Studies on lignin carbohydrate linkages in milled wood lignin preparations

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SUMMARY: Milled wood lignin (MWL) preparations with very low carbohydrate content have been investigated. Residual carbohydrates in MWL of birch were found to be almost completely removed by a mild alkaline treatment. A corresponding acid treatment did not lower the carbohydrate content significantly. These observations suggest the occurrence of lignin carbohydrate linkages of benzyl ester type.

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Soluble fractions of milled wood consist of lignin and related materials, lignin-carbohydrate complexes and carbohydrates. Lignin preparations with a low carbohydrate content and lignin-carbohydrate compounds with comparatively low relative molecular mass could be isolated from milled wood (1,2). Both types of materials are useful for studies of lignin-carbohydrate linkages. The primary concern of the present paper is a study of such linkages in lignin preparations with a low carbohydrate content. It seems reasonable to assume that the number of lignin-carbohydrate linkages per carbohydrate unit is relatively high in such materials. If this is the case, there is a good chance that the nature of the lignin-carbohydrate connections can be derived from the properties of residual carbohydrates.

Spruce lignin preparations with very low carbohydrate content have been prepared (1). We have reexamined the carbohydrates in so-called extraction lignin (2) from spruce, using an improved hydrolysis procedure (see Experimental). The carbohydrate content was determined as 0.2—0.3% (relative carbohydrate composition: arabinose, 17%; xylose, 16%; mannose, 25%; galactose, 14%; glucose, 28%). The carbohydrate analyses indicate that the relative amounts of the different monosaccharides undergo changes when the carbohydrate content is lowered; e.g., the proportion of arabinose tends to increase. Similar changes in relative carbohydrate composition were observed by Björkman (3) and have been encountered by several workers (4, 5) who have examined the carbohydrate composition in hemicelluloses and lignin-rich materials from spruce. The relative increase in arabinose content may reflect a preferential tendency of this type of sugar unit to be attached to the lignin. Evidence for the occurrence of arabinose-lignin linkages has recently been presented (6).

The amount of residual carbohydrates in milled wood lignin (MWL) of birch has been found to be considerably larger than in a corresponding preparation from spruce (2). Repeated purification by liquid-liquid extraction diminished the carbohydrate content, but it was still of the order of 1—2% (2). The changes in carbohydrate content were followed by ^{1}H NMR spectroscopic examinations, methoxyl analyses, and hydrolysis studies (2). Infrared (IR) and proton magnetic resonance (^{1}H NMR) studies gave additional information about the residual carbohydrates. IR spectra of MWL preparations from hardwoods usually exhibit an absorption band at about 1735 cm^{-1} (7, 8). This is also true for MWL of birch. However, it was found that the absorbance at 1735 cm^{-1} was very small in repeatedly purified lignin preparations, which suggests that the current absorption band is associated with the carbohydrate moiety of the lignin preparation. Xylan strongly dominates as the carbohydrate constituent in birch lignin. Acetyl and uronic acid groups are attached to the xylan, and these groups cause an IR absorption around 1735 cm^{-1} (C=O stretching). The occurrence of acetyl groups in the carbohydrate portion of the lignin preparations was supported by ^{1}H NMR spectroscopy. A signal appeared at chemical shift $\delta \approx 2.0$ ppm which could be attributed to acetyl groups; the intensity of this signal varied with the carbohydrate content of the lignin sample.

