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STRUCTURE OF THE REDUCING END-GROUPS IN SPRUCE XYLAN

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ABSTRACT

Borohydride reduction of spruce meal followed by mild hydrolysis with acid gave a large proportion of 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylitol. When the reduction was preceded by mild treatment with alkali, galactonic acid end-groups were formed. Enzymic degradation of holocellulose from reduced spruce-meal followed by ion-exchange chromatography led to the isolation of a tetrasaccharide having a xylitol end-group. Its structure revealed that the reducing end-group in spruce xylan has the structure β-D-Xyl*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xyl, which is the same as that in hardwood xylan.

INTRODUCTION

The isolation of large amounts of 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose[1] and fractionation studies[2] of the isolated birch xylan (*Betula verrucosa*) indicate that a major portion of the non-reducing galacturonic acid is a constituent of xylan. This has been confirmed for *Betula platyphylla* xylan by studies of products obtained by enzymic hydrolysis[3]. It was found that galacturonic acid is linked to a rhamnosyl residue, which in its turn is linked to a xylosyl group in the following manner: β-D-Xyl*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xyl. Analysis of the monocarboxylic acids formed by treatment of xylan with hypochlorite showed that this structure also occurs in the xylan present in *Betula verrucosa*[4]. Further studies revealed that reducing xylose end-groups are present in birch xylan and that these are linked directly to a galacturonic acid, as shown above. These and other results confirm that the illustrated structure represents the reducing end in birch xylan[5–7].

A subsequent investigation[8] showed that xylan in aspen wood (*Populus tremula*) contained the same end-group. The presence of reducing xylose end-groups in spruce has been established[9], but no detailed study of the structure of the sugar units that

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precede the reducing xylose end-group has been reported. In contrast to hardwood xylan, softwood xylan cannot be isolated in reasonable yield and purity without treatments that affect the structure seriously. The technique was therefore modified to permit a study of spruce xylan (*Picea abies, Karst.*).

RESULTS AND DISCUSSION

Partial hydrolysis of the reduced wood-meal under various conditions followed by anion-exchange chromatography[10] revealed that 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylitol was the only acid containing a xylitol residue that was present in the hydrolysate. The results show that, like hardwood xylan, spruce xylan contains a reducing xylose end-group glycosidically linked to a galacturonic acid residue by a (1→4) linkage. No higher oligomers containing a xylitol end-group were found in the hydrolysates, indicating that the galacturonic acid residue is linked to the rest of the polysaccharide by a bond that is unstable in acid.

Determination of the alditols after complete hydrolysis of the reduced wood-meal[11] confirmed that reducing xylose end-groups were present in spruce xylan (Table I). No galactonic acid was present. When the spruce meal was treated with 0.25M sodium hydroxide at 40° for 48 h under nitrogen and then reduced with boro-hydride, the loss of material was 9%. The hydrolysate contained a lowered proportion of xylitol and mannitol, and an appreciable amount of galactonic acid was present. Evidently, the mild treatment with alkali led to a loss of the reducing xylose end-group and the formation of a reducing galacturonic acid end-group by β-elimination. The results accord with those previously found for hardwood xylan and show that the reducing xylose end-group is linked to the galacturonic acid residue by a bond of much lower stability in alkali than that between the galacturonic acid and the rest of the polysaccharide. Since the galacturonic acid is linked to the reducing xylose end-group by a (1→4)-glycosidic bond, it can be assumed that the galacturonic acid is linked by a (1→2) bond to the rest of the polysaccharide.

The isolation of larger fragments from the reducing end by acid hydrolysis

TABLE I

ALDITOL AND GALACTONIC ACID END-GROUPS IN SPRUCE MEAL REDUCED BEFORE AND AFTER MILD TREATMENT WITH ALKALI

	No alkali treatment (mmol/100 g)	*Alkali-treated (mmol/100 g)*
Xylitol[a]	0.28	0.06
Galactonic acid	0.00	0.12
Mannitol	0.40	0.20
Glucitol	0.083	0.104

[a]Including a minor proportion of arabinitol, probably formed by isomerisation.

failed. Enzymic hydrolysis of holocellulose (50 g) isolated from reduced spruce-meal gave a large fraction (8.5 g) containing higher saccharides, including oligomeric uronic acids. A solution of the sample in dilute sodium hydroxide (pH 9) was kept at room temperature for 4 h and then chromatographed in 0.02M sodium acetate on a strongly basic, anion-exchange resin having a low degree of cross-linking (Dowex 1-X2). The fraction containing trisaccharides and tetrasaccharides (59 mg) having one carboxylic acid group was isolated. The column was over-loaded (2.5 g sample per 100-ml bed-volume), which means that these solutes appeared in an overlapping band. Chromatography of 2 mg of this fraction on Dowex 1-X8 in 0.02M sodium acetate gave two peaks recorded in the periodate–formaldehyde channel[12]. The first peak had a position of a tetrasaccharide containing one carboxylic acid group and was well separated from other acids present, while the second peak had the position of a trisaccharide and was incompletely separated from uronic acids not recorded in the periodate–formaldehyde channel.

