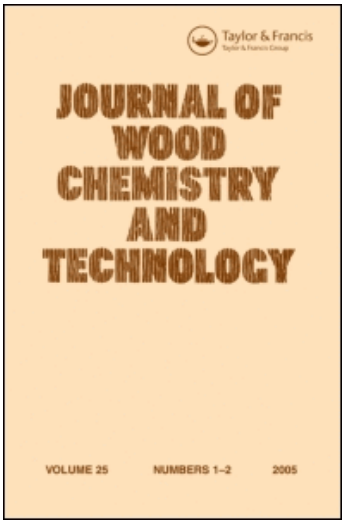


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# CHARACTERISATION OF OLIGOSACCHARIDES RELEASED BY STEAM EXPLOSION OF SULPHUR DIOXIDE IMPREGNATED PINUS RADIATA

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## ABSTRACT

The nature of the solubilized oligosaccharides released from the softwood Pinus radiata by the steam explosion process in the presence or absence of sulphur dioxide are described in this paper. Steam explosion in the absence of sulphur dioxide (215°, 3 min) resulted in partial solubilization of only the hemicelluloses (14.5 g of neutral sugars/100 g o.d. wood) to their respective oligomers, which ranged in degree of polymerization from 1 to at least 12. The effect of adding 2.5% sulphur dioxide to the substrate was to facilitate the removal and hydrolysis of both hemicellulose and cellulose components (29.5 g of neutral sugars/100 g o.d. wood) by acid catalysis. Steam explosion of the substrate at an elevated temperature of 248° in the presence of 2.5% sulphur dioxide, caused almost complete cellulose and hemicellulose solubilization, and subsequent degradation reflected in poor carbohydrate survival (24.7 g of neutral sugars/100 g o.d. wood). The addition of sulphur dioxide to the process results in enhanced hydrolysis of the solubilized material to mainly mono- and disaccharides. However, the acid reversion products such as isomaltose and

gentiobiose are formed, although their effect on total fermentable sugar yield is very small. Under normal steam explosion conditions the acidic aldooligouronic acid hydrolysis products represent only about 6% of total soluble carbohydrate. Interestingly, some demethylation of 4-0-methyl-D-glucuronic acid residues were observed as a consequence of sulphur dioxide catalysis.

## INTRODUCTION

The steam explosion of wood has received considerable attention in past years[1-12] as a pretreatment for wood prior to enzymatic hydrolysis. This process is the first step in the production of fuels and chemicals from wood via fermentation of the released sugars, therefore total saccharification of the biomass carbohydrate is desired. Our previous studies have focused on steam explosion of Pinus radiata, the most abundant source of renewable biomass available in New Zealand[2,10].



For hardwoods and agricultural residues, steam explosion is a highly effective pretreatment, however, with softwoods it is ineffective unless an acid catalyst such as sulphur dioxide is used[2,5-8,10,12]. Sulphur dioxide enhances carbohydrate hydrolysis while improving overall carbohydrate survival during the steam explosion process[1,4,10]. The main pretreatment mechanism appears to be the development of sufficient porosity in the fibre cell wall to allow adequate accessibility for cellulase enzymes, and carbohydrate hydrolysis is the major means by which cell wall porosity is increased[10,11,13-15]. Much more carbohydrate hydrolysis is required during steam-pretreatment of softwoods compared with hardwoods, to attain fibres of equal accessibility and enzymatic digestibility[10]. This is thought to be because of differences in the cell wall structure of the two wood types[14,15]. For P. radiata wood, all the hemicelluloses and about 25-30% of the cellulose must be solubilised to produce

fibre, where the residual cellulose is 80-90% digestible by cellulases[2,10]. In fact, in terms of the overall yield of total sugars, obtained from steam explosion and subsequent enzymatic hydrolysis of P. radiata, slightly more carbohydrate is solubilised during the pretreatment than is released by enzymatic hydrolysis of the pretreated fibre[2,10].

Clearly, carbohydrate solubilisation is an important aspect of the sulphur dioxide-catalysed steam-explosion pretreatment of P. radiata. Previous studies in this laboratory have described the effects of process conditions on the yield and, to some extent, on the nature of the soluble sugars[2,10]. Significantly, it was found that (1→6)-linked disaccharides, characteristic of acid reversion reactions, were detected. Such reversion products are non-fermentable by micro-organisms, used to ferment the sugars to products such as ethanol[16,17]. Therefore, their presence can significantly affect final product yields.



This paper describes a detailed examination of the soluble oligomeric sugars released during steam explosion of P. radiata. The neutral and acidic sugars were characterised, and the yields and identities of the reversion products, and the effects of some process conditions on their formation, were determined.

## EXPERIMENTAL

### Steam Explosion Substrate and Methodology

The P. radiata substrate used in this work was the same as described previously[2]. Its composition on an original oven dry (o.d.) wood basis was ash, 0.31%; extractives, 2.21%; Klason lignin, 26.16%; acid soluble lignin, nil; glucan, 43.31%; xylan, 5.27%; galactan, 2.89%; arabinan, 1.63%; and mannan, 10.71%; and 7.51% unaccounted (4-OMe-glucuronic anhydride and O-

acetyl)[2]. Techniques for $SO_2$-impregnation, steam explosion gun design and operation, and experimental methods have also been described[2].