In previous work it was found that borohydride reduction of MWL of birch resulted in a removal of residual carbohydrates (2). This observation prompted a study of the release of the carbohydrates from birch lignin by chemical means. Preliminary experiments showed that a mild alkaline treatment produced a lignin which was practically free from carbohydrates. Therefore, it seems plausible that the carbohydrate releasing effect of borohydride reduction is due to the alkaline conditions prevailing during this treatment. However, reductive cleavage of lignin-carbohydrate linkages of ester type may also contribute to the removal of the carbohydrates (9). Purified birch lignin (E 1, see Experimental) was subjected to treatments with dilute aqueous sodium hydroxide and hydrochloric acid at room temperature. The lignin was extracted from the crude reaction products obtained on neutralization and removal of solvents from the reaction mixtures. A reference lignin was prepared by applying the extraction procedure to a mixture of lignin E 1 and sodium chloride. The carbohydrate contents of the obtained lignin products were examined by hydrolysis and ^{1}H NMR studies. Results from hydrolysis studies are summarized in *table 1*. A mild alkaline treatment apparently removes the major part of the carbohydrates (table 1). This could be confirmed in ^{1}H NMR experiments. ^{1}H NMR spectra of the acetates of alkali treated lignin and reference lignin

Table 1. Carbohydrate contents of birch lignin subjected to various treatments.

Sample[1]	Carbohydrate content, %
Extraction lignin, purified (E 1)	2.1
E 1, treated with NaOH	0.2
E 1, treated with HCl	1.5
E 1, purified (NaCl present)	1.8
E 1, purified	1.4
E 1, purified (modified procedure)	1.3

[1] See Experimental.

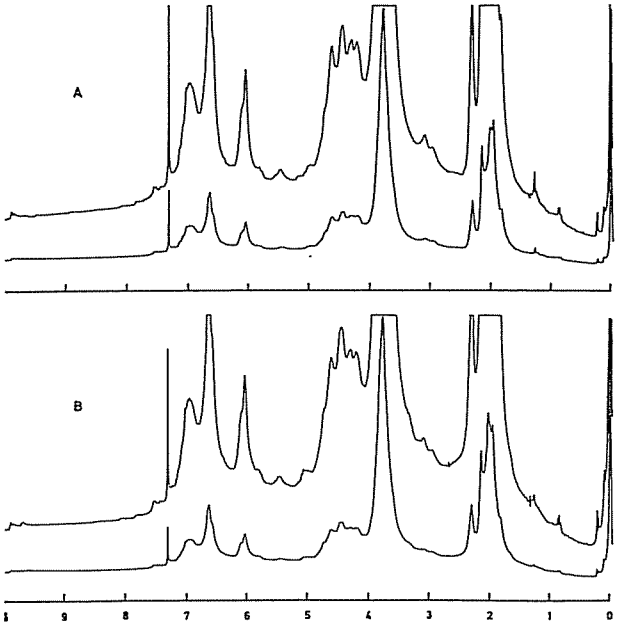
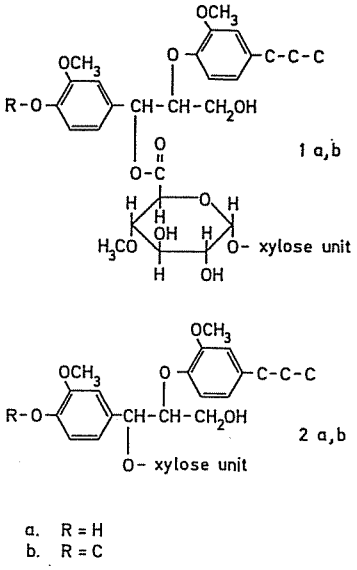


Fig. 1. ^1H NMR spectra of the acetate derivatives of alkali treated MWL of birch (spectrum A) and a reference lignin (spectrum B).



are shown in *fig. 1*. The presence of xylan in birch lignin preparations is revealed by signals at δ 2.02 and 5.04 (2). Signals at these positions are clearly detectable in the spectrum of the reference lignin while they are practically absent in the spectrum of alkali treated lignin. The two spectra in fig. 1 are very similar in other respects which suggests that the lignin is essentially unaffected by the alkaline treatment. However, a small peak at δ 9.66 in the reference lignin spectrum is lacking in the spectrum of alkali treated lignin. The 9.66 peak is attributed to formyl protons in cinnamaldehyde units (10); these units apparently undergo some kind of change during the alkaline treatment. (The weak signal at δ 5.76 has previously been attributed to the proton at C-1 in xylose endgroups (2). Borohydride reduction was found to affect this signal only slightly. This suggests that the 5.76 peak primarily is caused by other types of protons than those in xylose).

