arabinose (as internal standard) was hydrolysed in 0.25M sulphuric acid (3 ml) at 100° for 14 h. The hydrolysate was neutralised with barium carbonate, filtered, and concentrated to dryness. The resulting mixture was trimethylsilylated[16], dissolved in ethyl ether (10 ml), and treated with lithium aluminium deuteride (20 mg) at reflux temperature for 4 h. After processing, the reduced product was hydrolysed with 0.25M sulphuric acid for 14 h at 100°, and the acid was neutralised as above. The resulting sugars were converted into alditol acetates and analysed by g.l.c.[17]–m.s.[18].

Methylation and ethylation analyses. — The polysaccharide (10 mg) was dissolved in methyl sulphoxide (2 ml) in a flask sealed with a rubber cap. Nitrogen was flushed through the bottle, and 2M methylsulphinyl anion in methyl sulphoxide

(2 ml) was added. The gelatinous solution was agitated in an ultrasonic bath for 0.5 h and left at room temperature overnight. Methyl iodide (or ethyl iodide) (2 ml) was then added dropwise with external cooling, and the resulting turbid solution was agitated ultrasonically for 0.5 h, giving a clear solution. Methyl iodide was removed by distillation and the product (12 mg) recovered by dialysis and lyophilization.

One part (1/3) was treated with 90% formic acid (3 ml) at 100° for 2 h, the hydrolysate was concentrated to dryness, and the residue was hydrolysed with 0.25M sulphuric acid (3 ml) at 100° for 16 h. The resulting sugars were converted into alditol acetates and analysed by g.l.c.–m.s.[8] (Table I, *A*).

The remaining part (2/3) was dissolved in tetrahydrofuran (10 ml) and reduced at reflux temperature with lithium aluminium deuteride (20 mg) for 4 h. After processing, the reduced, methylated polysaccharide was hydrolysed and analysed as described above (Table I, *B*).

Elimination of 4-O-methyl-D-glucuronic acid residues. — Methylated polysaccharide [300 mg, prepared as described above and purified by chromatography on a column (50 × 5.5 cm) of Sephadex LH 20 using chloroform–acetone (2:1) as irrigant] was added to a solution of toluene-*p*-sulphonic acid (20 mg) in methyl sulphoxide–2,2-dimethoxypropane (19:1, 30 ml) contained in a serum vial sealed with a rubber cap. The vial was flushed with nitrogen and agitated ultrasonically for 0.5 h, and 2M methylsulphinyl anion in methyl sulphoxide (15 ml) was added with the aid of a syringe. The solution was agitated for 0.5 h and kept at room temperature overnight, and 50% aqueous acetic acid was then added with external cooling until pH 4 was reached. The mixture was poured into water (50 ml) and extracted with chloroform (3 × 25 ml). The combined extracts were washed with water (4 × 25 ml) and concentrated to dryness. The product was purified by elution from a column (50 × 5.5 cm) of Sephadex LH-20 with chloroform–acetone (2:1). The eluate was monitored polarimetrically. The product (230 mg), eluted with the void volume, was treated with 10% aqueous acetic acid (20 ml) for 2 h at 100°. The resulting suspension was dialysed against water overnight and lyophilized. The degraded material showed i.r.-absorption in the hydroxyl region (3600–3300 cm^{-1}) but none in the carbonyl region (1740–1700 cm^{-1}). Part (5 mg) of the product was ethylated, hydrolysed, and analysed by g.l.c.–m.s. as described before (Table I, *C*).

Oxidation of the modified polysaccharide. — An anhydrous M solution of chlorine in dichloromethane (25 ml) was placed under nitrogen in a serum flask sealed with a rubber cap and cooled to −55° with continuous stirring. Methyl sulphoxide (9 ml) was added dropwise to the solution, giving a white complex. The modified polysaccharide (100 mg), in dichloromethane (5 ml), was then added dropwise with a syringe while the reaction mixture was kept at −55°. After 5 h of continuous stirring, triethylamine (10 ml) was added, and the solution was allowed to attain room temperature (~0.5 h), then dialysed against water overnight, and concentrated to dryness. The dried material was fractionated on a column (30 × 3 cm) of Sephadex LH-20, using chloroform–acetone (2:1) as irrigant. The eluate was monitored polarimetrically. The oxidized product (80 mg), eluted with the void

volume, had i.r.-absorption in the carbonyl region (1710 cm^{-1}). Part (5 mg) of the product was hydrolysed, and analysed by g.l.c.–m.s. (Table I, *D*).