The basic methodology for each steam explosion run involved impregnation of the wet chips (300 g oven dry equivalent) with $SO_2$ to a predetermined level (percent dry weight basis). The impregnated chips were then heated with saturated steam at a known temperature for a controlled time, after which the chips were explosively discharged from the apparatus. The steam exploded wood was then water washed (5% consistency) to yield a water-soluble fraction and a water-insoluble fraction. The yields of individual carbohydrates and lignin in the two fractions were determined by methods previously described, and all yields are reported as g/100 g original o.d. wood. Monosaccharides in the water-soluble fraction were determined after post-hydrolysis in 4% sulphuric acid[2].



Gas Liquid Chromatography-Mass Spectrometry

G.l.c.-m.s. was conducted on a Hewlett Packard 5985B g.l.c.-m.s. using electron-impact at 70 e.V. or chemical ionization (ammonia) modes. The methylated disaccharide alditols were separated on an OV-1 (12m, 0.25mm) capillary column with a temperature program of 180-280° at 5°/min. The injection port was kept at 300°. Partially methylated alditol acetates were separated on a SP 2340 (25m, 0.25mm) capillary column with a temperature program of 100-200° at 2°/min. The injection port was kept at 250°. Samples were applied using the purged splitless injection mode, with helium as the carrier gas.

Separation of Neutral and Acidic Carbohydrates

An aliquot from each of the water soluble fractions was taken, lyophilized, redissolved in water (100 mL) and extracted with diethyl ether. Each of the aqueous fractions were then applied

to a column containing Dowex 1-X8 ($OAc^-$, 1.5 x 25 cm) anion exchange resin. The neutral components were eluted with water, and the acidic components were then eluted with 5M acetic acid. Both fractions were then lyophilized and the yields of each were determined as a percentage of the sample weight applied to the column.

Gel Permeation Chromatography

The neutral fractions were further fractionated on Bio Gel P-2 (200-400 mesh, two 1.6 x 100 cm) operated at 55° and eluted with water (0.5 mL/min). Components were detected by refractive index (Waters R401). Fractions (5 mL) were collected and pooled according to degree of polymerization (d.p.) and lyophilized. The dry weights of each fraction were determined and expressed as percentages of total dry matter in the soluble fraction.

Anionic Exchange Chromatography

Each of the acidic fractions was quantitatively analysed for uronic acid oligomers by anion exchange chromatography[18-20] on Aminex A-25 h.p.l.c. resin ($OAc^-$, guard column 0.4 x 5 cm and separation column 0.4 x 25 cm). The mobile phase was 0.08M sodium acetate buffer pH 5.9 (0.6 mL/min). The sugars were colorimetrically detected (at 546 nm) after post-column derivatisation[19,20]. The column eluate was mixed with the derivatisation reagent (copper (II) 2' 2' bicinchoninate) at 0.3 mL/min flow rate and the reaction passed through a reaction coil (Teflon, 0.5 mm i.d. x 30 m) at 110° to the detector cell. Preparative separation of the components from each of the acidic fractions was performed on Dowex 1-X8 ( ≤400 mesh, $OAc^-$, 1 x 50 cm) using the same mobile phase as above at 1 mL/min flow rate. Components were detected by refractive index (Waters R401).

Monosaccharide Analysis

Neutral sugars released after acid hydrolysis were determined by h.p.l.c. using two BioRad HPX-87P columns in series operating at 90°, which were eluted with water (0.4 mL/min). Components were detected by refractive index (Hewlett Packard 1037A). Erythritol was used as an internal standard[2].

$^{13}$C-N.m.r. Spectroscopy

Spectra were recorded on a Bruker AC-200 n.m.r. spectrometer operating at 50.33 MHz. Each sample (1-20 mg) was dissolved in deuterium oxide (99.5%, 0.5 mL) in a 5 mm tube. Chemical shifts were measured relative to acetone ($CH_3$, 30.8 p.p.m.) dissolved with the sample. The free induction decays were accumulated with a 40° pulse, an acquisition time of 1.34 s, and a pulse delay of 2 s. The spectral width was 12 kHz, and 32 K data points taken. The experiments were Waltz decoupled, and performed at 25°.



Separation of the Disaccharides

Separation of the disaccharides (from g.p.c.) was achieved by h.p.l.c. on an amino column (Alltech, econosphere $NH_2$) eluted with 80:20 acetonitrile-water at 1 mL/min flow rate. Components were detected by refractive index (Waters R401). Individual peaks were collected.

Methylation Analysis

Each of the disaccharide fractions (~1 mg, from g.p.c.) was reduced with sodium borodeuteride and desalted. The disaccharide alditols were then fully methylated by the Ciucanu-Kerek[21] method, extracted with dichloromethane and analysed by g.l.c.-m.s.

The isolated disaccharides (~0.2 mg, from h.p.l.c.) were fully methylated[21]. The methylated products were hydrolysed in 2M trifluoroacetic acid for 3 hours at 100°, and the products

reduced with sodium borodeuteride, acetylated, and analysed by g.l.c.-m.s.

