

# On the occurrence of carbohydrates in milled wood lignin preparations

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**KEYWORDS:** Milled wood lignins, *Betula verrucosa*, Carbohydrates, Nuclear magnetic resonance.

**SUMMARY:** Milled wood lignin with low carbohydrate content has been prepared from birch, using a method based on liquid-liquid extraction. The carbohydrate content of the final product was found to be considerably higher than that of the corresponding preparation from spruce.  $^1\text{H}$  NMR spectral technique for the analysis and characterization of carbohydrates in lignin preparations has been developed.

□ Malvedslignin med låg halt av kolhydrater har framställt från björk med användning av extraktionsteknik. Kolhydratinnehållet i den erhållna produkten visade sig vara betydligt större än i motsvarande preparat från gran. En metod för analys och karakterisering av kolhydrater i ligninpreparat baserad på kärnresonansspektroskopi har utvecklats.

□ Lignin aus Birkenholzmehl mit niederem Gehalt an Kohlenhydraten wurde mittels einer Extraktionstechnik hergestellt. Der Kohlenhydratgehalt des so erhaltenen Produktes war merklich höher als der eines entsprechenden Präparates aus Fichtenholz. Eine Methode zur Analyse und Charakterisierung von Kohlenhydraten in Ligninpräparaten mit Hilfe der Kernresonanzspektroskopie wurde ausgearbeitet.

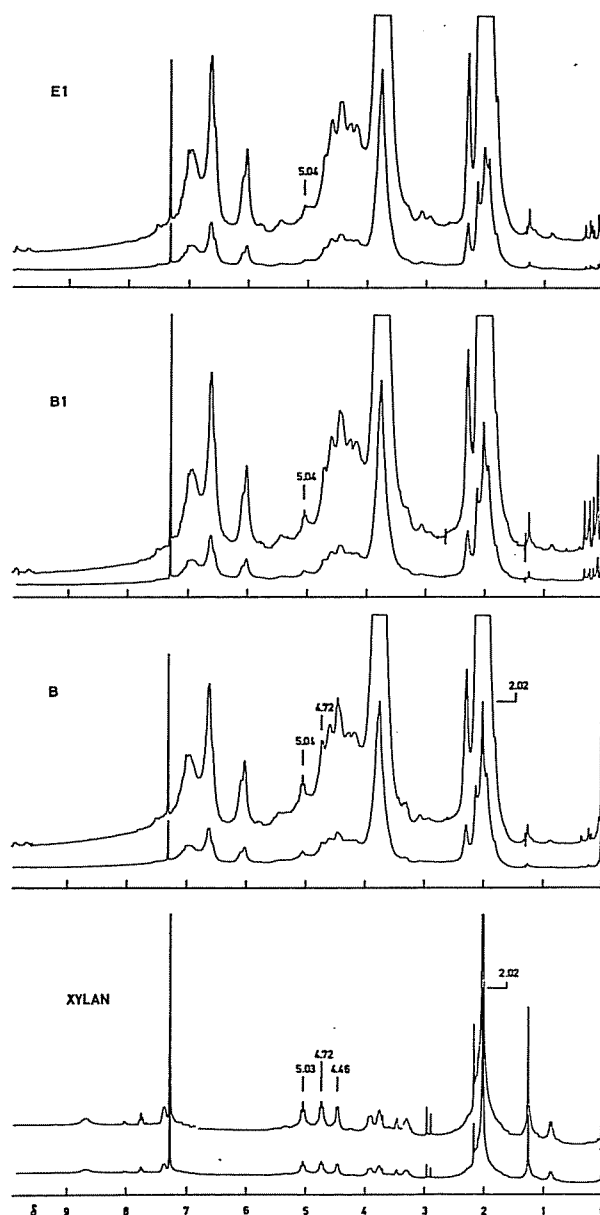
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In previous papers (1, 2), the preparation of milled wood lignin (MWL) from spruce (*Picea abies*) with very low carbohydrate content has been described, where a procedure involving liquid-liquid extraction was used for the isolation of the lignin. The same extraction technique has now been adopted for the preparation of MWL from birch (*Betula verrucosa*) with a small proportion of carbohydrates. However, the carbohydrate content of the birch lignin obtained (3.7%) was considerably higher than that of the corresponding product obtained from spruce [0.05%, (2)]. Similarly, the carbohydrate content in MWL from birch prepared according to Björkman [7.5%, (3)] has been found to be much larger than in the corresponding preparation from spruce [1.5%, (4)]. Several explanations seem possible for the relatively high carbohydrate content in MWL from birch, since MWL preparations from birch and spruce differ distinctly in composition. Spruce lignin (a typical softwood lignin) consists almost entirely of units of the guaiacylpropane type, while birch lignin (a typical hardwood lignin) contains about equal proportions of guaiacylpropane and syringylpropane units. Furthermore, xylose is the predominating constituent of the carbohydrates in MWL of birch, while the carbohydrate moiety in MWL from spruce has a more complex composition.

