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Characterisation of water-soluble galactoglucomannans from Norway spruce wood and thermomechanical pulp

S. Willför[a,*], R. Sjöholm[a], C. Laine[b], M. Roslund[c], J. Hemming[a], B. Holmbom[a]

[a]*Process Chemistry Group, Laboratory of Forest Products Chemistry, Åbo Akademi University, Porthansgatan 3, FIN-20500 Turku/Åbo, Finland*
[b]*KCL, P.O. Box 70, FIN-02151, Espoo, Finland*
[c]*Department of Organic Chemistry, Åbo Akademi University, Biskopsgatan 8, FIN-20500 Turku/Åbo, Finland*

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Abstract

Different fractions of water-soluble polysaccharides, all composed mainly of acetyl-galactoglucomannans, from native Norway spruce wood and thermomechanical pulp were isolated and characterised. About 65% of the mannose units in the glucomannan backbone were acetylated at either the C-2 or C-3 positions in a ratio of 2.2:1.0. Mainly the mannose units, but also some glucose units, were also partly substituted at C-6 by galactopyranose units. The number of galactose side groups at C-6 was considerably lower for acetyl-galactoglucomannans from TMP than from wood. The molar sugar unit ratios of the different fractions of dissolved acetyl-galactoglucomannans differed only slightly. Acetyl-galactoglucomannans dissolved at room temperature, as well as at 90 °C and during prolonged treatment time, contained a large proportion of mannose units. Acidic arabinogalactans were also dissolved at room temperature, while some xylans, pectins, and (1 → 5)-bonded arabinans were dissolved at higher temperatures and longer treatment times. Also the water-soluble xylans contained acetyl groups, about 0.6 per xylose unit, all attached to C-3.

Keywords: Hemicelluloses; Acetyl groups; Galactoglucomannans; Xylans; Arabinans; ^{13}C NMR; ^{1}H NMR; Methylation; Methanolysis; Gas chromatography; Apparent molar mass; Norwegian spruce; Wood; Thermomechanical pulp

1. Introduction

In the Nordic countries, mechanical pulp is predominantly produced from Norway spruce (*Picea abies*). Water-soluble polysaccharides are released and accumulate into process waters in the production of mechanical pulps and wood-containing papers. The dissolution of polysaccharides from Norway spruce wood and mechanical pulp has been reported in several publications (e.g. Örså, Holmbom, & Thornton, 1997; Thornton, Ekman, Holmbom, & Örså, 1994a; Thornton, Ekman, Holmbom, & Petterson, 1994b; Willför, 2000; Willför & Holmbom, 2002; Willför, Sjöholm, Laine, & Holmbom, 2002). The main dissolved spruce polysaccharides are acetyl-galactoglucomannans. There are two main types of acetyl-galactoglucomannans in softwoods, one rich and the second poor in galactose (Sjöström, 1993). The main chain is believed to be linear or possibly slightly branched and consists of β-D-(1 → 4)-glucopyranose and β-D-(1 → 4)-mannopyranose units. α-D-galactopyranose is bonded as single-unit side chains to the main chain by (1 → 6)-bonds. An important structural feature is the hydroxyl groups at C-2 and C-3 positions in the main chain units being partially substituted by *O*-acetyl groups, on the average one group per 3–4 hexose units. Acetyl-galactoglucomannans isolated from spruce by microwave oven treatment (Lundqvist, Teleman, Junel, Zacchi, Dahlman, Tjerneld, & Stalbrand, 2002) or isolated from spruce wood holocellulose (Capek, Alföldi, & Lišková, 2002) were recently shown to be partially substituted at the C-2 and C-3 positions on the β-D-(1 → 4)-mannopyranose units and not on the β-D-(1 → 4)-glucopyranose units. So far, no detailed and comparative structural studies have been presented on different fractions of water-soluble acetyl-galactoglucomannans from native Norway spruce wood and mechanical pulp.

In this study, three different fractions of water-soluble polysaccharides, composed of mainly acetyl-galactoglucomannans, in native Norwegian spruce wood and four

* Corresponding author. Tel.: +358-2-215-4729; fax: +358-2-215-4868.
E-mail address: stefan.willfor@abo.fi (S. Willför).

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different fractions from thermomechanical pulp (TMP) were isolated and characterised. The main structural features of the polysaccharides were determined and compared.

2. Materials and methods

2.1. Wood and pulp material

Two healthy Norway spruce trees, grown in southern Finland, were felled in May and stem cross sections at 1.5 m height were sawn out and stored at −24 °C. The number of growth rings was 48 and 56, respectively, while the number of heartwood growth rings was about 20 and 25, respectively. A knot-free sector with no visible reaction wood was cut from the two sampled cross sections and the pith and the two outermost growth rings were removed (Fig. 1). The wood sectors were splintered, freeze-dried, and ground in a Cyclo-Tec mill (Tecator Inc.) producing particles passing a 30-mesh screen. The resulting wood meals were blended to give a composite sample.

TMP, with a consistency of about 40%, was obtained from a mill in Finland using two-stage refining of Norway spruce. The TMP was stored at −24 °C before freeze-drying. The freeze-dried TMP sample (TMP) and the composite wood meal (Wood) were extracted in a Soxhlet apparatus with acetone (p.a. grade) to remove lipophilic materials.

2.2. Isolation of water-soluble polysaccharide fractions from wood meal and TMP

Distilled water was added to 88 g (o.d.) of acetone-extracted composite wood meal or TMP to a total weight of 3000 g (Fig. 2). The suspension was stirred vigorously at 23 ± 2 °C for 3 h. The wood suspension was stirred with a Vibro-mixer (Chemap AG), while the TMP suspension needed stirring with a blade propeller. The pH was measured, but not adjusted, at the beginning and the end of the stirring. The suspension was vacuum-filtered on a paper machine wire (<400 mesh) and the resulting fibre pad was washed with about 120 ml of distilled water. The washing water was added to the supernatant. The supernatant was vacuum-filtered on a GF 52 glass fibre filter (Schleicher and Schuell) and then concentrated to about one litre by vacuum evaporation in a water bath set at 45 °C. The concentrate was filtered on a 0.2 μm pore size Polycap™ 75AS filter (Whatman) to remove colloidal substances. A small volume of the filtered concentrate was removed for carbohydrate analysis. The supernatant was further concentrated to 300 ml by vacuum evaporation in a water bath set at 45 °C. The concentrated supernatant was then added to technical grade ethanol, the volume percentage of ethanol being at least 90, and the polysaccharides were allowed to precipitate overnight in a cold room. The samples were centrifuged at 500 g for 25 min. The precipitated polysaccharides were collected and washed twice with ethanol, twice with methanol and once with methyl *tert*-butyl ether (MTBE). The precipitates, here called Wood (25 °C, 3 h) and TMP (25 °C, 3 h), were finally dried in a vacuum drier and weighed.

Distilled water was added to the filtered fibres to a total weight of 3000 g (Fig. 2). The suspension was then stirred vigorously for 1 h using a glycol bath set at 90 °C. The polysaccharides were then isolated as described above. The precipitates are here called Wood (90 °C, 1 h) and TMP (90 °C, 1 h).

Distilled water was again added to the filtered fibres to a total weight of 3000 g (Fig. 2). The suspension was then stirred vigorously for 12 h using a glycol bath set at 90 °C. The polysaccharides were then isolated as described above. The precipitates are here called Wood (90 °C, 12 h) and TMP (90 °C, 12 h).