## RESULTS AND DISCUSSION

The effects of temperature, treatment time and level of sulphur dioxide on the yield of carbohydrate obtained from the water soluble and enzyme (cellulase) digested insoluble fractions of steam exploded P. radiata have been established by Clark et al[2,10]. The steam explosion conditions resulting in best carbohydrate recovery had been determined at 215° for 3 min., with a level of sulphur dioxide impregnation of 2.5% based on dry wood weight, and were used as standard conditions (b) in this study. The nature of the solubilized carbohydrate released under standard conditions was therefore examined, to determine the mode in which wood carbohydrates were solubilized by this process. The influence of extreme treatment conditions (undercooking and overcooking) on the nature of the water soluble oligosaccharides released by the steam explosion process was also investigated. Undercooking of the steam exploded substrate was achieved in the absence of sulphur dioxide (conditions (a) 215°, 3 min). Overcooking of the substrate was achieved by elevation of the treatment temperature to 248° in the presence of 2.5% sulphur dioxide (conditions (c)).

Process conditions, and the yields of lignin and carbohydrates in the soluble and insoluble fractions are given in Table 1. Carbohydrate analysis of the soluble and insoluble fractions generated from steam explosion of P. radiata showed that: (i) In the absence of sulphur dioxide (conditions (a)) only the hemicellulose components were solubilized. The glucose in the water soluble fraction originated from glucomannan solubilization, since the glucose:mannose ratio is 1:3.4, which is

typical of P. radiata glucomannan[22]. Total carbohydrate survival under these conditions was 92% (that is, total carbohydrate in the soluble and insoluble fractions as a percentage of that in the original wood). (ii) In the presence of sulphur dioxide (conditions (b)) all the hemicelluloses and 25% of the cellulose were solubilised. The total soluble sugar yield was 29.5 g/100 g o.d. wood, greatly increased over the yield of 14.5 g/100 g o.d. wood obtained in the absence of sulphur dioxide. Total carbohydrate survival was still relatively high (89%) showing that sulphur dioxide catalysis can greatly enhance hydrolytic reactions without resulting in a corresponding increase in degradative reactions[10]. The soluble sugars were a mixture of those derived from hemicellulose and cellulose hydrolysis (Table 1). (iii) When the process temperature was elevated to 248°, an extremely severe process resulted, with 94% hydrolysis of cellulose. Total carbohydrate survival was, however, low at only 38.6%, and the yield of water soluble sugars was only 24.7 g/100 g o.d. wood. The main soluble sugar was glucose.



As has been previously described[2,10], lignin remains, almost entirely, in the insoluble fraction (compare total lignin yield in the insoluble fraction with the original wood lignin content of 26.2%). In fact, under the severe process conditions (c), some carbohydrate degradation products analyse as Klason lignin, resulting in an erroneously high apparent lignin yield.

In order to better understand the mode of solubilization of carbohydrate during the steam explosion process, the oligosaccharide components in the water soluble fractions were analysed. The soluble fractions from treatments (a), (b) and (c) were separated into neutral and acidic carbohydrate fractions on Dowex 1-X8 anion exchange resin. Yields based on % soluble material are given in Table 2.

**TABLE 1**

Component Yields for the Water Insoluble and Soluble Fractions from Steam Exploded P. radiata.

| Steam explosion conditions | Insoluble fraction | | | | | Soluble fraction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Component Yields (g/100 g o.d. wood) | | | | | Component Yields (g/100 g o.d. wood) | | | | | |
| | total dry matter | total[a] lignin | glc[b] | xyl[c] | man[f] | glc[b] | xyl[c] | gal[d] | ara[e] | man[f] | total soluble sugars |
| (a) 215°, 3 min, 0% $SO_2$. | 76.8 | 27.0 | 44.3 | 2.5 | 4.0 | 2.0 | 3.0 | 1.8 | 0.9 | 6.8 | 14.5 |
| (b) 215°, 3 min, 2.5% $SO_2$. | 60.1 | 27.6 | 33.9 | - | - | 12.2 | 4.2 | 3.1 | 1.2 | 8.7 | 29.5 |
| (c) 248°, 3 min, 2.5% $SO_2$. | 38.7 | 35.8 | 2.7 | - | - | 18.0 | 1.2 | 1.1 | 0.7 | 3.8 | 24.7 |

[a]Klason plus acid-soluble lignin, [b]glc = glucose, [c]xyl = xylose, [d]gal = galactose, [e]ara = arabinose, [f]man = mannose (all sugar yields expressed as free monomeric sugars, after acid hydrolysis and correction for hydrolysis loss)

**TABLE 2**

Yields of Soluble Neutral and Acidic Carbohydrates from Steam Exploded Pinus radiata.

| Steam explosion conditions | yield (% of total dry matter in the soluble fraction) | | |
|---|---|---|---|
| | neutrals | acidics | losses |
| (a) 215°, 3 min, 0% $SO_2$. | 92.9 | 8.7 | -1.6 |
| (b) 215°, 3 min, 2.5% $SO_2$. | 88.9 | 5.5 | 5.6 |
| (c) 248°, 3 min, 2.5% $SO_2$. | 80.7 | 12.8 | 6.5 |