Methoxyl analyses, examination of carbohydrates by hydrolysis studies, and  $^1\text{H}$  NMR spectroscopy were used for the characterization of birch lignin preparations with respect to carbohydrate content. A corresponding  $^1\text{H}$  NMR study of spruce lignin is included in the present paper.

## Investigation of carbohydrates in MWL preparations

The presence of xylan (xylan fragments attached to lignin, see below) in MWL preparations from birch could



**Fig. 1.**  $^1\text{H}$  NMR spectra of acetylated MWL preparations from birch (cf. table 1) and acetylated birch xylan. Peaks due to contaminants are relatively prominent in the xylan acetate spectrum; this is related to the facts that xylan acetate is difficult to purify and only slightly soluble in chloroform. The spectrum of the acetate of extraction lignin resembled closely that of purified Björkman lignin (B1). Similarly, the spectrum of repeatedly purified Björkman lignin (acetate) resembled that of purified extractive lignin (acetate) (E1).

be very well illustrated by  $^1\text{H}$  NMR spectroscopy. *Fig. 1* shows  $^1\text{H}$  NMR spectra of a series of acetate derivatives of birch lignins and acetylated birch xylan. The occurrence of xylan in the lignin preparations is most clearly demonstrated by the signal at  $\delta = 5.04$ , but the signal at  $\delta = 2.02$  is also indicative. These signals can be

