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The occurrence of p-hydroxyphenylpropane units in the middle-lamella lignin of spruce (*Picea abies*)

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Summary. A sieving technique has been developed for the separation of middle-lamella fragments. The middle-lamella fraction as well as the whole wood and compression wood from *Picea abies* have been analysed by nitrobenzene oxidation and acidolysis in order to determine the content of p-hydroxyphenylpropane units in the middle-lamella lignin. These analyses revealed only traces of p-hydroxyphenylpropane units in the whole wood and in the middle-lamella fraction but considerable amounts were found in compression-wood lignin. This points to the fact that middle-lamella lignin is of guaiacyl nature and that earlier results reporting high proportions of p-hydroxyphenylpropane units in the middle lamella-lignin may be due to the inclusion of compression wood in the fraction studied. The acidolysis experiments further indicate that the middle-lamella lignin has fewer uncondensed β-0-4 aryl ether structures than the whole wood lignin.

Introduction

Especially in hardwoods a clear morphological variation can be noted in the structure of lignin. The ratio of guaiacyl to syringyl building units varies considerably between the middle-lamella, the secondary-wall and the ray-cell lignin (Fergus, Goring 1970; Musha, Goring 1975; Hardell et al. 1980a). Structural differences in lignin have also been found in softwoods. UV-microscopy indicated that the phenolic hydroxyl content of the secondary-wall lignin from spruce was almost twice as high as that of the middle-lamella lignin (Yang, Goring 1978). This was also found when a fraction enriched in middle lamella obtained by a sieving technique was analysed by chemical methods (Hardell et al. 1980b). Another technique for the isolation of middle lamella material is based on the fact that lignin has a lower density than carbohydrates and that lignin-rich particles can be separated in a medium having a density between the densities of lignin and

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carbohydrates (Whiting et al. 1981 a). The lignin-rich particles obtained are assumed to come from the middle lamella since this is the most lignin-rich part of the fiber. Using this technique, Whiting et al. (1971) isolated from *Picea mariana* a fraction which constituted 0.4% of the wood and had a lignin content of 52%. This fraction was further separated and fractions with 55–60% lignin were obtained. The technique has also been used by Sorvari et al. (1983) who could at best obtain a fraction containing 49.8% lignin.

The density-gradient-separated material has been used in a series of studies to characterize the chemical composition and reactivity of the middle lamella (Whiting et al. 1981 b, c, 1982 a, b, c, 1983; Saka et al. 1982; Sorvari et al. 1983).

The most striking feature of the density-gradient-separated middle lamella was the low content of methoxyl groups in the lignin (Whiting, Goring 1982 a, c). It was found that the ratio of methoxyl groups in the secondary-wall lignin to methoxyl groups in the middle-lamella lignin was 1.7–1.8. This was interpreted together with results from pyrolysis experiments (Whiting, Goring 1982 c) as indicating that the middle-lamella lignin contained a substantial amount of p-hydroxyphenylpropane units, assumed to be of the order of 43% of the total building units in the middle-lamella lignin. Other characteristics of the middle-lamella fraction were a high content of carboxylic groups (Whiting, Goring 1982 a) and a low reactivity to bromination (Saka et al. 1982). If the density gradient technique gives a proper isolation of the middle lamella it is obvious that the middle lamella-lignin has a structure which differs considerably from that of the secondary wall lignin.

A prerequisite for the density gradient technique to give a good separation of the middle lamella is that the middle lamella is the only lignin-rich part in the wood. Softwood, however, contains abundant quantitites of compression wood which has a lignin content considerably higher than that of the normal wood (Timell 1981). The cell wall of compression wood contains an especially lignin-rich layer in the outer part of the middle secondary wall. This layer, called S_2(L) (Timell 1981), is isotropic in polarized light as is the middle lamella (Bailey, Kerr 1937). Compression wood can sometimes be quite difficult to recognize since it can vary extensively in intensity from very mild to fully developed with spiral grooves. In the transition between compression wood and normal wood the unique character of compression-wood lignin is the last feature to disappear (Yumoto et al. 1983). There seems therefore to be a potential risk that compression wood present in the wood can escape notice and be enriched in the low density fraction when a technique that separates middle lamella according to the lignin content of the particles is used. The lignin in the middle lamella fraction isolated by Whiting and Goring (1982 a) has many similarities to compression-wood lignin. Characteristic of compression-wood lignin is a low methoxyl content and a high content of p-hydroxyphenylpropane units. Latif (1968) has estimated that the lignin in compression wood contains about 30% p-hydroxyphenylpropane units.

