Lignin carbohydrate linkages in milled wood lignin preparations from spruce wood

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SUMMARY: A mild alkaline treatment was found to release about 50 per cent of the carbohydrates in lignin samples obtained from spruce wood. Xylan was removed to a greater extent than the other carbohydrate constituents. The results are interpreted in terms of the occurrence of alkali labile (ester linkages) as well as alkali stable (ether linkages) lignin carbohydrate bonds. The results are compared with those obtained in a similar study on birch lignin.

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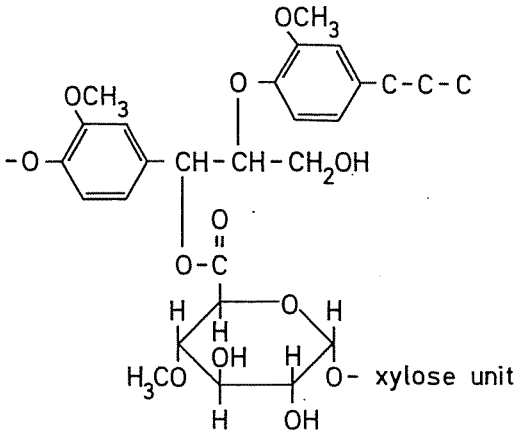
In a previous paper (1) the removal of the major part of the carbohydrates (primarily xylan) from a milled wood lignin (MWL) sample of birch wood by a mild alkaline treatment was reported. It was suggested that this was a result of a saponification of lignin carbohydrate linkages of the ester type. In connection with studies on the preparation of lignin from wood pretreated with alkali, Bland and Menshun (2) observed that a mild alkaline treatment released the major part of the xylan in a MWL sample of *Eucalyptus regnans* and gave hydrolysis of ester linkages as a conceivable explanation. Their findings are in line with those of our studies on birch lignin. Several other authors (3; cf also 4) discuss the occurrence of alkali labile lignin carbohydrate linkages on the basis of hydrolysis studies. However, these studies have generally involved samples of high carbohydrate content, which, in our opinion, makes an interpretation of hydrolysis results in terms of cleavage of lignin carbohydrate bonds rather uncertain.

On the basis of the composition of the wood components and biosynthetic considerations, benzyl ester groups of type *1* are likely candidates for alkali labile lignin carbohydrate linkages in hardwoods (5). They can be expected to be formed by the addition of the carboxyl group in 4-*O*-methylglucuronic acid units attached to the xylan to quinone methide intermediates generated during lignin biosynthesis [or during the "aging" processes in the wood (6)]. Higuchi and co-workers (7) have in model experiments demonstrated that the formation of this type of lignin carbohydrate linkages is possible. Additional support for the occurrence of such linkages comes from studies showing that a large portion of the 4-*O*-methylglucuronic acid units in these woods is esterified (although not necessarily attached to the lignin). This can be concluded in an unambiguous way from studies of the formation of 4-*O*-methylglucose on sodium borohydride reduction and subsequent hydrolysis (8, 9).



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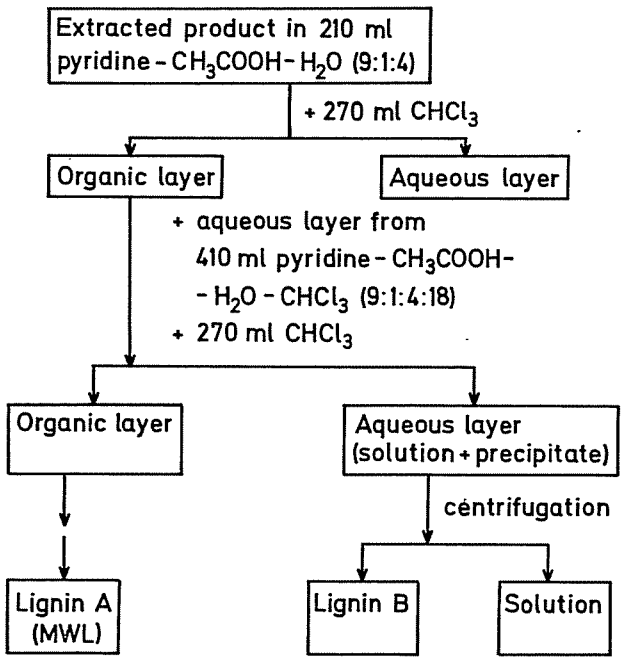
A study of the removal of carbohydrates from lignin samples of spruce on mild alkaline treatment is described in the present paper. The existence and nature of the lignin carbohydrate linkages in spruce wood are discussed on the basis of hydrolysis results. The compositions of MWL of spruce (a softwood lignin) and MWL of birch (a hardwood lignin) differ considerably. Consequently, the frequency and nature of lignin carbohydrate linkages might be different in these two types of woods. Results from experiments with spruce and birch lignin samples are therefore compared and an attempt is made to explain differences found as a result of the dissimilarities in chemical composition of the lignin samples.