Analysis of the Neutral Soluble Oligosaccharides

The neutral products were fractionated into oligomers by gel permeation chromatography (g.p.c.) on Bio Gel P-2. The g.p.c. chromatograms are shown in Figure 1. The yields and carbohydrate compositions of the g.p.c. oligomeric fractions are given in Table 3. Steam explosion in the absence of sulphur dioxide, (conditions (a)) resulted in substantial extraction of hemicelluloses with little or no cellulose removal. The neutral products were mainly monosaccharides (16%), oligomers d.p. ≥10 (32%), and a spectrum of saccharides ranging in d.p. from 2 to 9. Carbohydrate analysis of these collected g.p.c. fractions revealed them to be partial hydrolysis products from galactoglucomannan and xylan. The xylan oligomers were relatively evenly distributed throughout the molecular weight fractions, although with the highest amount in the monomeric fraction I. The galactoglucomannan hydrolysis products, on the other hand, were mainly present in the higher molecular weight

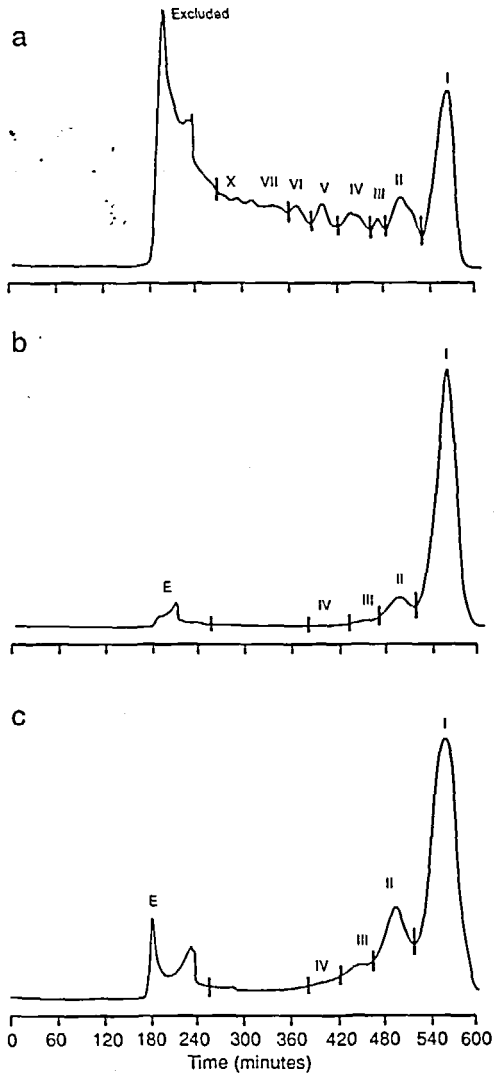



**FIGURE 1** : Bio Gel P-2 chromatograms of soluble neutral oligosaccharides from steam exploded P. radiata under treatment conditions : (a) 215°, 3 min, 0% $SO_2$; (b) 215°, 3 min, 2.5% $SO_2$ and (c) 248°, 3 min, 2.5% $SO_2$.

**TABLE 3**

Yields and Carbohydrate Compositions for the Sub-fractions Obtained by g.p.c. Separation of the Neutral Oligosaccharides in the Water Soluble Fraction from Steam-Exploded P. radiata.

| Steam explosion conditions | g.p.c. Fraction No. | Yield (% of total dry matter in the soluble fraction) | Carbohydrate Composition of g.p.c. fractions (%). | | | | |
|---|---|---|---|---|---|---|---|
| | | | glc[a] | xyl[b] | gal[c] | ara[d] | man[e] |
| (a) | I | 16.3 | 4.1 | 33.0 | 19.4 | 27.9 | 15.7 |
| 215°, | II | 6.1 | 9.7 | 41.7 | 12.8 | 4.9 | 30.9 |
| 3 min, | III | 4.0 | 7.1 | 54.3 | 10.3 | 3.4 | 24.8 |
| 0% SO2 | IV | 4.2 | 9.2 | 36.8 | 15.7 | - | 38.3 |
| | V | 5.4 | 13.3 | 11.0 | 13.4 | 5.4 | 56.8 |
| | VI | 4.9 | 10.0 | 19.1 | 13.1 | - | 57.8 |
| | VII-X | 24.7 | 14.5 | 8.5 | 12.7 | 3.6 | 60.6 |
| | E | 31.8 | 19.9 | 4.6 | 11.0 | 1.9 | 62.6 |
| (b) | I | 75.6 | 43.7 | 12.9 | 9.5 | 4.9 | 29.0 |
| 215°, | II | 10.7 | 47.1 | 10.9 | 10.3 | 5.1 | 26.5 |
| 3 min, | III | 1.6 | 50.5 | 8.7 | 8.3 | 5.5 | 27.0 |
| 2.5% SO2 | IV | 0.3 | 47.3 | 9.2 | 8.8 | 6.2 | 28.5 |
| | E | 0.5 | 26.4 | 9.1 | 10.2 | 18.5 | 35.8 |
| (c) | I | 54.1 | 76.0 | 5.9 | 4.7 | 2.2 | 11.2 |
| 248°, | II | 11.9 | 67.6 | 6.7 | 7.7 | 5.5 | 12.5 |
| 3 min, | III | 4.8 | 66.5 | 6.2 | 7.5 | 4.9 | 14.9 |
| 2.5% SO2 | IV | 2.9 | 62.9 | 5.6 | 6.0 | 7.0 | 18.4 |
| | E | 7.0 | 59.1 | 5.9 | 9.3 | 7.1 | 18.6 |

[a]glc = glucose, [b]xyl = xylose, [c]gal = galactose, [d]ara = arabinose, and [e]man = mannose.

fractions, especially fractions VII-X and the excluded fraction E (d.p. ≥10).