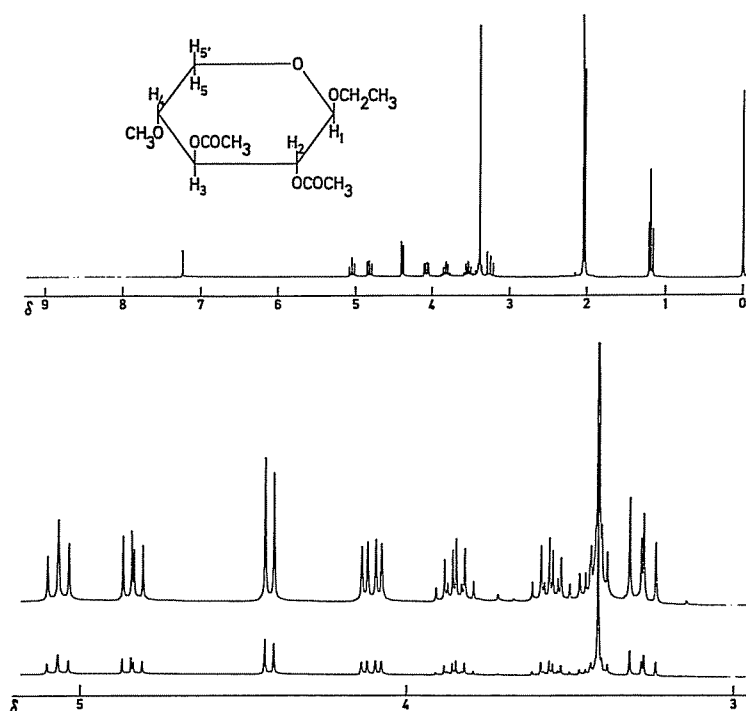


Fig. 2.  $^1\text{H}$  NMR spectrum of ethyl 2,3-di-*O*-acetyl-4-*O*-methyl- $\beta$ -D-xylopyranoside. Partly on the basis of decoupling experiments, the spectrum could be interpreted as follows ( $\delta$  units): 1.19 (3 H, triplet,  $J = 7.0$  Hz;  $\text{CH}_3\text{-CH}_2$ ), 2.04 (3 H, singlet;  $\text{CH}_3\text{CO}$ ), 2.06 (3 H, singlet;  $\text{CH}_3\text{CO}$ ), 3.28 (1 H, doublet of doublets,  $J = 9.6$  and  $11.5$  Hz;  $\text{H}_5$ ), 3.41 (3 H, singlet;  $\text{CH}_3\text{O}$ ), 3.42 (1 H, multiplet;  $\text{H}_4$ ), 3.56 (1 H, doublet of quartets,  $J = 7.0$  and  $9.9$  Hz;  $\text{CH}_3\text{-CH}_2$ ), 3.86 (1 H, doublet of quartets;  $J = 7.0$  and  $9.9$  Hz;  $\text{CH}_2\text{-CH}_3$ ), 4.11 (1 H, doublet of doublets,  $J = 4.9$  and  $11.5$  Hz;  $\text{H}_5$ ), 4.42 (1 H doublet,  $J = 7.3$  Hz;  $\text{H}_1$ ), 4.84 (1 H, doublet of doublets,  $J = 7.3$  and  $9.2$  Hz;  $\text{H}_2$ ), 5.07 (1 H, triplet,  $J = 8.8$  Hz;  $\text{H}_3$ ).

attributed to the proton at the C-3 atom and the protons in the acetyl groups, respectively. The assignments of the xylan acetate signals have been derived from a comparison with the  $^1\text{H}$  NMR spectrum of a model compound, ethyl 2,3-di-*O*-acetyl-4-*O*-methyl- $\beta$ -D-xylopyranoside (fig. 2). A second model compound, methyl 2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranoside, which is representative of end groups in xylan with a free 4-position, was also examined. The signal with the highest  $\delta$  value appeared at  $\delta = 5.17$  and could be attributed to the proton at the C-3 atom. No signal could be detected around this  $\delta$  value in the lignin spectra, and, consequently, no evidence of the occurrence of such end groups was obtained. The protons at the C-1 atoms in the acetylated anomers of D-xylopyranoside gave signals at  $\delta = 6.25$  ( $\alpha$  form) and  $\delta = 5.72$  ( $\beta$  form). The occurrence of a small peak at  $\delta = 5.75$  in the lignin spectra (fig. 1) may therefore reflect the presence of xylose end groups with a free 1-position.

Free carbohydrates should be completely removed by the procedures used for purification of the lignin preparations. Therefore, the  $^1\text{H}$  NMR spectral investigation, together with the fact that xylose is the predominating sugar liberated on hydrolysis, suggests that the carbohydrates in the MWL preparations essentially consist of xylan fragments attached to the lignin.

The differences in xylan content of the MWL preparations examined are apparent from inspections of the spectra (fig. 1). Of course, signals caused by protons in lignin units dominate the spectra. It is of interest to note that the appearance of these signals seems to be very similar in all the lignin preparations examined. This

similarity suggests that no fractionation of the lignin component occurs on purification by the extraction procedure.

Results from methoxyl and carbohydrate analyses are summarized in table 1. A lower carbohydrate content should result in an increased methoxyl content, since the methoxyl groups are a part of the lignin units. (The contribution from methoxyl groups present in 4-*O*-methylglucuronic acid units linked to xylose is assumed to be negligible.) The data given in table 1 are largely in accordance with the results obtained by NMR spectral studies. The carbohydrate content in the extraction lignin was found to be 3.7%. Purification by repeated liquid-liquid extraction lowered the percentage to 2.0%.