The fraction isolated on Dowex 1-X2 was rechromatographed on a preparative scale in 0.5M acetic acid on the same resin. Two fractions that gave responses in the periodate–formaldehyde channel were collected. The first fraction (3.8 mg) contained a tetrasaccharide having one carboxylic acid group and one alditol group. No other compounds were present. An aldotriouronic acid containing xylose and 4-*O*-methylglucuronic acid was present in the second fraction together with the acid recorded in the periodate–formaldehyde channel.

Only the acid contained in the first fraction was therefore analysed in detail. Hydrolysis in 0.05M sulphuric acid at 130° for 4 h gave xylose, rhamnose, galacturonic acid, and xylitol in the molar proportions 1 : 1 : 0.3 : 1. This shows that the reducing end in spruce xylan is built up from the same monosaccharides as those present in hardwood xylan. The low proportion of galacturonic acid is explained by losses during the hydrolysis.

The mass spectrum of the permethylated[13] sample (Fig. 1, Scheme 1) contained a peak from the molecular ion (*m/z* 774) of low intensity. The interpretation of the mass spectrum was confirmed by comparison with that of the tetrasaccharide isolated from birch[3] and reduced with sodium borodeuteride before methylation. Fragment ions are denoted by symbols suggested by Chizhov and Kochetkov[14,15]. The ions of

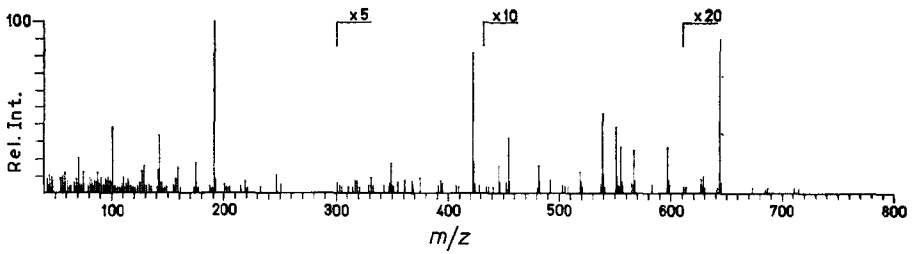
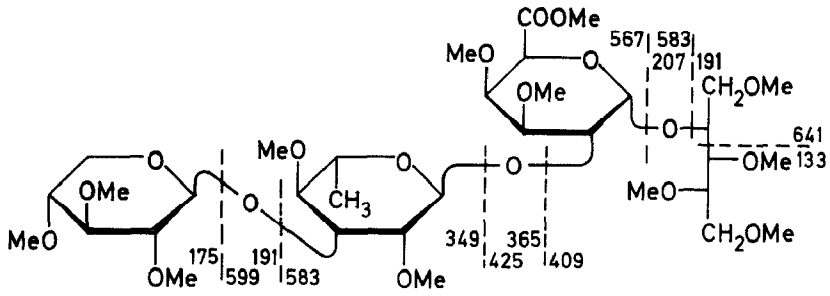


Fig. 1. Mass spectrum of permethylated β-D-Xyl*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xylitol.



Scheme 1. Primary, mass-spectral fragmentation of permethylated β-D-Xyl*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xylitol.

m/z 643 (abcdJ_1), 583 (bcdA_1), 567 (cabA_1), 551 (bcdA_2), 349 (baA_1), 251 (cdJ_1), 191 (dA_1), 175 (aA_1), 143 (aA_2), and 111 (aA_3) indicate that the sequence is Xyl*p*→Rha*p*→Gal*p*A→Xylitol.

The formation of an *m/z* 641 (M − 133) ion is consistent with the fact that the galacturonic acid is linked to xylitol by a (1→4) linkage. The observation that *m/z* 191 (dA_1), and not *m/z* 88 (H_1), is the base peak indicates that the other linkages are (1→2) or (1→3)[16,17]. The absence of the ion *m/z* 469 (bcdJ_1) demonstrates[17] that xylose is linked to the rhamnose residue at O-3. The presence of an intense ion at *m/z* 129 and the occurrence of the ion *m/z* 539, corresponding to *m/z* 365 for trisaccharide alditols[17], confirm this type of linkage.