Alkaline treatment of lignin samples from spruce

The fractionation procedure shown in *scheme 1* was used for the isolation of MWL (lignin A) from spruce



Scheme 1.

Table 1. Carbohydrate contents and carbohydrate compositions of MWL from spruce (lignin A, see scheme 1) subjected to various treatments.

Sample*	Carbohydrate content (%)	Relative carbohydrate composition (%) Ar	Xyl	Ma	Ga	Glu
Lignin A	0.30	15	16	18	20	31
Lignin A, purified	0.30	15	16	19	20	30
Lignin A, purified (NaCl present)	0.25	16	16	19	20	28
Lignin A, treated with NaOH	0.14	14	9	21	22	34

*See "Experimental" and scheme 1.

wood. It has been shown that this fractionation method efficiently separates lignin from carbohydrates and other components of the wood extract (10). Carbohydrate content and relative carbohydrate composition are given in *table 1*. Repeated purification according to scheme 1 did not affect the carbohydrate content (table 1). Lignin that had been repeatedly purified was used as a starting material in this study. Alkaline treatment (0.05 M NaOH in dioxane—water [1:5], 24 h; room temperature), neutralization with dilute hydrochloric acid, evaporation of solvents, and purification of the product according to scheme 1 gave a lignin sample with a lower carbohydrate content (0.14%) than the starting material (0.3%). The relative carbohydrate composition did not differ drastically from the one of the starting material, but a preferential release of xylan had occurred (table 1). A reference sample was prepared by subjecting the starting material and added sodium chloride to the work-up procedure used in the hydrolysis experiment. It appears from the data in table 1 that the work-up procedure alone did not appreciably affect the carbohydrate content or carbohydrate composition.

MWL from birch prepared by the same procedure (scheme 1) as used for the spruce lignin examined in this study contains about 2% carbohydrate (xylan) (11). For a comparison of the results from spruce and birch lignin studies, it was desirable to examine a spruce lignin sample containing a few per cent carbohydrates. The lignin precipitate denoted lignin B in scheme 1 constituted such a lignin sample. Alkaline hydrolysis experiments were performed in analogy with the experiments with MWL from spruce described above. The results are summarized in *table 2*. Although the carbohydrate content of lignin B is about ten times higher than in MWL (lignin A), the hydrolysis results are quite analogous with those obtained when MWL was examined (tables 1 and 2).

Table 2. Carbohydrate contents and carbohydrate compositions of lignin B subjected to purification and alkaline treatment.

Sample*	Carbohydrate content (%)	Relative carbohydrate composition (%) Ar	Xyl	Ma	Ga	Glu
Lignin B	3.5	12	21	25	22	20
Lignin B, purified (NaCl present)	3.5	14	20	26	22	19
Lignin B, treated with NaOH	1.6	15	12	23	24	27

*See "Experimental" and scheme 1.