Table 1. Methoxyl and carbohydrate contents of MWL preparations from birch.

Sample	$\text{OCH}_3$ , %	Carbohydrate content, % <sup>1</sup>
Extraction lignin (see Exp) (E)	21.1	3.7
Extraction lignin, purified <sup>2</sup> (E1)	21.5	2.0
Björkman lignin (B)	19.9	5.7
Björkman lignin, purified <sup>2</sup> (B1)	20.5	3.8
Björkman lignin, purified twice <sup>2</sup> (B2)	21.2	2.5

<sup>1</sup> Hydrolysis was performed by treatment with 1 M  $\text{H}_2\text{SO}_4$ -dioxane (1:1) at  $100^\circ\text{C}$  for 4h; only very small amounts of monosaccharides other than xylose were found.

<sup>2</sup> Purification was accomplished by liquid-liquid extraction according to the procedure described in (2).

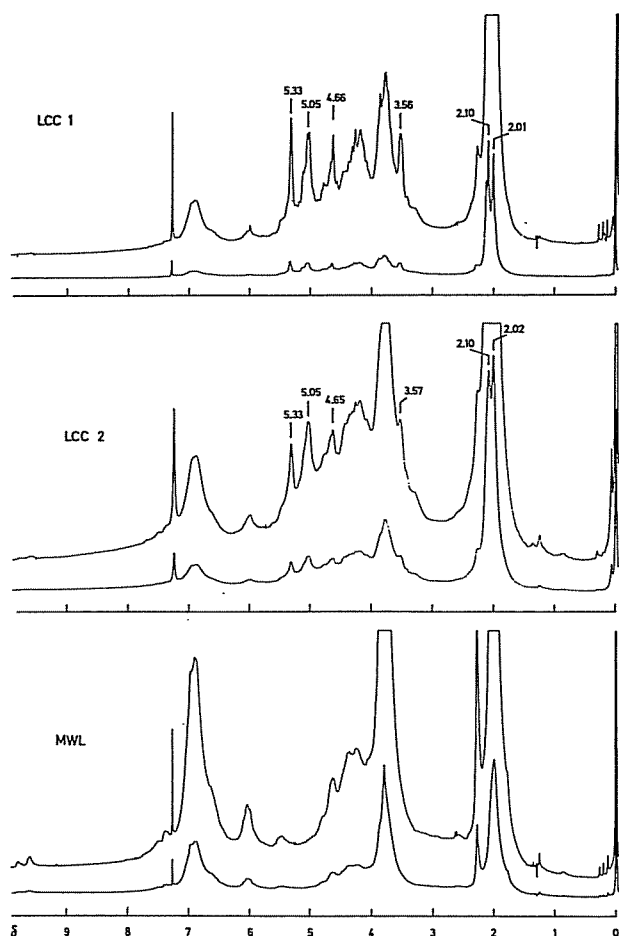


Fig. 3.  $^1\text{H}$  NMR spectra of acetylated LCC samples (LCC 1, lignin content 30%; LCC 2, lignin content 44%) and MWL from spruce [extraction lignin, (2)].

For comparison, a Björkman lignin preparation (B) was subjected to purification by the extraction procedure. The product obtained (B1) had about the same carbohydrate content as the extraction lignin (E). An additional purification by the same method gave a product (B2) with properties similar to those of purified extraction lignin (E1). The carbohydrate content could be further lowered by a third purification. However, the carbohydrate content was still on the order of 1–2%.