Alkaline degradation. — The oxidized material (60 mg) was dissolved in dichloromethane (4 ml), and ethanolic M sodium ethoxide (2 ml) was added. The reaction mixture was kept at room temperature for 1 h with continuous stirring, then neutralised with acetic acid, and evaporated to dryness. The residue was dissolved in methanol, and Dowex-50(H^+) resin was added to pH ~4. The solution was filtered and concentrated to dryness. This material (50 mg) was treated with 50% aqueous acetic acid for 2 h at 100° and evaporated to dryness, yielding 40 mg of degraded material. Part (5 mg) of this product was ethylated, recovered by partition between chloroform–water, hydrolysed, and analysed by g.l.c.–m.s. (Table I, *E*).

Distribution analysis of methylated oligosaccharides. — The alkali–mild acid-degraded material (30 mg) was methylated, and the resulting methylated oligosaccharides were analysed by high-pressure liquid chromatography.

The eluant (flow rate: 1 ml/min) was analysed colorimetrically, using anthrone[19] (Fig. 1) as follows. The fractions were evaporated to dryness, and then the reagent (2 ml, 0.2% of anthrone in 70% aqueous sulphuric acid) was added. Samples were heated (100°, 10 min), cooled, and diluted with ethanol (4 ml), and the absorbances measured at 620 nm. Reference oligosaccharides ($n = 2$–8) were prepared by partial hydrolysis[15] of native birch-xylan and methylated[7], and their elution volumes determined in the above chromatographic system. Elution volumes (ml) were: $X_2 = 7.1$, $X_3 = 7.3$, $X_4 = 7.4$, $X_5 = 7.6$, $X_6 = 7.7$, $X_7 = 7.9$, and $X_8 = 8.0$; X_2 = permethylated β-D-Xyl*p*-(1→4)-D-Xyl, etc.

ACKNOWLEDGMENTS

The skilled technical assistance of Miss Birthe Abrahamsson is acknowledged. The authors are indebted to Cellulosaindustriens Stiftelse för Teknisk och Skoglig Forskning samt Utbildning for financial support, to Dr. Kirsti Granath (Pharmacia AB) for estimation of the molecular weight distribution of native birch-xylan, to Professor Bengt Lindberg for his interest, and to Dr. Olle Larm for valuable discussions.

REFERENCES

1 T. E. Timell, *Advan. Carbohyd. Chem.*, 19 (1964) 247–302.
2 B. Lindberg, K.-G. Rosell, and S. Svensson, *Sv. Papperstidn.*, 76 (1973) 30–32.
3 H. O. Bouveng, P. J. Garegg, and B. Lindberg, *Acta Chem. Scand.*, 14 (1960) 742–748.
4 J. Havlicek and O. Samuelson, *Carbohyd. Res.*, 22 (1972) 307–316.
5 C. P. J. Glaudemans and T. E. Timell, *Sv. Papperstidn.*, 23 (1957) 869–871.
6 M. Han and B. Swan, *Sv. Papperstidn.*, 71 (1968) 552–557.
7 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
8 H. Björndal, C. G. Hellerqvist, B. Lindberg, and S. Svensson, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610–619.
9 B. Lindberg, J. Lönngren, and J. L. Thompson, *Carbohyd. Res.*, 28 (1973) 351–357.
10 E. J. Corey and C. H. Kim, *Tetrahedron Lett.*, 12 (1973) 919–922.

11 L. KENNE AND S. SVENSSON, *Acta Chem. Scand.*, 26 (1972) 2144–2146.
12 E. F. L. J. ANET, *Chem. Ind. (London)*, (1963) 1035–1036.
13 G. O. ASPINALL, R. KHAN, R. R. KING, AND Z. PAWLAK, *Can. J. Chem.*, 51 (1973) 1359–1362.
14 L. KENNE, K.-G. Rosell, AND S. SVENSSON, unpublished results.
15 R. L. WHISTLER AND C. C. TU, *J. Amer. Chem. Soc.*, 74 (1952) 3609–3612.
16 G. O. ASPINALL, B. GESTETNER, J. A. MOLLOY, AND M. UDDIN, *J. Chem. Soc.*, (1968) 2554–2559.
17 J. S. SAWARDEKER, J. H. SLONEKER, AND A. R. JEANES, *Anal. Chem.*, 37 (1965) 1602–1604.
18 O. S. CHIZHOV, L. S. GOLOVKINA, AND N. S. WULFSSON, *Bull. Acad. Sci. (USSR)*, 11 (1966) 1853–1863.
19 T. A. SCOTT AND E. H. MELVIN, *Anal. Chem.*, 25 (1953) 1656–1661.