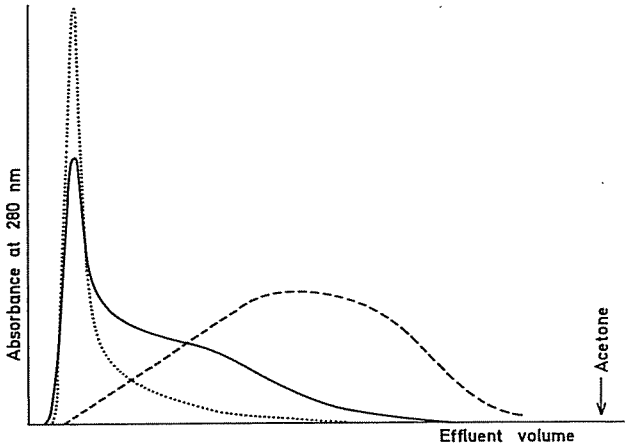
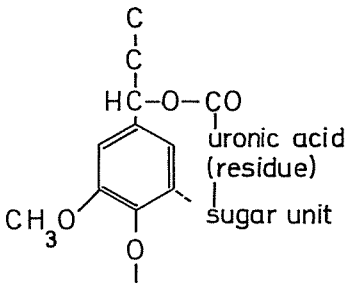


Fig. 1. Gel permeation chromatography of lignin carbohydrate complexes from spruce (. . . . .), lignin B (————), and lignin A (————————). The elution volume of a small molecule, acetone, is indicated.

The molecular mass distribution of lignin A, lignin B, and lignin carbohydrate complexes from spruce (12) is shown in *fig 1*. Lignin carbohydrate complexes are practically excluded from the gel, and a substantial fraction of lignin B is also excluded from the gel. The molecular mass of MWL is apparently lower than that of lignin B. To further characterize lignin B, this material was examined by spectroscopical methods (UV and IR). The spectra of lignin B exhibited striking similarities with those of MWL (lignin A).

Discussion

From studies of the dehydrogenation of coniferyl alcohol in the presence of sucrose, Freudenberg and co-workers (13, 14) concluded that benzyl ethers probably constitute the most important type of lignin carbohydrate linkages. Such linkages are formed when hydroxyl groups in carbohydrates are added to quinone methides. It is known that organic acids react rather rapidly with quinone methides (14). On the basis of this observation, Lai and Sarkanen (5) suggested the occurrence of lignin carbohydrate linkages of the benzyl ester type *1, 2,* formed by the addition of carboxylic acid groups in uronic acid units to quinone methides. The frequency of such linkages was presumed to be particularly high in



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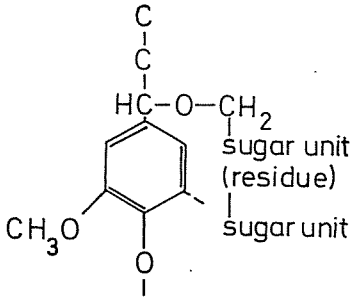
hardwoods, since xylan with attached 4-*O*-methylglucuronic acid groups is the predominating hemicellulose in hardwoods (lignins are always associated with the hemicelluloses). Higuchi and co-workers (7) have made experiments which show that the formation of benzyl esters is a favored reaction when models for hemicelluloses (D-glucuronic acid, D-glucose and methyl-D-glucoside) react with quinone methides. The reaction of D-glucose and methyl-D-glucoside with a quinone methide resulted in a preferential etherification of the primary alcohol group at C-6 (7). Leary and co-workers (6) made model experiments which similarly show that the primary alcohol group in glucose and other hexoses is most reactive in the addition reaction with quinone methides. They also found that the primary alcohol group at C-5 in arabinose (furanose form) was comparatively reactive. The anomeric hydroxyl groups at C-1 were also found to be rather reactive. Reaction with such alcohol groups will result in glycosidic linkages. One may, however, assume that the number of such linkages is small, since there are only a few units in the hemicelluloses with a free hydroxyl group at C-1 (end groups).

As judged from the present knowledge about the biosynthesis of lignin carbohydrate linkages, benzyl esters of type *2* and benzyl ethers of type *3* represent the most probable arrangements for such linkages. The following discussion is therefore based on the assumption that these two types of linkages essentially are responsible for the lignin carbohydrate connections.