2.3. Isolation of 'standard' TMP acetyl-galactoglucomannans

Two different batches of standard TMP acetyl-galactoglucomannans were prepared (Fig. 3). Extracted TMP (hexane/acetone 9:1) from Norway spruce was suspended in distilled water at 2% consistency (Sundberg, Holmbom, Willför, & Pranovich, 2000a). The suspension was stirred with a blade propeller at about 200 min^{-1} and 60 °C for 3 h, after which the suspension was filtered on a paper machine wire. The TMP was again suspended in distilled water, stirred as above, and filtered. The pH in the suspensions was about 5.5. The filtrates from the first and second stirrings were mixed and centrifuged at 500 g for 30 min. The

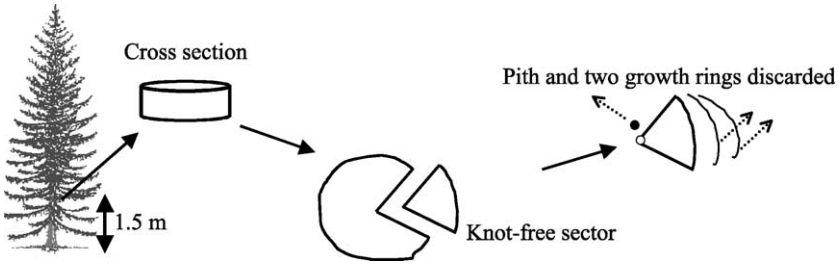

Fig. 1. A knot-free sector, with no visible reaction wood, was cut from the cross section of the trees, and the pith and the two outermost growth rings were removed.

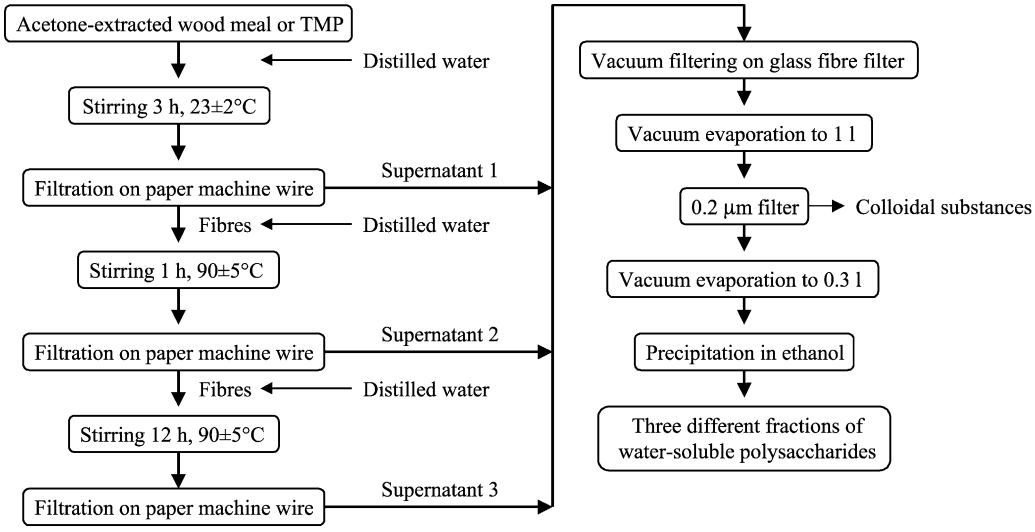

Fig. 2. Scheme for sequential extraction and isolation of water-soluble polysaccharide fractions from ground wood and TMP.

supernatant was collected and concentrated by vacuum evaporation in a water bath set at 40 °C. The concentrate was filtered on a PolycapTM 75AS filter obtained from Whatman with a 0.2 μm pore size to remove colloidal substances. Ethanol was added to the filtrate, the volume percentage of ethanol being at least 80. The polysaccharides were allowed to precipitate. The precipitated polysaccharides were collected and washed twice with ethanol, twice with methanol and once with MTBE. The precipitates, mainly composed of acetyl-galactoglucomannans and here called GM1 (60 °C, 3 h) and GM2 (60 °C, 3 h), were finally dried in a vacuum drier.

2.4. Water solubility of wood and pulp

The freeze-dried TMP and Wood samples were also treated according to TAPPI T207 cm-99, as a reference to the other methods. The TAPPI cold water solubility (CWS) was determined so that 2 g of the TMP and Wood samples were extracted with distilled water at 23 ± 2 °C for 48 h, after which the suspensions were vacuum-filtered on a GF 52 glass fibre filter. The starting pH was 5.9 for the Wood and 5.4 for the TMP sample. An additional syringe filtering with a 0.2 μm nylon 25 ml filter unit was performed to resemble the treatment during the isolation procedure above. The resulting supernatants are here called Wood CWS and TMP CWS. The TAPPI hot water solubility (HWS) was determined so that 2 g of the TMP and Wood samples were extracted with distilled water under reflux for 3 h using a glycol bath set at 100 °C. The starting pH was the same as for the CWS. The suspensions were filtered as above and the resulting supernatants are here called Wood HWS and TMP HWS. In contrast to the TAPPI method the carbohydrate sugar composition was determined as below.

2.5. Total carbohydrate sugar composition

The total sugar composition of the isolated polysaccharides and the sugar composition and amount of the dissolved carbohydrates, including monosaccharides, oligosaccharides, and polysaccharides, were analysed by acid methanolysis followed by silylation and gas chromatography (GC) of the silylated sugar monomers, according to Sundberg, Sundberg, Lillandt, and Holmbom (1996).

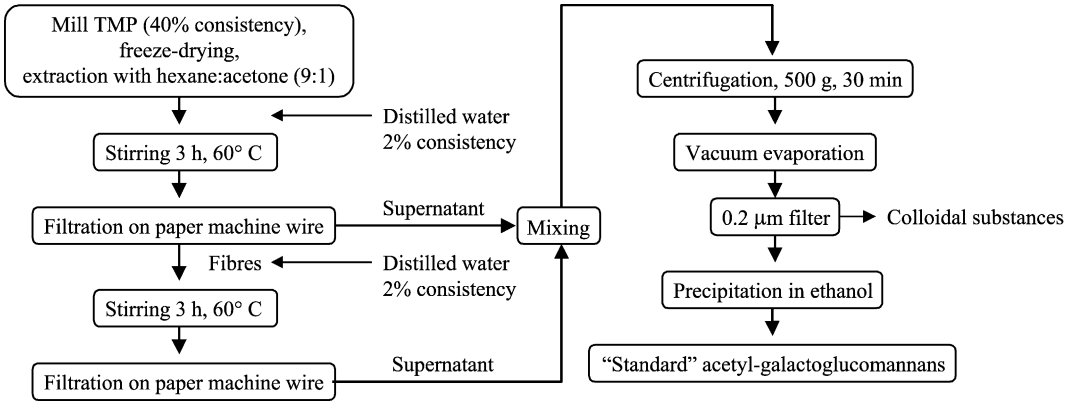

Fig. 3. Scheme for isolation of standard Norway spruce TMP acetyl-galactoglucomannans.

Free monosaccharides in the water samples were determined by GC after direct silylation.