Steam explosion at the standard conditions (b), in the presence of 2.5% sulphur dioxide, removed all of the hemicelluloses and 25% of the cellulose as already described. The major neutral products were monosaccharides (75%), disaccharides (11%), and minor amounts of oligosaccharides of d.p. 3, 4 and greater than 10, being also present. Monosaccharide analysis of these collected g.p.c. fractions showed that they contained cellulose, galactoglucomannan and xylan hydrolysis products. The high monosaccharide content of the soluble material confirms that sulphur dioxide is an excellent catalyst for hemicellulose and cellulose removal[5,6,23]. However, a subsequent hydrolysis step will be necessary to convert the oligomeric sugars to monomers, to maximise the fermentable sugar yield.



High temperature treatment (248°) in the presence of sulphur dioxide (conditions (c)) caused total removal of the hemicelluloses and also 94% solubilisation of cellulose from the wood substrate. The neutral products obtained were mainly monosaccharides (54%) and disaccharides (12%). Minor amounts of oligosaccharides d.p. ≥10, trisaccharides and tetrasaccharides were also present in the hydrolysate. Monosaccharide analysis of the g.p.c. oligosaccharide fractions gave conclusive evidence that extensive cellulose solubilization and hydrolysis had occurred, and showed that approximately 60% of the products came from cellulose. The large amount of disaccharides present, under these harsh acidic conditions, strongly suggests the presence of acid reversion products[16,17].

Acid reversion products had previously been detected in the soluble fraction obtained under the standard steam explosion conditions[10]. However, only some of the products were

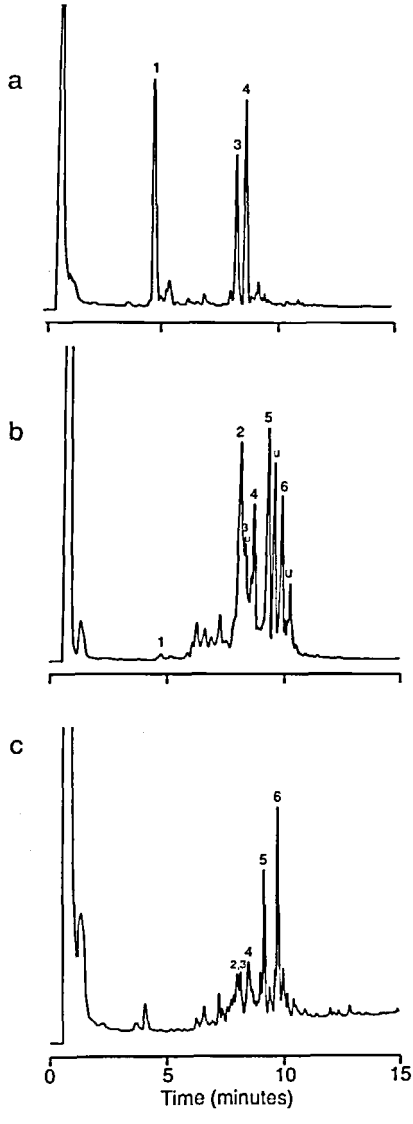
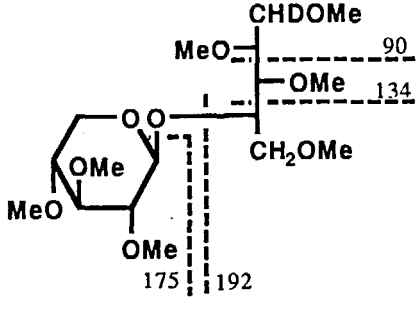



**FIGURE 2** : G.l.c. chromatograms of the neutral disaccharide fractions (as their methylated disaccharide alditols) from steam exploded P. radiata under treatment conditions (a) 215°, 3 min, 0% $SO_2$, (b) 215°, 3 min, 2.5% $SO_2$, and (c) 248°, 3 min, 2.5% $SO_2$.

(1) (1→4)-β-D-xylobiose, (2) cellobiose, (3) 4-O-β-D-glucosyl-D-mannose, (4) (1→4)-β-D-mannobiose, (5) isomaltose, (6) gentiobiose and (u) unknown.