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The alkaline treatment results in a release of about 50% of the carbohydrates in spruce lignin samples. The residual carbohydrates would thus be linked to the lignin by linkages resistant to the alkaline treatment. Benzyl ethers of type *3* constitute such linkages (15) and could explain the attachment of galactoglucomannan residues (tables 1 and 2) to the lignin, since all the sugar units in this type of hemicellulose contain primary alcohol groups. Xylan in spruce wood consists of a xylan backbone with attached 4-*O*-methyl glucuronic acid units and glycosidically bound furanosidic arabinose units (16). The latter type of units contain primary alcohol groups, and, therefore, xylan could be connected to the lignin by linkages of type *3* via the arabinose units. Thus, the presence of glucose, galactose, mannose, xylose, and arabinose, in the alkali-treated lignin could be explained by linkages of type *3*. A fraction of the xylan may be linked to the lignin via 4-*O*-methylglucuronic acid residues by linkages of type *1*. Liberation of xylan by saponification of such linkages may explain the comparatively low xylan content in the alkali-treated sample.

Xylan is the predominating carbohydrate associated with birch lignin. About 90% of the xylan was found to be removed by a mild alkaline treatment. This result is in accordance with a prevalence of lignin carbohydrate bonds of type *2* in birch wood. Xylan from birch does not contain arabinose units of the type present in spruce xylan (17). Therefore, birch xylan is lacking primary alcohol groups and cannot be attached to lignin by linkages of type *3*. Residual xylan in the alkali-treated birch lignin may be linked to the lignin by other types of benzyl ether linkages.

The carbohydrate content of spruce MWL (0.3%) is considerably lower than that of birch MWL (2%). One explanation would be a higher frequency of lignin carbohydrate linkages in birch lignin. A reason for this might be the high reactivity of carboxylic groups in uronic acids in addition reactions with quinone methides, since birch hemicelluloses contain a relatively large number of uronic acid units.

In connection with studies on lignin carbohydrate complexes from birch, it was found that certain lignin-rich fractions contain rather large amounts of arabinose (18). Since birch xylan does not contain arabinose units, this indicates that other types of carbohydrates than those discussed above (galactoglucomannan, different types of xylan) are associated with the lignin. This fact complicates the interpretation of the changes in carbohydrate composition caused by fractionation or alkaline treatment.

Eriksson et al. (19) have recently studied lignin carbohydrate connections in lignin carbohydrate complexes from spruce wood using other approaches than those in the present work. Their results seem to match the data obtained in the present work fairly well. Erins and co-workers (20) have studied the hydrolysis of lignin carbohydrate complexes from spruce and birch by a mild alkaline treatment and

concluded that lignin carbohydrate linkages of the ester type occur in both types of wood, which is in agreement with the results obtained in this study.

It is well known that the lignin can be extracted from certain plants, e.g. grasses, by mild alkaline extraction. Since the lignin as well as hemicelluloses in such plants resembles those of hardwoods, it seems possible that the liberation of lignin is due to saponification of ester linkages of type *2*. However, other explanations for the alkali extractability of the lignin from such plant materials have been presented (21).

Experimental

Analyses

Carbohydrate analyses were performed according to the procedure described in (11). Molecular mass distributions were examined by gel permeation chromatography on Sephadex LH 60, using dimethyl formamide—acetic acid (200:1) as eluent (cf. 22).

Isolation of lignin samples from milled spruce wood

Milled wood lignin (MWL, denoted lignin A in this paper) was isolated from a dioxane-water extract of milled spruce wood by liquid-liquid extraction according to the procedure described in (10); cf. scheme 1. A second lignin preparation (denoted lignin B in this paper) was isolated from the aqueous layer obtained in one of the extraction steps involved in the extraction procedure (scheme 1). When isolated lignin B was dissolved in pyridine-CH_3COOH-H_2O (9:1:4) and subjected to the procedures in scheme 1, about one half of the material was found in the fraction defined as lignin B (mechanical losses probably lowered the yield considerably). Lignin B constituted about 2% of the original dioxane-water extract.

Alkaline treatment of lignin samples

The lignin samples were dissolved in 0.05 M NaOH-dioxane (5:1) and the solutions stored at room temperature for 24 h (nitrogen atmosphere). Work-up procedures were essentially those applied earlier (1). Reference samples were prepared as described in (1).

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