Isolation of lignin by means of liquid-liquid extraction

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SUMMARY: A convenient procedure for the isolation of lignin is described. Lignin and low molecular weight lignin-related phenols were separated from sugars and lignin-carbohydrate complexes by liquid-liquid extraction. The lignin was freed from low molecular weight materials by precipitation into ether. A number of low molecular weight aromatic compounds were identified in the supernatant ether solution.

□ En enkel och effektiv metod för isolering av lignin har utvecklats. Lignin och lågmolekylära, ligninbesläktade fenoler separerades från socker och ligninkolhydratföreningar genom vätske-vätskeextraktion. Genom utfällning i eter erhölls ligninet fritt från lågmolekylärt material. I eterlösningen påvisades ett antal lågmolekylära aromatiska föreningar.

□ Eine einfache und effektive Methode zur Isolierung von Lignin wurde ausgearbeitet. Lignin und niedermolekulare, mit Lignin verwandte phenolische Substanzen wurden von Zuckern und Lignin-Kohlenhydratverbindungen durch Flüssigkeits-Flüssigkeitsextraktion getrennt. Durch Fällung in Äther wurde das Lignin frei von niedermolekularen Stoffen erhalten. In der Ätherlösung könnte dadurch eine Anzahl von niedermolekularen, aromatischen Vereinigungen identifiziert werden.

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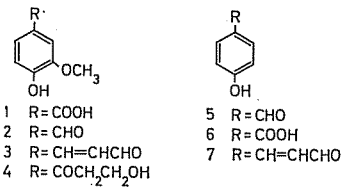
"Milled wood lignin" as well as "lignin-carbohydrate complexes" have been prepared by Björkman (1) by procedures involving solvent extraction of extensively milled wood. In recent studies (2, 3) extracts from milled spruce wood have been subjected to more detailed examinations. In addition to lignin and lignin-carbohydrate complexes, minor amounts of lignin-related low molecular weight phenols, sugars, and low molecular weight lignin-carbohydrate compounds have been found to be constituents. In the course of this work, it was found that a practically carbohydrate-free fraction, containing lignin and lignin-related phenols, could be separated from other constituents by liquid-liquid extraction. The present paper describes a method for the isolation of lignin based on these findings. Lignin-related phenols extracted together with the lignin have also been investigated to some extent.

The product obtained on extraction of milled spruce wood with dioxane-water (100:4) [cf. (1)] was dissolved in pyridine-acetic acid-water (9:1:4). This solution was extracted with chloroform. Essentially all the lignin was transferred to the organic layer. In earlier extraction experiments (2), using a dioxane-water-chloroform system, large amounts of lignin precipitated during extraction; with the pyridine-acetic acid-water-chloroform system only small amounts of precipitate formed, tentatively characterized as lignin-carbohydrate complexes.

The major part of the solvent was removed from the organic layer by film evaporation, following which the lignin was precipitated by dripping the residual solution into ether. The lignin precipitate had to be washed carefully with ether to completely remove the pyridine. Alternatively, this could be effected by reprecipitation into ether [e.g. from an ethanol-1,2-dichloroethane solution, cf. (1)].

The precipitated lignin constituted 62% of the original extract fraction. The sugar content was 0.05%, which is considerably less than that found for Björkman lignin preparations from spruce. Consistent with this fact, the methoxyl content tended to be slightly higher than the average for the Björkman lignin preparations. The PMR (acetyl derivative) and IR spectra were in good agreement with the corresponding spectra from the Björkman lignin preparations. These comparisons of analytical data corroborate the lignin nature of the product obtained and also indicate that the lignin product had remained unaffected by the purification procedure. To avoid chemical changes of the lignin all operations were performed within one day and the temperature was not permitted to exceed 30°C. It may be pointed out in this connection that the pH of the aqueous layer was about 5.

The ether solution (including washings) from the lignin precipitation gave a residue on evaporation of solvents constituting 11% of the extract fraction. Fractions of monomers (1.1%) and dimers (1.3%) were separated by gel permeation chromatography. These fractions were silylated and examined by gas chromatography and gas chromatography-mass spectrometry resulting in the identification of the low molecular weight aromatic compounds shown in *fig. 1.*



1 R = COOH
2 R = CHO
3 R = CH=CHCHO
4 R = $COCH_2CH_2OH$

5 R = CHO
6 R = COOH
7 R = CH=CHCHO

Fig. 1. Lignin-related phenols present in extracts from milled spruce wood.