A mild acid treatment does not change the carbohydrate content of birch lignin significantly (table 1). Within the error limits the amounts of carbohydrates are the same as in the reference lignin. This is also true for lignin E 1 purified by liquid-liquid extraction as described in ref. (1), or by a modified procedure [the sample was dissolved in pyridine-acetic acid-3 molar hydrochloric acid (9:1:4) instead of pyridine-acetic acid-water (9:1:4)] (table 1).

Assuming that the removal of carbohydrates on alkaline treatment is due to cleavage of lignin-carbohydrate linkages, it follows from the investigations made that these linkages must be alkali labile but able to withstand a mild acidic treatment. Benzyl ester linkages of type *1* (in the scheme) can be expected to have properties which are in line with the observations made. (Esters of type *1a* are particularly alkali labile, since a decomposition of their phenolic anions to a quinone methide and carboxylate is favoured.) The formation of such linkages during the biosynthesis can be explained by an addition of uronic acids to quinone methide intermediates. This mode of formation is strongly supported by the fact that organic acids are known to add rapidly to quinone methides (11). It is plausible that lignin-carbohydrate linkages of type *1* are prevalent in birch wood which is rich in glucuronoxylan (12). Lignin-carbohydrate linkages of benzyl ether type *2* have been considered (12). However, such ethers can be expected to be comparatively resistant to alkaline hydrolysis (13). Our results therefore, point to a low frequency of lignin-carbohydrate linkages of type *2* in birch wood.

Experimental

Spectroscopic analyses

IR spectra were recorded using KBr pellets, with a Beckman IR 9 instrument. ^1H NMR spectra were recorded with a 270 MHz instrument working in the pulse Fourier mode (Bruker WH 270). Deuteriochloroform (internal reference TMS) and dioxane-d_8 — D_2O (5:1) were used as solvent (internal reference was the sodium salt of 3-(trimethylsilyl)propanesulfonic acid).

Carbohydrate analyses

Lignin preparations were hydrolysed by heating (100°C) with 1 molar H_2SO_4 — dioxane (1:1) for 4 h in sealed glass ampoules. The reaction mixture was neutralized by addition of an anion exchange resin in the bicarbonate form, and the liberated monosaccharides were analysed

by means of partition chromatography on an anion exchanger (Durrum DA-X8 Fine) in the sulfate form, using 85% ethanol as eluent.

Chemical treatments of milled wood lignin (MWL) from birch

MWL was prepared from birch wood according to the directions given in (2) and subjected to an additional purification by liquid-liquid extraction. The lignin preparation obtained is designated E 1, cf. (2), and was used as starting material in the experiments described below.

Alkaline treatment. Lignin E 1 (200 mg) was dissolved in 30 ml 0.05 molar NaOH — dioxane (5:1). The solution was stored at room temperature for 24 h (nitrogen atmosphere). The reaction mixture was acidified (pH 5) with dilute hydrochloric acid and solvents removed by film evaporation (25°C).

Acid treatment. Lignin E 1 (200 mg) was dissolved in 50 ml 0.05 molar HCl — dioxane (1:1). After 24 h storage at room temperature, the pH was adjusted to 5 with aqueous NaOH and solvents evaporated as described above.

The products obtained on alkaline and acid treatment as well as untreated E 1 (200 mg) with added NaCl (1.25 mmol) were each dissolved in 7 ml pyridine-acetic acid-water (9:1:4) and the solutions were extracted as described in (1). The lignin preparation obtained from untreated E 1 mixed with NaCl served as a reference sample.

Purification of lignin E 1

Lignin E 1 (200 mg) was dissolved in 7 ml pyridine-acetic acid-water (9:1:4) and the solution was extracted with chloroform as described in (1). Lignin E 1 was also purified by a modified procedure in which the sample was dissolved in pyridine-acetic acid-3 molar HCl (9:1:4).

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