2.6. Methylation analysis

Methylation analysis was performed using a modification of the method of Ciucanu and Kerek (Ciucanu & Kerek, 1984; Laine, Tamminen, Vikkula, & Vuorinen, 2002; Willför et al., 2002). About 20 mg ground sodium hydroxide and 100 μl methyl iodide were added to 3–5 mg sample in 500 μl dimethylsulfoxide (DMSO). The sample was kept for 30 min in an ultrasonic bath at room temperature. Distilled water was added to the sample and the water phase was extracted with dichloromethane (DCM). The organic phase was extracted three times with distilled water, dried and evaporated. After methylation, acid methanolysis was performed as described by Sundberg et al. (1996). After that the samples were silylated with 250 μl *N*,*O*-bis(trimethylsilyl)trifluoroacetamide containing 5% trimethylchlorosilane and the samples were analysed by GC/MS (Laine et al., 2002; Willför et al., 2002).

2.7. ^{1}H NMR and ^{13}C NMR spectroscopic analyses

The dried precipitates were dissolved in D_2O after which the solutions were frozen and freeze-dried. This treatment was repeated four times. TSP (sodium 3-trimetylsilyl-propionate-2,2,3,3-D(4)) and acetone were added as internal standards ($\delta = 0.0$ and 33.2 ppm, respectively). ^{1}H and ^{13}C NMR spectra were recorded at 323 K with a JEOL JNM-A 500 NMR spectrometer at 500 and 125 MHz, respectively. For quantitative ^{13}C NMR spectroscopy an inverse-gated pulse sequence, which suppressed the nOe enhancement, was used. The pulse angle was 45° and the pulse interval ca. 11 s. These parameters facilitated the use of the signal intensities for the determination of the relative amounts of different types of carbon atoms in the samples. Thus, a rough estimation of the relative amounts of the units building up the polysaccharides could be made.

2.8. Apparent molar mass

High-performance size-exclusion chromatography (HPSEC) was used to determine the apparent molar masses of the isolated polysaccharides. The following system was used: Waters Linear Ultrahydrogel Column, Waters guard column, and a differential refractometric detector (Shimadzu Corp., Japan). The eluent used was 0.2 M NaCl in distilled water, with a flow rate of 0.3 ml/min. The samples were filtered through a 0.2 μm filter before injection. Dextran standards were used for apparent molar mass calibration.

3. Results and discussion

3.1. Dissolved carbohydrates from spruce wood and TMP

The starting pH was 5.9 ± 0.2 in all suspensions, except for TMP (23 °C, 3 h) where it was 5.2, and no pH adjustment was performed. A small measured drop of about 0.5 pH units after stirring was probably due to other effects such as matrix effects of the fibre suspensions rather than an actual drop in the pH. For the Wood (90 °C, 12 h) and the TMP (90 °C, 12 h) samples a clear drop in pH of about 1.5 units was observed. This was probably due to the prolonged treatment time at elevated temperature causing hydrolysis of some ester bonds and the release of small amounts of acetic acid (Thornton, Eckerman, & Ekman, 1991; Thornton et al., 1994a). The mild pH conditions (slightly acidic) ensured a minimum degree of hydrolysis and deacetylation of the released polysaccharides. Analysis of the released free monosaccharides confirmed that most carbohydrates were present as oligo- or polysaccharides in all samples.

The wood meal released about 0.4% (w/w) of oligo- and polysaccharides on treatment at room temperature (Table 1), which corresponds to earlier findings (Willför & Holmbom, 2002). The TMP (23 °C, 3 h) sample was not analysed for the total dissolved amount of carbohydrates. It is however clear that the released amount was much higher than for the wood sample. The longer treatment time used in the TAPPI standard method released only slightly more oligo- and polysaccharides. We can therefore also assume that the amount of carbohydrates released from the TMP sample was about 1% (w/w). The sugar composition, as determined by acid methanolysis and GC, revealed that the main oligo- and polysaccharides released were galactoglucomannans together with some acidic arabinogalactans and small amounts of other carbohydrates, which also agrees with earlier findings (Willför & Holmbom, 2002).

The treatment at elevated temperature (90 °C) for 1 h released about 0.2% (w/w) of a very pure fraction of galactoglucomannans (Table 1). The continued treatment for 12 h at elevated temperature released additional galactoglucomannans, some pectins, and xylans. The total amount released was between 0.4 and 0.5% (w/w). The amount of oligomeric arabinose units was surprisingly high. Örså et al. (1997) reported similar results but did not try to explain the origin of these non-monomeric arabinose units. It is possible that spruce wood contains, in addition to the cold-water-soluble arabinogalactans, high-molar-mass arabinogalactans that are not so easily accessible for water extraction of lignified fibres. It has also been suggested that spruce wood may contain small amounts of arabinans (Willför & Holmbom, 2002). Over 80% of the total amount released in the TAPPI HWS method was released during the first two extraction steps in our experiments. Especially the second extraction step gave galactoglucomannans of higher purity than the TAPPI method.

Table 1
Total amount of sugar units and sugar composition expressed in mol%, determined by acid methanolysis and GC, of the dissolved oligosaccharides and polysaccharides

	Total amount (mg/g dry fibre)	Man (mol%)	Glc (mol%)	Gal (mol%)	Ara (mol%)	Xyl (mol%)	Rha (mol%)	GlcA (mol%)	GalA (mol%)	4-*O*-MeGlcA (mol%)
Wood (23 °C, 3 h)	3.8	43	19	20	6	4	<1	4	3	1
Wood (90 °C, 1 h)	2.4	65	17	9	2	2	<1	<1	3	<1
Wood (90 °C, 12 h)	4.7	45	10	8	14	8	2	1	10	2
TMP (23 °C, 3 h)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TMP (90 °C, 1 h)	2.4	64	19	8	2	2	<1	<1	4	1
TMP (90 °C, 12 h)	4.1	46	7	7	15	9	2	1	10	2
Wood CWS	4.1	49	19	19	5	<1	<1	3	3	1
Wood HWS	6.9	57	20	15	3	<1	<1	2	2	<1
TMP CWS	10.0	50	17	14	9	3	<1	2	4	1
TMP HWS	13.5	55	18	14	4	2	<1	2	4	<1

n.d. = not determined.

The presence of arabinogalactans, starches, arabinans and xylans in the dissolved polysaccharides of the (23 °C, 3 h) and (90 °C, 12 h) samples makes an estimation of an average molar ratio of the galactoglucomannans, based on the acid methanolysis and GC analysis, difficult. For the Wood (90 °C, 1 h) and the TMP (90 °C, 1 h) dissolved galactoglucomannans it is possible to estimate an average ratio for Man/Glc/Gal of about 3.8:1:0.4 and 3.4:1:0.4, respectively. It is also possible to estimate an average ratio for Man/Glc of about 4.5:1 and 6.6:1 of the Wood (90 °C, 12 h) and the TMP (90 °C, 12 h) dissolved galactoglucomannans. The estimated ratios are an average of the total dissolved oligo- and polysaccharides released from the fibres.