In the monomer fraction vanillic acid **1** and vanillin **2** dominated. The fraction also contained *p*-hydroxybenzaldehyde **5**, *p*-hydroxybenzoic acid **6**, coniferaldehyde **3**, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone **4** and, possibly, *p*-cumaraldehyde **7**. While compounds **1—3** and **5—7** are well-known as lignin degradation products and as constituents of wood extracts, compound **4** has hitherto only been found in extractives from pine bark (4). The dimer fraction was examined for the presence of a series of lignin-related phenols (lignin degradation products, lignols, and lignans). There were no peaks corresponding to the derivatives of pinoresinol, dehydrodiconiferyl alcohol, conidendrin, and 2,3-divanillyl-1,4-butanediol. The derivatives of 3,4-divanillyltetrahydrofuran and 4,4′-dihydroxy-3,3′-dimethoxystilbene had retention times which were close to, but different from, the major peaks of the dimer fraction; the presence of small amounts of the latter two compounds can therefore not be excluded. Only a few peaks appeared in the chromatogram from the dimer fraction; estimates indicated that these peaks corresponded only to a small portion of the materials present in the fraction.

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Preextracted wood was used in the present studies. Whether low molecular weight phenolic products had become accessible to extraction during the milling or whether they formed during milling has not been determined.

Experimental

Preextracted (acetone, Soxhlet) wood chips from spruce were milled and, subsequently, extracted with dioxane-water (100:4), in similar way to the procedures described by Björkman (1). Extracted solids constituted 11% of the milled wood. The extracted product (6.80 g) was dissolved in 210 ml pyridine-acetic acid-water (9:1:4). The solution was extracted with 540 ml of chloroform. To avoid contamination due to incomplete separation of the organic and aqueous layers, the extraction procedure was divided into two steps as shown in *fig. 2*. The layers separated reasonably rapidly, but it was possible to speed up the rate considerably by centrifugation. The aqueous layers contained 1.0 g product (0.37 g of which was in the form of a precipitate). The organic layer was a clear solution, free from precipitated materials. The major part of the solvents were removed by film evaporation. The lignin was precipated by slowly dropping the residual solution (about 250 ml) into stirred ether (1.6 liters). The precipitate was washed with 3×325 ml ether. The lignin precipitate weighed 4.2 g after drying over P_2O_5 *in vacuo*. The ether solutions from precipitation and washings of lignin were collected and the solvent removed by film evaporation. The residue weighed 0.78 g. [The total weight of fractions obtained constituted 5.98 g; samples were taken for various analyses which explain about one half of the weight deficit (0.82 g)].

Investigation of the residue from ether solutions

Gel permeation chromatography on Sephadex G-25 with dioxane-water (1:1) as eluent gave fractions of monomers (76 mg) and dimers (92 mg). (A portion of the fraction was insoluble in dioxane-water (1:1); the insoluble materials were not investigated). Samples of monomers and dimers were silylated by treatment with BSA in pyridine and examined by gas chromatography and gas chromatography-mass spectrometry [cf. (5)]. Retention times were calculated relative that of docosane (monomers) and dotriacontane (dimers). Docosane was also used as internal standard for quantitative estimations of monomers.

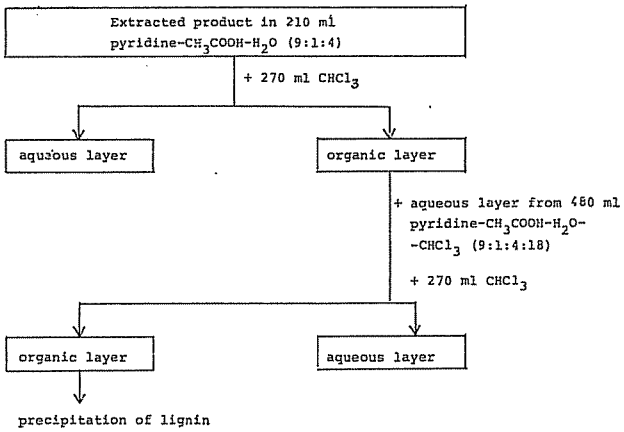

Fig. 2. Fractionation scheme for the separation of lignin from materials extracted from milled wood.

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