The ion *m/z* 251 (cdJ_1) indicates[18] that the rhamnosyl residue is linked to the galacturonic acid residue at O-2 or O-4. The abundance of the ion *m/z* 435 (cabF_1) was approximately the same as that of *m/z* 503 (cabA_3). This suggests a (1→2) linkage[19]. A cleavage of this linkage at C-1 with the uptake of one hydrogen[20] would lead to a fragment that would produce prominent ions from the galacturonic acid moiety at *m/z* 219, 187, and 155. These ions were also significant for the permethylated, reduced tetrasaccharide from birch xylan. The same ions and the same ratios of intensities were also recorded for the methylated 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylopyranose[21] obtained by mild, acid hydrolysis of permethylated birch xylan and hence containing OH at C-2 in the galacturonic acid moiety. These results confirm the conclusion drawn from the alkali stability that the rhamnosyl residue is (1→2)-linked to the galacturonic acid residue.

The ^{1}H-n.m.r. spectrum of the reduced tetrasaccharide showed three signals for anomeric protons. The signal at δ 5.33 ($J_{1,2}$ 3.5 Hz) agreed with that of a reference spectrum of 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylitol. This confirmed the presence of an α linkage between the galacturonic acid and xylitol residues. The second signal at δ 5.20 ($J_{1,2}$ 1.3 Hz) indicated an α linkage between rhamnose and galacturonic acid, while the third signal at δ 4.77 ($J_{1,2}$ 7.6 Hz) demonstrated the presence of a β linkage between the xylose and rhamnose residues[22].

The results permit the conclusion that the reducing end-group in spruce xylan has the same structure as that in birch xylan.

EXPERIMENTAL

Spruce meal, supplied by Mo och Domsjö AB (Örnsköldsvik, Sweden), was extracted for 24 h with acetone and for 48 h with water. It was then kept at pH 8 for 24 h at room temperature while sodium hydroxide was added with an automatic titrator. The meal was reduced with potassium borohydride, washed with water, and finally air-dried.

After hydrolysis in 0.125M sulphuric acid for 15 h at 90° followed by anion-exchange chromatography[10], 30 mg of 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylitol was isolated per 100 g of reduced spruce-meal. The same acid was present after hydrolysis in trifluoroacetic acid at room temperature for 12, 16, and 20 days. No other acid giving a response in the periodate–formaldehyde channel[12] was recorded on the chromatograms.

Holocellulose was prepared by treating the reduced spruce-meal 10 times with an aqueous solution of chlorine dioxide (6.4 g of active chlorine per litre) at pH 4.75. After each treatment, the meal was washed thoroughly with water. The yield was 69% calculated on dry, reduced spruce-meal, and the lignin content was 4%.

An extracellular enzyme was prepared from the culture filtrate of *Tyromyces palustris* as described in a previous paper[23], with the modifications that kraft pulp (0.5%) was used as an inducer instead of the hardwood meal and that the pH was kept at 2 by addition of sodium hydroxide until the glucose was consumed. The crude enzyme preparation was applied to a column (50 × 820 mm) of Sephadex G-100, and the fraction rich in xylanase activity was collected. This partially purified enzyme-preparation also contained mannanase, but was almost free from cellulase, carboxymethylcellulase, and such glycosidases as mannosidase, xylosidase, and cellobiase.

The spruce holocellulose (50 g) was hydrolysed with the enzyme preparation (500 mg) in 0.1M acetate buffer (pH 4) at 40° for 16 h. After filtration, the enzyme was recovered from the hydrolysate by ultrafiltration through a hollow-fiber membrane (H1P5 Amicon Ltd.; cut-off molecular mass, 5000). After removal of sodium ions and acetic acid, the hydrolysate was freeze-dried (yield, 8.5 g).

The mass spectrometer was a VG Analytical ZAB 2F with a direct-inlet system. The electron energy was 30 eV, the acceleration voltage 8 kV, and the trap current 500 μA. Spectra were recorded at a probe temperature of 155°.

N.m.r. spectra (^{1}H, 270 MHz) were recorded in the pulsed Fourier-transform mode with a Bruker WH 270 spectrometer. The sample (1.5 mg, sodium salt) was dissolved in 0.5 mL of D_2O. No internal standard was added and the signal from HDO (δ = 4.74) was used to calculate the δ values. The temperature was 309 K.

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