3.2. Total sugar composition of the isolated polysaccharides from spruce wood and TMP

The Wood and TMP (23 °C, 3 h) precipitates were recovered, after freeze-drying, as light-white-brownish powders. The Wood and TMP (90 °C, 1 h) precipitates were recovered as pure white powders, while the (90 °C, 12 h) samples were dark-white-brownish powders. Especially the precipitates from the 12-h experiments contained some non-carbohydrate impurities, probably mainly lignin-derived compounds, which explains the gravimetrically determined amounts of precipitate (Table 2) being higher than the amounts of dissolved carbohydrates (Table 1). Two different batches of standard TMP acetyl-galactoglucomannans were also prepared. Such galactoglucomannans have been used in previous studies (Hannuksela & Holmbom, 2001; Sihvonen, Sundberg, Sundberg, & Holmbom, 1998; Sundberg et al., 2000a; Sundberg, Holmbom, Willför, & Pranovich, 2000b; Willför et al., 2002), and it was therefore of interest to compare such galactoglucomannans to the different isolated fractions. The precipitates, GM1 (60 °C, 3 h) and GM2 (60 °C, 3 h), were recovered as pure white powders.

The total sugar composition, as determined by acid methanolysis and GC, showed that the purest galactoglucomannan fractions were the two (90 °C, 1 h) precipitates (Table 2). We can assume that all of the mannose, glucose, and almost all of the galactose units analysed can be assigned to galactoglucomannans in these two fractions,

Table 2
Gravimetrically determined total amount and sugar composition expressed in mol%, determined by acid methanolysis and GC, of the isolated polysaccharides. The gravimetric value also contains small amounts of co-isolated impurities such as lignin

	Isolated amount (mg/g dry fibre)	Man (mol%)	Glc (mol%)	Gal (mol%)	Ara (mol%)	Xyl (mol%)	Rha (mol%)	GlcA (mol%)	GalA (mol%)	4-*O*-MeGlcA (mol%)
Wood (23 °C, 3 h)	3.5	50	11	21	6	4	<1	4	2	1
Wood (90 °C, 1 h)	2.3	68	16	9	1	3	<1	<1	3	<1
Wood (90 °C, 12 h)	5.0	54	12	9	4	8	1	<1	11	1
TMP (23 °C, 3 h)	10.0	61	15	15	3	2	<1	2	3	<1
TMP (90 °C, 1 h)	2.5	66	18	7	1	3	<1	<1	3	<1
TMP (90 °C, 12 h)	4.3	54	8	7	6	10	1	<1	12	1
GM1 (60 °C, 3 h)	–	63	17	13	2	<1	<1	2	3	<1
GM2 (60 °C, 3 h)	–	59	18	15	2	<1	<1	2	2	<1

which gives a galactoglucomannan purity of more than 90%. More than half of the galactose units, and all glucuronic acid units, in the (23 °C, 3 h) samples can be assigned to acidic arabinogalactans, mainly derived from the heartwood portion (Willför & Holmbom, 2002; Willför et al., 2002). A small amount of the glucose in the Wood (23 °C, 3 h) sample is probably also derived from starches or other easily soluble glucans. The galactoglucomannan portion was about 60% in the Wood and about 80% in the TMP sample. Some partial hydrolysis occurred during the 12 h treatment time at 90 °C for the Wood and TMP samples. The amount of galacturonic acid and xylose has clearly increased, indicating the release of pectins and xylans. The relative amount of non-monomeric arabinose units is lower than it was before precipitation, indicating the loss of some oligomeric compounds, but still higher than what can be explained by the xylans alone. It is most likely that some kind of arabinogalactans and arabinans occurred also in these samples. The galactoglucomannan purity was about 70% in the Wood and 65% in the TMP sample. The galactoglucomannan purity of the standard samples was about 85%, with some acidic arabinogalactans as the main impurity.

A rough estimation of an average molar ratio of the isolated galactoglucomannans, based on the acid methanolysis and GC analysis, is possible since the average molar ratio of isolated arabinogalactans is known (Willför et al., 2002). The ratio was close to 4.5:1:0.5 for the Wood samples, while the variation was larger for the TMP samples (Table 3). The relatively high portion of galactose in the Wood and TMP (23 °C, 3 h) galactoglucomannans promotes the water-solubility, since the introduction of branches, or side-groups, into polysaccharide backbones results in changes in the solution properties. The ratio for the two standard galactoglucomannans (3.3–3.7:1:0.6–0.7) differed slightly from what has earlier been reported (4:1:0.5–1.1) (Thornton et al., 1994a,b; Sundberg et al., 2000a,b).

Table 3
Average molar sugar unit ratios of the isolated galactoglucomannans determined with the different analytical techniques

	Man/Glc/Gal		
	Acid methanolysis and GC	Methylation analysis	^{13}C NMR
Wood (23 °C, 3 h)	4.5:1:1.0	4.8:1:0.4	n.d.
Wood (90 °C, 1 h)	4.3:1:0.5	3.4:1:0.3	4.0:1:0.3
Wood (90 °C, 12 h)	4.5:1:0.6	3.8:1:0.3	n.d.
TMP (23 °C, 3 h)	4.1:1:0.8	3.3:1:0.3	n.d.
TMP (90 °C, 1 h)	3.7:1:0.4	3.0:1:0.1	n.d.
TMP (90 °C, 12 h)	6.8:1:0.5	3.7:1:0.2	n.d.
GM1 (60 °C, 3 h)	3.7:1:0.6	n.d.	n.d.
GM2 (60 °C, 3 h)	3.3:1:0.7	n.d.	n.d.

n.d. = not determined.

3.3. Apparent molar mass of the isolated polysaccharides from spruce wood and TMP

The apparent molar mass and corresponding degree of polymerisation (DP) of the isolated polysaccharides are presented in Table 4. The absolute molar mass can unfortunately not be determined using HPSEC with dextran standards for calibration, since these have slightly different structure, and therefore also different hydrodynamic volumes, compared to the isolated polysaccharides. The molar masses of the isolated polysaccharides from the Wood samples are smaller than from the TMP samples (Table 4). This can be explained by the external and internal fibrillation caused by the TMP process, which facilitates the dissolution of larger molecules. The molar masses of the (23 °C, 3 h) samples are smaller than the ones of the (90 °C, 1 h) samples. Smaller molecules and polysaccharides in the outer layers of the cell wall, and also some extracellular polysaccharides, are naturally easily accessible for cold-water extraction. The molar masses of the (90 °C, 12 h) samples are the smallest because of the prolonged treatment time at elevated temperature causing hydrolysis and cleavage of larger molecules in the cell wall. This was also seen as an increase in the amount of monosaccharides present before isolation of the polysaccharides. It is probable that the same phenomenon has occurred during the isolation of the standard galactoglucomannans.

Sjöström (1990) reported molar masses of 56–60 kDa (DP 310–330) for dissolved (50 °C, 1.5 h dissolution time) neutral polysaccharides from Norway spruce stone groundwood. Pranovich (1995) reported molar masses for fractionated standard galactoglucomannans isolated from Norway spruce TMP. The total amount of dissolved polysaccharides was divided into a high molar mass (HMM) and a medium molar mass (MMM) fraction using a 50 nm membrane filter. The MMM fraction, constituting 75% of the total amount of dissolved polysaccharides, had a molar mass of about 21 kDa (DP 130). The HMM fraction, constituting 25% of

Table 4
Apparent molar masses of the isolated polysaccharides. The apparent molar mass range was between 4 and 300 kDa

	Apparent molar mass[a] (kDa)	Average degree of polymerisation (DP)[a]
Wood (23 °C, 3 h)	33	200
Wood (90 °C, 1 h)	48	300
Wood (90 °C, 12 h)	29	180
TMP (23 °C, 3 h)	55	340
TMP (90 °C, 1 h)	64	400
TMP (90 °C, 12 h)	45	280
GM1 (60 °C, 3 h)	41	250
GM2 (60 °C, 3 h)	45	280

[a] Range of all samples: 4–300 kDa, DP 25–1850.

the total amount of dissolved polysaccharides, had a molar mass of about 59 kDa (DP 360). The apparent molar masses reported by both Pranovich (1995) and Sjöström (1990) are well in agreement with the present results (Table 4).