Since  $^1\text{H}$  NMR spectral technique successfully could be used to illustrate the presence of carbohydrates in birch lignins, a study of this type also was made with MWL preparations and lignin carbohydrate complexes (LCC) from spruce. Fig. 3 shows NMR spectra of LCC and extraction lignin from spruce. The spectra of LCC preparations exhibit several peaks, which can be attributed to protons in carbohydrate units. Signals at  $\delta = 5.33$  and  $5.05$  seem to be well suited for the detection of carbohydrates in spruce lignin samples. On the basis of spectral data for acetylated sugars, these signals can primarily be ascribed to protons at the C-2 and C-3 atoms in carbohydrate units. Signals at these positions

could not be detected in the spectrum of extraction lignin from spruce (fig. 3). This was expected since extraction lignin from spruce has a very low carbohydrate content [0.05%, (2)]. Some spectra of Björkman lignin preparations from spruce exhibited a very weak signal at  $\delta = 5.05$ , which can be attributed to the presence of carbohydrates.

## Discussion

Apparently the carbohydrate content in extraction lignin as well as Björkman lignin from spruce is much lower than in the corresponding preparations from birch. A conceivable explanation is that the frequency of lignin carbohydrate linkages is comparatively high in birch wood. As already pointed out in the introductory section, there are pronounced differences in composition between spruce and birch lignin preparations, and other reasons for the dissimilarities in carbohydrate content therefore seem possible.

It was found that the percentage of carbohydrates in MWL from birch could be lowered by repeated purification by liquid-liquid extraction. However, the carbohydrate content in the final products was still much larger than in extraction lignin from spruce. Losses of material during the purification steps were small; the yield of the final product after two purifications was about 80% of the starting material. The reason for the effect of repeated purifications has not been clarified. However, it seems in this connection relevant to report some results from examinations of the "aqueous layers" obtained on fractionation of lignins by the extraction procedure (2) used for purification [the purified lignin is obtained from the "organic layer", cf. (2)]. The materials in the aqueous layers have been found to have a high carbohydrate content. Minor amounts of precipitates are present in the aqueous layers. These precipitates also contain a relatively large proportion of carbohydrates. According to GPC experiments, the relative molecular masses of the precipitates are higher than those of the purified lignins. (GPC was performed on Sephadex LH-60 with DMF as a solvent; it was demonstrated that lignins, lignin carbohydrate compounds, and carbohydrates were separated according to molecular size under these conditions). Thus, as far as it is known, the fractionation is determined by carbohydrate content and, possibly, to some extent by relative molecular mass.

The effects of repeated purifications may be due to a cleavage of very weak lignin carbohydrate linkages in connection with the purification procedure. This explanation seems unlikely, since very mild conditions are prevailing during the purification. On the other hand, it was found that borohydride reduction—a fairly mild treatment—resulted in the removal of carbohydrates from MWL of birch. This conclusion was derived from  $^1\text{H}$  NMR spectroscopic studies and methoxyl analysis ( $\text{OCH}_3$ , 21.8%). Research into the removal of carbohydrate residues from MWL preparations by chemical means is in progress.

## Experimental

### *<sup>1</sup>H NMR spectra*

<sup>1</sup>H NMR spectra were recorded with a 270 MHz instrument working in the pulse Fourier mode (Bruker WH 270). Deuteriochloroform was used as a solvent (internal reference TMS). In most experiments the concentration was 50–100 mg in 0.5 ml of solvent. Temperatures were in the range of 300–302 K.

### *Acetate derivatives*

Milled wood lignin (MWL) and lignin carbohydrate complexes (LCC) (5) were acetylated by treatment with acetic anhydride-pyridine (1:1) for 24 h. The acetates were precipitated by dropping the reaction mixture into ether under stirring. The products were purified by precipitation in ether from a chloroform solution. Acetylated xylan was prepared according to (6).

### *Milled wood lignin (MWL) from birch*

MWL from birch was prepared according to the procedure involving liquid-liquid extraction described in (2).

The yield of MWL was 54% of the crude product obtained when milled birch wood was trituated with dioxane-water (96:4) [cf. (4)]. Calculated on the same basis, the yield of materials in the "aqueous layers" [cf. (2)] was 20% (carbohydrate content 37%); in addition to dissolved substances this figure includes a small amount of a precipitate (carbohydrate content 18%). MWL isolated according to the procedure described in (2) is termed extraction lignin in this paper.

## Literature

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