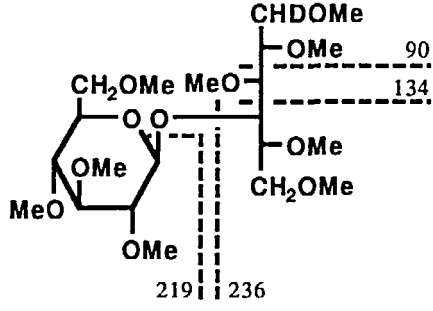
identified and their yields, and the effects of process conditions on their formation, were not determined. Since these reversion products, typically (1,→6) linked disaccharides, are undesirable, especially as they are not utilized in fermentation, the neutral disaccharides from the three treatment conditions were further examined. The disaccharide mixtures from treatments (a), (b) and (c) were analysed by g.l.c.-m.s. as their per-methylated disaccharide alditols, Figure 2. Some of the disaccharides were identified by g.l.c.-m.s. (e.i. and ammonia c.i.) and spiking with authentic samples.

The mass spectra of the methylated disaccharide alditols were used to distinguish between pentosyl-pentitol and hexosyl-hexitol disaccharides, and (1→4) and (1→6) linkages. The c.i.-m.s. of methylated (1→4)-β-D-xylobiose alditol (1) gave $(M+1)^+$and $(M+18)^+$ ions at m/z 384 and 401 respectively, and show that this disaccharide contained one pentosyl and one pentitol residue. The e.i.-m.s. ion at m/z 175 identifies the pentosyl (1→?) sequence. The three other e.i.-m.s. ions at m/z 192, 134 and 90 show that the pentitol residue was substituted through position 4, as shown in the following structure.



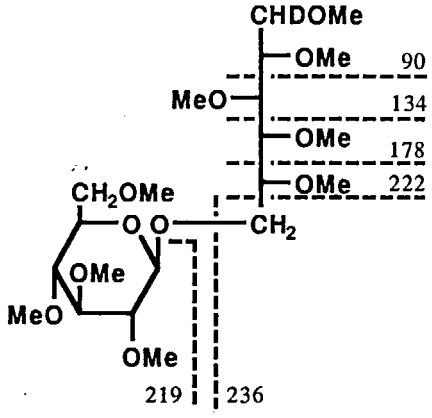

(1)

The c.i.-m.s. of the methylated disaccharide alditols of cellobiose (2), (1→4)-β-D-mannosyl-D-glucose (3) and (1→4)-β-D-mannobiose (4) were identical. The ion at m/z 472 and 489 corresponded to $(M+1)^+$ and $(M+18)^+$ respectively from a methylated disaccharide containing one hexosyl and one hexitol residue. The e.i.-m.s. ion at m/z 219 identifies the hexosyl (1→?) sequence. The other e.i.-m.s. ions at m/z 236, 134 and 90 show that the hexitol residue was substituted through position 4, as shown in the following structure.




(2)

The c.i.-m.s. of the methylated disaccharide alditols of isomaltose (5) and gentiobiose (6) were identical. The ions at m/z 472 and 489 correspond to $(M+1)^+$ and $(M+18)^+$ respectively. The e.i.-m.s. ion at m/z 219 identifies the hexosyl (1→?) sequence. The following e.i.-m.s. ions at m/z 236, 222, 178, 134 and 90 demonstrated the hexitol residue was substituted through position 6, as shown in the following structure.




(5)



Steam explosion using conditions (a), released three disaccharides (1→4)-β-D-xylobiose (1), 4-0-β-D-glucosyl-D-mannose (3) and (1→4)-β-D-mannobiose (4). The presence of these products shows that with no sulphur dioxide addition, only hemicellulose hydrolysis occurs on steam explosion (Figure 2a). Using the standard steam explosion conditions (b), significant wood hydrolysis had occurred, producing a multitude of disaccharides. Some of these disaccharides were identified as: (1→4)-β-D-xylobiose (1), cellobiose (2), 4-0-β-D-glucosyl-D-mannose (3), (1→4)-β-D-mannobiose (4), isomaltose (5), and gentiobiose (6). The first four disaccharides originate from hemicellulose and cellulose hydrolysis. The latter two disaccharides (isomaltose and gentiobiose) were formed by acid reversion reactions of D-glucose in the hydrolysate. The other disaccharides present, which were presumably acid reversion products, were not positively identified (Figure 2b). Reverse phase h.p.l.c. using an amino column was also used to separate the individual disaccharides in order to identify the unknown products by methylation analysis (as per methylated alditol acetate derivatives) and g.l.c.-m.s. Only one of the unknowns

was isolated and tentitively identified as 6-0-D-mannosyl-D-glucose, confirming it as a reversion product although the configuration of the glycosidic linkage could not be determined unequivocally. The total amount of the reversion products was estimated to be from 2.6-4.0% of the total soluble carbohydrates.

The more severe steam explosion conditions (c) gave a predominance of isomaltose and gentiobiose over other disaccharides (Figure 2c), representing about 5% of the total soluble sugars. Since the amount of reversion products was always less than 5% of the total soluble carbohydrate, the effect of these undesirable reactions on fermentable sugar yield is relatively small. Certainly, the beneficial effects of sulphur dioxide catalysis, in terms of improved total sugar yields from a combined steam-explosion, enzymatic hydrolysis process, far outweigh the effects of the reversion reactions.