In the literature the DP given for hemicelluloses in general is under 200 and for softwood glucomannans between 70 and 250 (e.g. Capek et al., 2000, 2002; Croon & Lindberg, 1958; Katz, 1965; Sjöström, 1993; Timell, 1965). These DP/s have however been determined on polysaccharides isolated from wood holocellulose. During the delignification necessary to obtain holocellulose there will always occur depolymerisation to some extent (Browning, 1967; Timell, 1965). The galactoglucomannans have also usually been extracted from the holocellulose using alkaline solutions, which causes not only deacetylation, but also depolymerisation to some extent.

3.4. Methylation analysis of isolated polysaccharides from wood

The results from the methylation analysis (Table 5) supported the main conclusions drawn from the sugar composition determined by acid methanolysis and GC (Table 2). In addition to the galactoglucomannans, the Wood (23 °C, 3 h) sample contained some arabinogalactans (Table 5). The arabinogalactans show some solubility problems in the alkaline solvent (NaOH–DMSO) used during methylation (Willför et al., 2002). Consequently, the results may not be representative for the whole arabinogalactan fraction or the total polysaccharides of the sample. The solubility of the galactoglucomannans in DMSO was good. The Wood (90 °C, 1 h) sample contained the purest galactoglucomannans, while the Wood (90 °C, 12 h) sample also contained some xylans as impurities. It is notable that the Wood (90 °C, 1 h) galactoglucomannans contained the lowest relative amount of mannose. The methylation analysis did not give any information about acidic groups, which were not identified in the gas chromatograms. Especially the Wood (90 °C, 12 h) sample contained some (1 → 5)-bonded arabinose units that can be assigned to pure arabinans. Arabinans with (1 → 5)-bonded arabinose units have earlier been reported in maritime pine (Roudier, 1964), southern pine kraft pulp (Minor, 1983), radiata pine high temperature TMP (McDonald, Clare, & Meder, 1999), Norway spruce kraft pulp (Laine, Haakana, Hortling, & Tamminen, 1999) and Scots pine and silver/pubescent birch kraft pulps (Laine and Tamminen, 2001). Some of the (1 → 5)-bonded arabinans appear to be linked to lignin in so-called lignin–carbohydrate complexes (LCC).

All samples also contained two structural units that could not be identified, even though the library used contained a wide choice of possible structural units (Laine et al., 2002). The structural unit 'Unknown 1' (Table 5) had a mass spectrum similar to 2,4,6-bonded glucose, but the retention time was rather close to that of 1,4,6-bonded hexose units. It is possible that it was a 1,2,6-bonded hexose unit. The structural unit 'Unknown 2' might have been a 1,3,6-bonded hexose unit other than galactose or glucose. These two unidentified structural units could not be assigned to any specific type of polysaccharides based on the methylation data available.

The average sugar unit ratios for the galactoglucomannans differed slightly from those obtained by acid methanolysis and GC (Table 3). The galactose content was higher for the acid methanolysis and GC method of the Wood (23 °C, 3 h) sample. The difference can be due to an overestimation of the methanolysis results or due to the solubility problem of the arabinogalactan-rich sample in the methylation analysis. For the two other wood samples the glucose content was higher as determined by the methylation analysis. It is interesting to notice that the ratio of 1,4-bonded to 1,4,6-bonded units was between 11 and 13 for mannose and between 10 and 11 for glucose. This indicates that glucose was slightly more often the branching point than mannose, taking the ratio of glucose and mannose into account. Approximately every 12th unit was branched and the non-reducing galactose end units corresponded approximately to the branching units in all samples. However, it is also possible that there might be a small amount of other sugar units or side-chains attached to the branching points

Table 5
Sugar composition, expressed in mol%, as determined by methylation analysis

	Man (mol%)			Glc (mol%)			Gal (mol%)		Ara (mol%)		Xyl (mol%)				Unknown 1[a]	Unknown 2[a]
	T[b]	1,4	1,4,6	T[b]	1,4	1,4,6	T[b]	1,3,6	T[b]	1,5	T[b]	1,4	1,2,4	1,3,4	?	?
Wood (23 °C, 3 h)	1.0	58.5	4.6		12.3	1.1	5.2	6.8	2.3			2.7			3.3	2.1
Wood (90 °C, 1 h)	1.4	56.7	5.3	0.3	16.5	1.6	5.0	0.9	0.3	0.1		1.4	0.1	0.1	5.3	4.9
Wood (90 °C, 12 h)	1.2	52.6	4.3	0.3	13.7	1.3	4.1	1.0	1.0	1.4	2.1	6.7	0.8	0.8	5.0	3.9
TMP (23 °C, 3 h)	1.1	53.4	5.2	0.3	16.1	1.6	4.8	1.6	0.8	0.3		0.5			6.9	7.5
TMP (90 °C, 1 h)	1.3	58.7	2.7	0.4	19.6	0.9	2.5	1.4	0.4	0.2	0.6	1.5			5.3	4.3
TMP (90 °C, 12 h)	1.1	52.7	3.1	0.3	14.1	0.9	3.1	1.1	1.4	2.8	1.4	7.9	0.9	0.9	4.8	3.5

[a] Unidentified structural units.
[b] T = terminal non-reducing end unit.

in the main chain of the galactoglucomannans. Literature data are somewhat vague, but several authors have shown the presence of both 2,3-di-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-glucose units after methylation of softwood galactoglucomannans (e.g. Aspinall & Wood, 1963; Brasch, 1983; Capek et al., 2000; Lindberg, Rosell, & Svensson, 1973; Timell, 1965). A general conclusion has been that the non-reducing galactose end units are bonded to both mannose and glucose units in the main chain. Brasch (1983) and Brasch and Wilkins (1985) suggested the same after ^{13}C NMR analysis of radiata pine galactoglucomannans. However, the ^{13}C NMR analysis in this work did not show the presence of any signals that could be assigned to galactose units bonded to glucose units. This may have been due to the relatively small amount of 1,4,6-bonded glucose units present in the galactoglucomannans (Table 5). More mannose than glucose units occurred as non-reducing end groups, but exact quantitative interpretation should not be made due to the small amounts determined.

3.5. Methylation analysis of isolated polysaccharides from TMP

The TMP (23 °C, 3 h) sample contained, in addition to the galactoglucomannans, lower amounts of arabinogalactans and xylans and much more of the two unidentified structural units, than the Wood (23 °C, 3 h) sample (Table 5). The TMP (90 °C, 1 h) sample contained slightly more xylans, while the xylan content in the TMP (90 °C, 12 h) sample was almost 10%. The TMP (90 °C, 12 h) sample also contained twice the amount of arabinans as the Wood (90 °C, 12 h) sample. The fibrillation caused by the TMP process and the partial hydrolysis occurring during the treatment can promote the dissolution of LCC, which in this case is corroborated by the presence of arabinans and by the dark-white-brownish colour of the precipitate.

As for the Wood samples, the average sugar unit ratios for the galactoglucomannans differed slightly from those obtained by acid methanolysis and GC (Table 3). The methylation analysis gave a lower ratio of galactose and mannose, especially in the TMP (90 °C, 12 h) sample, than the methanolysis and GC analysis. The explanation for the difference is probably the same as for the wood samples. Again the (90 °C, 1 h) galactoglucomannans contained the lowest relative amount of mannose. The ratio of 1,4-bonded to 1,4,6-bonded units was about the same for mannose and glucose in all TMP samples. Compared to the Wood samples, the degree of branching was about the same for the TMP (23 °C, 3 h) sample, while it was much lower for the TMP (90 °C, 1 h) and TMP (90 °C, 12 h) samples. Every 22nd and 17th unit, respectively, was branched in these samples. Why the degree of branching is lower for the TMP sample is not clear. A possible explanation is the thermal treatment and fibrillation caused by the TMP process facilitating the release of a slightly different galactoglucomannan from, for example, the secondary cell wall. It is also possible that the TMP process causes a loss in the amount of side-chains in the galactoglucomannans. Another explanation, although less likely, is that the TMP was not produced from similar trees as the tree sample, and there can be small differences between different trees.

3.6. NMR studies of isolated polysaccharides from wood and TMP

The six fractions of isolated polysaccharides were studied by ^{13}C NMR spectroscopy. Quantitative calculations were done only for the Wood (90 °C, 1 h) sample, which also was additionally studied by ^{1}H NMR spectroscopy. The partial ^{13}C NMR spectra displaying the ring carbon signals are shown in Fig. 4 and the signals of the acetyl carbons in Fig. 5. The ^{1}H NMR spectrum is shown in Fig. 6. It was not subjected to detailed interpretation.

The ^{13}C NMR signal assignments were made from a higher resolution (non-quantitative) spectrum shown in Fig. 7 and were based on literature data (Brasch & Wilkins, 1985; Capek et al., 2000; Eda, Akiyama, & Katō, 1984; van Hazendonk, Reinerink, de Waard, & van Dam, 1996). The chemical shifts are shown in Table 6.

The ^{13}C NMR spectra showed signals from several different carbonyl carbons ($\delta = 176.2–175.6$ ppm) as well as methyl carbons assigned to acetyl groups based on their chemical shifts ($\delta = 21.6$ and 21.3 ppm). The signals of the anomeric carbons appeared in a range from $\delta = 107.2$ to 95.2 ppm. The Wood and TMP (90 °C, 12 h) samples also showed signals from carbonyl and methoxyl groups in lignin ($\delta = 173.5$ and 56.0 ppm, respectively), which supports the assumptions of small amounts of lignin impurities in these samples.

The ratio of the basic units identified by ^{13}C NMR spectroscopy was determined by integration of signals of carbon atoms assigned to the different monosaccharides units. The ratio of Man/Glc/Gal/Xyl was about 4.0:1:0.3:0.2 for the Wood (90 °C, 1 h) sample.

β-D-Mannose units and acetyl groups. Several signals of anomeric carbons of both non-reducing ($\delta = 103.0–101.9$ ppm) and reducing units ($\delta = 96.6$ ppm) were detected. The ratio of non-reducing to reducing mannose units was about 26.5:1 for the Wood (90 °C, 1 h) sample. Based on literature data (van Hazendonk et al., 1996) it was concluded that the acetyl groups were bound to C-2 and C-3 in the mannose units. Possibly, part of the acetyl groups were bound to xylose units, as the intensities of the signals of the acetyl groups were somewhat higher than those of the signals assigned to acetylated mannose. This was also supported by the fact that the differences in the signal intensities corresponded well with the amount of xylose units, as determined by acid methanolysis and GC. The native softwood xylans have been considered to be non-acetylated (Fengel & Wegener, 1984; Sjöström,

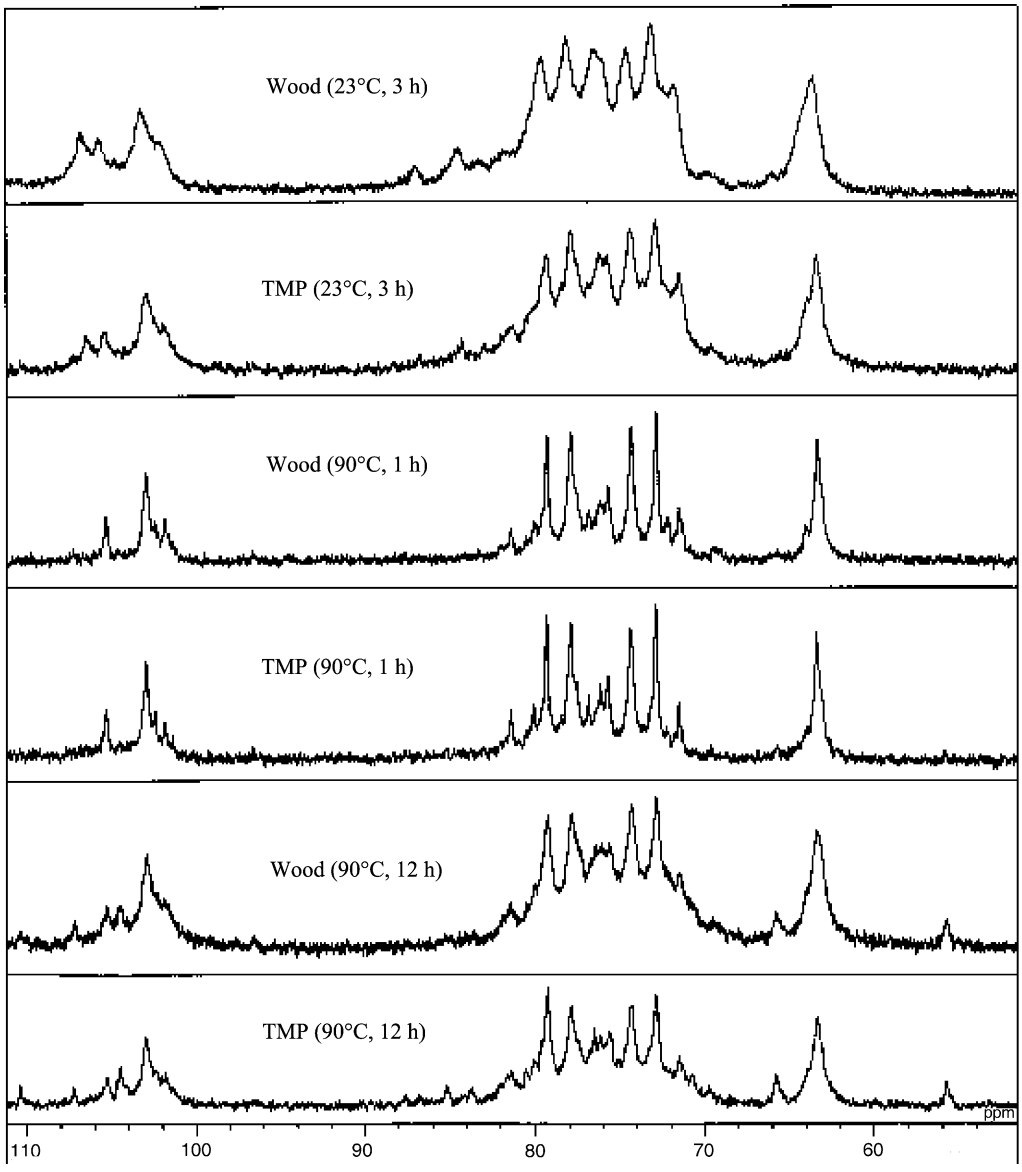

Fig. 4. Partial ^{13}C NMR spectra of the isolated polysaccharides.