Analysis of the Soluble Acidic Oligosaccharides

The acidic fractions from each treatment were quantitatively analysed by anionic exchange chromatography[18,19,20], on a column containing Aminex A-25 resin and preparatively on a column containing Dowex 1-X8 resin, eluted with acetate buffer. The chromatograms are shown in Figure 3. The identity of the acids were determined by chromatographic comparison with reference substances[18,19,20] and $^{13}$C-n.m.r. spectroscopy. Yields of the oligomers as a percentage of total dry matter in the soluble fraction are given in Table 4.

The identity of the acids were confirmed by $^{13}$C-n.m.r. spectroscopy. The carbon chemical shift assignments of 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylose (7), 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylobiose (8), 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylotriose (9), D-glucuronic acid (10) and 4-0-methyl-D-glucuronic acid (11) are given in Table 5.

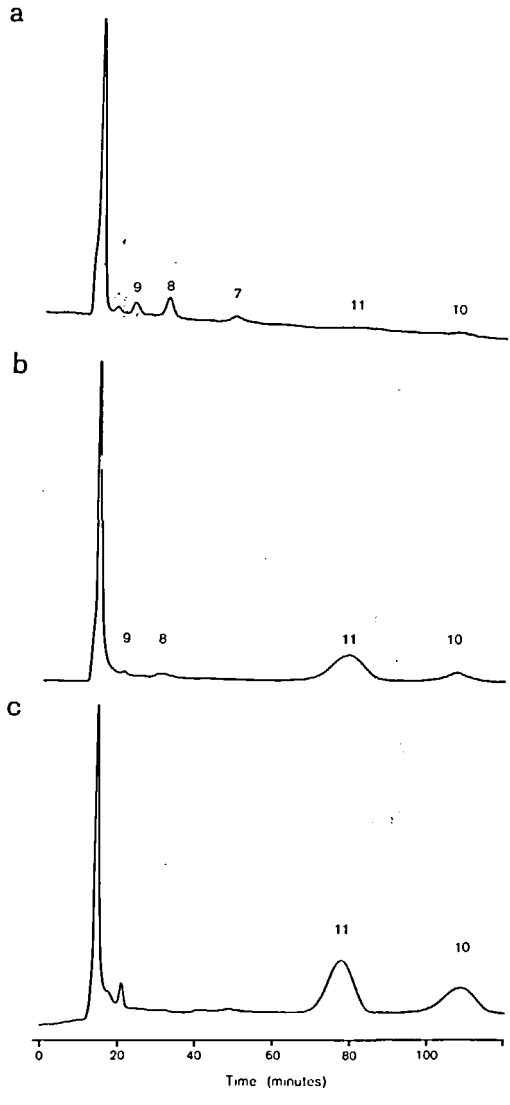



**FIGURE 3** : Anion exchange chromatograms of soluble acidic oligosaccharides from steam exploded P. radiata under treatment conditions (a) 215°, 3 min, 0% $SO_2$, (b) 215°, 3 min, 2.5% $SO_2$ and (c) 248°, 3 min, 2.5% $SO_2$.
(7) 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylose, (8) 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylobiose, (9) 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylotriose, (10) D-glucuronic acid, and (11) 4-0-methyl-D-glucuronic acid.

**TABLE 4**

Yields (%) of Uronic Acid Oligomers in the Water Soluble Fraction from Steam Exploded P. radiata.

| Steam explosion conditions | yield of uronic acid oligomers in the water soluble fraction (% of total dry matter in the soluble fraction) | | | | | | |
|---|---|---|---|---|---|---|---|
| | glcA (10) | 4OMeglcA (11) | 4OMeglcA[X] (7) | 4OMeglcA[X]$_2$ (8) | 4OMeglcA[X]$_3$ (9) | 4OMeglcA[X]$_4$ | O4MeglcA[X]$\geq_5$ |
| (a) 215°, 3 min, 0% $SO_2$. | - | - | 0.32 | 0.70 | 0.36 | 0.24 | 7.06 |
| (b) 215°, 3 min, 2.5% $SO_2$. | 0.50 | 1.60 | - | 0.11 | 0.02 | - | 3.27 |
| (c) 248°, 3 min, 2.5% $SO_2$ | 0.50 | 6.43 | - | - | 0.29 | - | 5.57 |