1993). At least the water-soluble xylans seem to contain acetyl groups according to our results. The mannose units gave several sets of signals due to the presence of non-acetylated as well as two types of acetylated units. The ratio of Man to *O*-Ac-Man was about 1.9:1 for the Wood (90 °C, 1 h) sample. The ratio of 2-*O*-Ac-Man to 3-*O*-Ac-Man was about 2.2:1. According to Capek et al. (2002), about half of the mannose units in spruce galactoglucomannans are *O*-acetylated with a ratio of 2-*O*-Ac-Man to 3-*O*-Ac-Man of about 1.7:1. Lundqvist et al. (2002) reported that about one third of the mannose units are *O*-acetylated with a ratio of 2-*O*-Ac-Man to 3-*O*-Ac-Man of about 1:1.

β-D-Glucose units. Signals of anomeric carbons of both non-reducing ($\delta = 105.3$ ppm) and reducing units ($\delta = 95.2$ ppm) were detected. The ratio of non-reducing to reducing glucose units was about 7.0:1 for the Wood (90 °C, 1 h) sample. A signal of C-6 in the reducing units is expected at $\delta = 69.5$ ppm. However, it is overlapped by a signal possibly originating from C-6 of a mannose unit bound to 6-*O*-Gal (Brasch & Wilkins, 1985). The signal of C-6 of the non-reducing unit was found at $\delta = 64.0$ ppm.

Galactose units. For the Gal units, signals from the anomeric carbons of α- ($\delta = 107.2$ ppm), as well as, β-units ($\delta = 101.4$ ppm) were detected. The β/α ratio was about 1.2:1.0 for the Wood (90 °C, 1 h) sample. Capek et al. (2000) also suggested the presence of β-glycosidic linkages of D-Gal*p* units in galactoglucomannans from Norway spruce. Recently Capek et al. (2002) and Lundqvist et al. (2002) suggested that only α-Gal*p* units were present in galactoglucomannans from Norway spruce. Enzymatic hydrolysis with α-galactosidase was shown to remove virtually all galactose from the polysaccharides (Lundqvist et al., 2002). On the other hand, Tenkanen, Puls, Rättö, and Viikari (1993) managed to remove only 8%, and even after

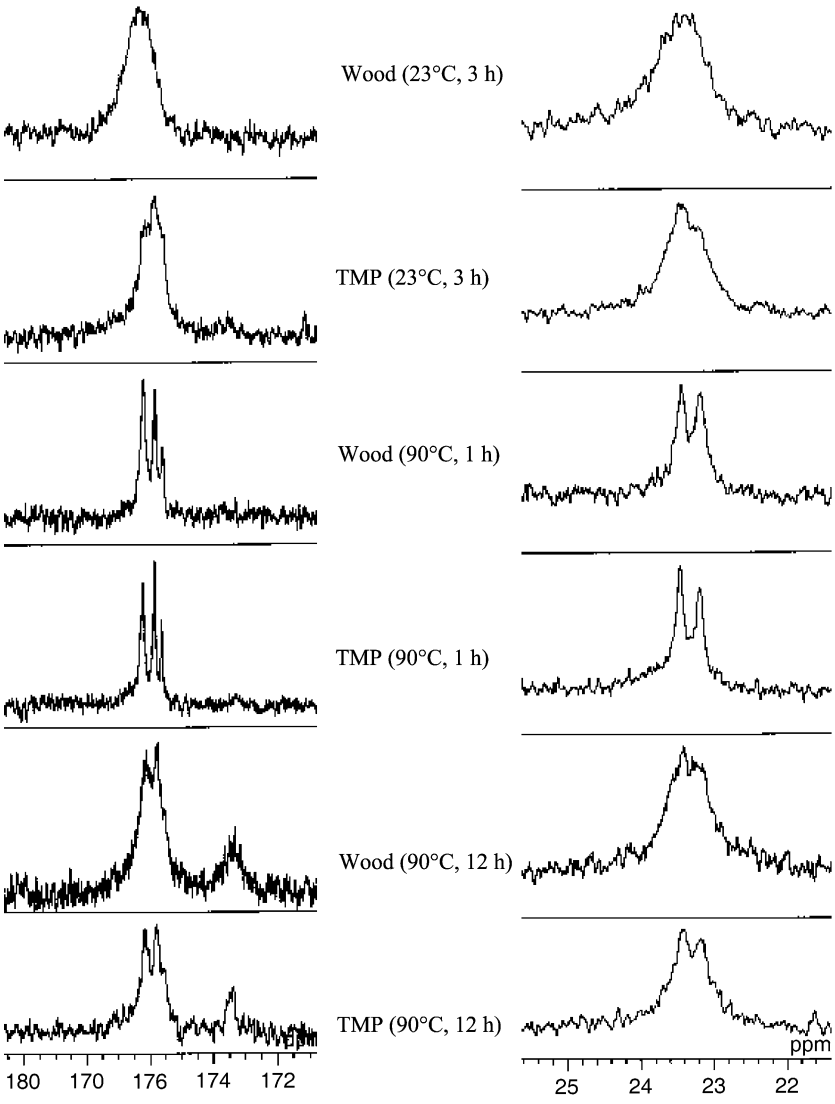

Fig. 5. Partial ^{13}C NMR spectra showing the signals of the carbonyl and methyl carbons of the acetyl groups of the isolated polysaccharides.

addition of an esterase and a β-glucosidase only 31%, of the galactose units from spruce galactoglucomannans using a similar α-galactosidase enzyme. The matter of the glycosidic character of the galactose units in spruce galactoglucomannans should obviously be further investigated.

In the spectral region $\delta = 72.3-71.3$ ppm several signals from Gal C-2, C-3, C-4 and Man C-2 were observed. The signal of C-6 was observed at $\delta = 63.0$ ppm. The intensities of the signals of the anomeric carbons matched that of the signal at 69.5 assigned mainly to C-6 in Man units binding Gal units. This indicates that all Gal units were bound to C-6 in mannose units in the main chain. It also indicates the presence of both α- and β-glycosidic bonds of D-Gal*p* units in galactoglucomannans.

β-D-xylose units. A weak signal at $\delta = 104.5$ ppm was assigned to Xyl C-1. According to van Hazendonk et al. (1996) this signal could also contain the peak from C-1 of 3-*O*-Ac units. Based on the signal intensities the amount of acetyl groups bound to xylose was ca. 0.6 per xylose unit for the Wood (90 °C, 1 h) sample. No specific signals from the acetyl groups could be assigned to the Xyl units. The signals of Xyl C-3, C-4 and C-5 were detected at $\delta = 76.5$, 79.7 and 65.8 ppm, respectively.