glcA = D-glucuronic acid, 4OMeglcA = 4-0-methyl-D-glucuronic acid, X = D-xylose

**TABLE 5**

$^{13}$C-N.M.R. Chemical Shifts (p.p.m.[a]) of Uronic Acid Oligomers

| Compound | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6O$ | Me |
|---|---|---|---|---|---|---|---|---|
| **glcA** (10) | β | 96.6 | 75.0 | 75.8 | 74.2 | 71.8 | 172.9 | |
| | α | 92.9 | 71.6 | 72.4 | 72.0 | 71.0 | 173.8 | |
| **4-0-Me-glcA** (11) | β | 96.5 | 74.3 | 75.5 | 82.1 | 74.3 | 174.4 | 60.6 |
| (pH 4) | α | 92.7 | 71.6 | 72.6 | 82.0 | 70.0 | 173.7 | |
| **aldobiouronic acid** (7) | | | | | | | | |
| 4-0-Me-glcA | β | 97.4 | | | | | | |
| | α | 97.3 | 71.5 | 72.7 | 82.2 | 70.0 | 174.3 | 60.6 |
| xylose (r) | β | 98.3 | 79.2 | 74.8 | 70.0 | 65.5 | | |
| | α | 90.2 | 77.0 | 71.7 | 70.2 | 61.3 | | |
| **aldotriouronic acid** (8) | | | | | | | | |
| 4-0-Me-glcA | | 98.1 | 71.5 | 72.8 | 82.7 | 69.9 | 173.9 | 60.4 |
| xylose (c) | | 102.1 | 77.0 | 74.8 | 69.9 | 65.7 | | |
| xylose (r) | β | 97.0 | 74.7 | 74.7 | 76.6 | 63.4 | | |
| | α | 92.5 | 71.8 | 71.7 | 76.7 | 59.2 | | |
| **aldotetraouronic acid** (9) | | | | | | | | |
| 4-0-Me-D-glcA | | 98.1 | 71.8 | 72.8 | 82.9 | 69.9 | 174.0 | 60.5 |
| xylose (c) | | 102.2 | 77.0 | 74.9 | 69.9 | 65.5 | | |
| xylose (r) | β | 98.0 | 74.5 | 74.5 | 76.6 | - | | |
| | α | 92.4 | 71.8 | 71.8 | 76.6 | 59.2 | | |

[a] chemical shifts are given relative to acetone ($CH_3$, 30.8 p.p.m.)
glc A = D-glucuronic acid, (r) = reducing end, (c) = centre residues.

Compounds 7, 8, 9 and 10 were assigned and identified from known spectral assignments of these compounds[24-27]. Assignments of the carbon resonances of 4-0-methyl-D-glucuronic acid were based on known assignments for D-glucuronic acid[24], 4-0-methyl glucose[28] and 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylobiose[24].

Steam exploded P. radiata in the absence of sulphur dioxide at 215° for 3 min (conditions (a)) gave the following acidic sugars: 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylose (7), 2-0-α(4-0-methyl-D-glucuronic acid)-D-xylobiose (8), 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylotriose (9), 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylotetraose and higher aldooligouronic acids, with trace amounts of D-glucuronic acid (10) and 4-0-methyl-D-glucuronic acid (11) . The higher aldooligouronic acids (d.p. ≥6) were the predominant products, representing 7% of the total water soluble material. Steam explosion in the presence of 2.5% sulphur dioxide, conditions (b) and (c) produced substantial amounts of d.p. ≥6 aldooligouronic acids (3.3-5.6%), 4-0-methyl-D-glucuronic acid (1.6-6.4%) and D-glucuronic acid (0.5%) with minor amounts of 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylobiose and 2-0-α-(4-0-methyl-D-glucuronic acid)-D-xylotriose in the water soluble acidic fraction.

In the absence of sulphur dioxide the acidic hydrolysis products were typically aldooligouronic acids of d.p. 2 and greater. Addition of sulphur dioxide changed the acidity of the wood media to low pH, resulting in more effective hydrolysis of the xylan to 4-0-methyl-D-glucuronic acid and to its constituent neutral monosaccharides. Interesting, also the presence of D-glucuronic acid was observed in the hydrolysate. This appears to be an artefact of the steam explosion process (with sulphur dioxide addition). This causes demethylation of the 4-0-methyl-D-glucuronic acid residue. Roudier[29] has also shown that

demethylation of uronic acid residues occurs during sulphite pulping.

## CONCLUSIONS

Steam explosion of P. radiata in the absence of sulphur dioxide (215°, 3 min) resulted in solubilization of only the hemicelluloses (14.5g of neutral sugars/100g o.d. wood) yielding oligomers (d.p. 1 to ≥12) with no cellulose attack. Addition of sulphur dioxide (2.5%) to the process facilitated the solubilization and hydrolysis of the hemicelluloses and about 25% of the cellulose (29.5g of neutral sugars/100g o.d. wood) yielding mainly mono- and disaccharides. Temperature elevation (248°) and sulphur dioxide (2.5%) resulted in almost complete hemicellulose and cellulose solubilization, and subsequent degradation reflected in poor carbohydrate survival (24.7g of neutral sugars/100g o.d. wood) during the process, to form mainly mono- and disaccharides. The acidic hydrolysis products were a mixture of xylooligouronic acids from d.p. 2 to ≥6, which were more resistant to hydrolysis than the neutral oligomers, even in the presence of sulphur dioxide. Under the standard steam explosion conditions the acidic products account for only about 6% of the total soluble carbohydrate. Interestingly, some demethylation of 4-O-methyl-D-glucuronic acid residues was observed as a consequence of sulphur dioxide catalysis. The major benefit of sulphur dioxide addition to the steam explosion of P. radiata is to acidify the wood and thus promote hydrolysis of the polysaccharides to monosaccharides. However, acid reversion products are formed (typically (1→6) linked disaccharides) although their concentration relative to total soluble carbohydrate, is very low. Therefore, the effect on fermentable sugar yield of these undesirable reversion reactions is insignificant.

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