4. Conclusion

Acetyl-galactoglucomannans are the main polysaccharides dissolved into water at 23–90 °C from both native Norway spruce wood and TMP. Native spruce wood releases about 1% (w/w) and the more fibrillated TMP releases over 1.5% (w/w) of carbohydrates altogether. The apparent degree of polymerisation was 180–300 for polysaccharides dissolved from spruce wood and 280–400 for polysaccharides dissolved from TMP. The acetyl-galactoglucomannans consist of a backbone of β-D-(1 → 4)-glucopyranose and β-D-(1 → 4)-mannopyranose units. About 45% of the mannose units are naturally

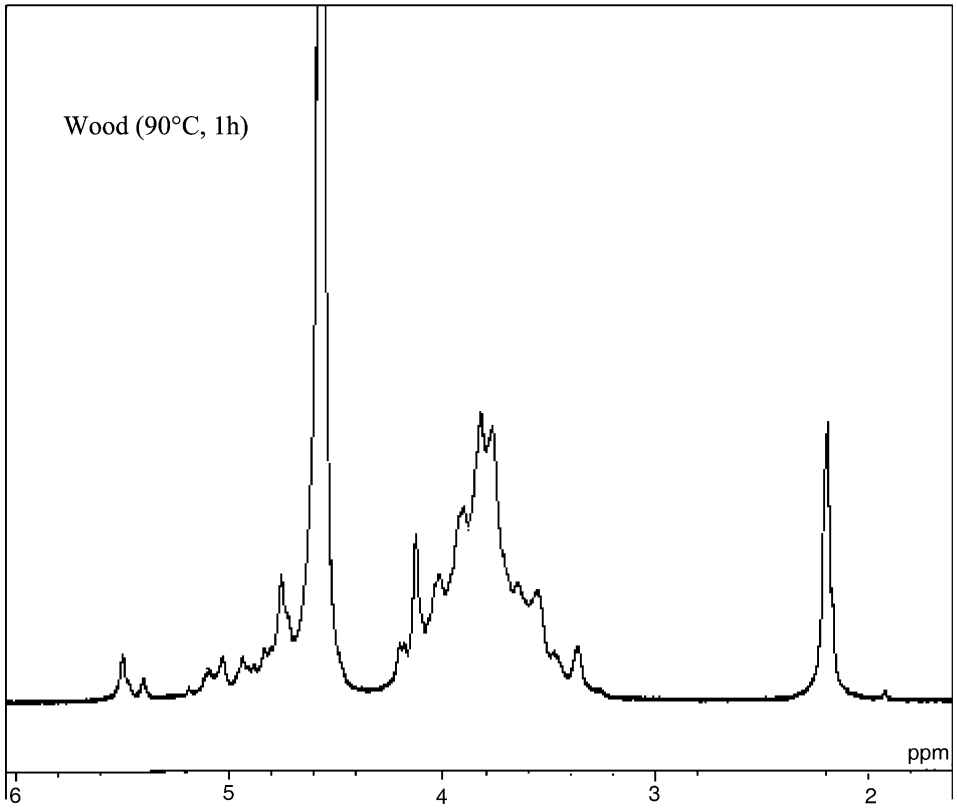

Fig. 6. ^{1}H NMR spectrum of the isolated polysaccharides in the Wood (90 °C, 1 h) sample.

acetylated at C-2 and 20% at C-3, while the glucose units are non-acetylated. Mainly the mannose units, but also some glucose units, in the backbone are also partly substituted at C-6 by galactopyranose units. The number of galactose side groups at C-6 is considerably lower for acetyl-galactoglucomannans from spruce TMP than from wood. The reason for this is not clear. The molar sugar unit ratios of different fractions of dissolved acetyl-galactoglucomannans differ only slightly. Acetyl-galactoglucomannans dissolved at room temperature contain more mannose units than

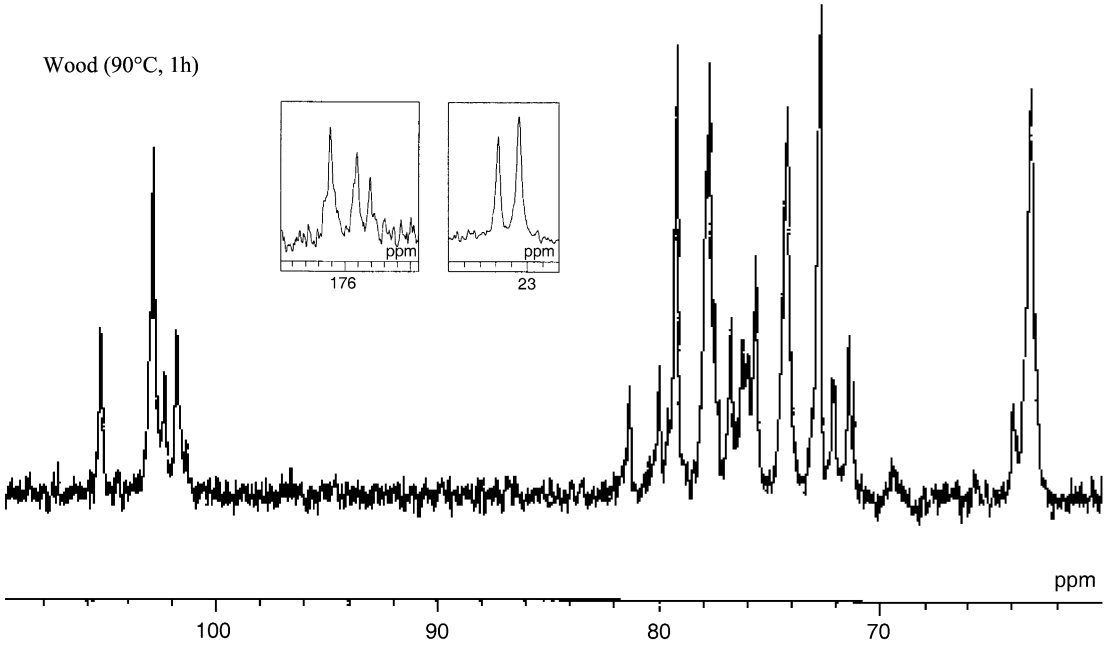

Fig. 7. Partial high-resolution (non-quantitative) ^{13}C NMR spectrum, of the isolated polysaccharides in the Wood (90 °C, 1 h) sample, used for signal assignment.

Table 6
^{13}C NMR chemical shifts of the most significant signals in the spectra of the isolated polysaccharides

Residue	C=0	CH_3	C-1	C-2	C-3	C-4	C-5	C-6
Gal			107.2 (β-), 101.4 (α-)	72.3–71.3	72.3–71.3	72.3–71.3	74.1	63
Glc			105.3, 95.2 (red.)	75.7	77.5	81.4	76.9	69.5 (red.), 64.0 (non-red.)
Man	176.2 (2-Ac), 175.8 (3-Ac)	21.6 (2-Ac), 21.3 (3-Ac)	103.0-101.9, 96.6 (red.)	74.3, 72.8	76.3, 74.3, 72.8, 72.3–71.3	80.8, 79.3, 76.1	78.0, 77.9	69.5, 63.4
Xyl	175.6?	n.d.	104.5	n.d.	76.5	79.7	65.8	

n.d. = not detected.

acetyl-galactoglucomannans dissolved at 90 °C and one hour treatment time. However, the mannose to glucose ratio increases for acetyl-galactoglucomannans dissolved at 90 °C and prolonged treatment time.

Acidic arabinogalactans are also dissolved from both spruce wood and TMP at room temperature. At higher temperature and during longer treatment time some xylans, pectins, and (1 → 5)-bonded arabinans dissolve, mainly due to partial hydrolysis, which renders polysaccharide fragments more easily soluble from the cell walls. The (1 → 5)-bonded arabinans partly occur as lignin-carbohydrate complexes as may also be the case for the xylans. The water-soluble xylans also bear acetyl groups, about 0.6 per xylose unit, all attached to C-3.

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