This paper reports the results of an analysis of a middle lamella fraction isolated by a different technique. The fraction was analysed by two procedures, acidolysis and nitrobenzene oxidation in an attempt to elucidate whether the middle-lamella lignin possesses a "compression wood character" or whether the high p-hydroxyphenylpropane content indicated in the density-gradient-separated middle lamella can be due to enrichment of compression wood by this technique.

Experimental

Wood samples

Acetone-extracted Norway spruce (*Picea abies*) was used for all preparations.

Wood meal for analysis was prepared by grinding the wood in a Wiley mill.

Compression wood was obtained from the stem of a heavily leaning spruce. The lignin content of the compression wood was 36%.

Isolation of middle lamella

A procedure for enrichment of material from the middle lamella and primary wall has been described by Hardell et al. (1980 b). A further development of this procedure is described under "Results" in the present paper. Nylon sieves with different openings were obtained from Bigman AB, Gothenburg, Sweden.

Chemicals

Dioxane was purchased from Merck, Darmstadt, West Germany and further purified by the method of Kraus and Vingee (1934). Vanilin, p-hydroxybenzaldehyde, syringaldehyde and methyl-vanillate were purchased from Sigma Chemical Co. 3-hydroxy-1-(4-hydroxyphenyl)-2-propanone, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone and 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone were kindly supplied by Dr. Knut Lundquist, Gothenburg, Sweden. Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from Pierce, Rockford, Ill., USA. Nitrobenzene was purchased from BDH Chemicals Ltd., Poole, England.

Chemical analysis

Nitrobenzene oxidation

The oxidation with nitrobenzene was performed according to the method of Hardell et al. (1980 b). Trimethylsilyl derivatives (TMS ethers) of the degradation products were made by addition of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA with 1% TMCS). The TMS ethers were quantified by gas liquid chromatography with methyl-vanillate-TMS as internal standard. A 20 m BP 1 fused silica capillary column from J. W. Inc. Rancho Cordova CA. USA with 1.5 ml/min helium as carrier gas was used for the gas chromatography. Injector temperature 230 °C. Oven temperature 100–240 °C with 5 °C/min. Detector 240 °C.

The contents of p-hydroxybenzaldehyde, p-hydroxybenzoic acid, vanillin, vanillic acid and syringaldehyde were calculated using relative response factors which had been determined separately.

Acidolysis

Acidolysis was carried out mainly according to the procedure of Lundquist (1970) and Lundquist, Lundgren (1972). The wood material (5–20 mg) was suspended in 1 ml of dioxane: 0.2 N hydrochloric acid 9:1 (v/v) in a 1.5 ml reaction flask and the flask was heated for 4 h at 100 °C. After the acidolysis, the solid was removed and washed with three aliquots of dioxane. Distilled water (2 ml) was added to the combined solutions and the pH was then adjusted to 3 with sodium hydrogen carbonate. The solution was extracted with 3×20 ml chloroform. Methyl-vanillate (internal standard) was added to the chloroform solution which then was dried with sodium sulfate and evaporated to dryness. The residue was further dried over phosphorus pentoxide in a vacuum desiccator. TMS derivatives of the acidolysis products were prepared and analysed in the same manner as after the nitrobenzene oxidation.

Mass spectra

Mass spectra were run on a Finnigan mass spectrometer 3200 F.

Results

Isolation of middle-lamella fragments

A procedure for isolation of middle-lamella and primary-wall material from wood has been published earlier (Hardell et al. 1980 b). In that work very fine material from disintegrated wood and thermo-mechanical pulp was recovered and assigned to be an enrichment of middle-lamella and primary-wall material on the basis of its lignin content and microscopic properties.

In the disintegrated wood quite large fragments of loose middle lamella could be seen. To ensure a good representation of the whole compound middle lamella these fragments were isolated and used in this study. Isolation and purification of the middle-lamella fragments is summarized in Fig. 1. The appearance of the isolated fragments can be seen in Figs. 2 and 3. The majority of the particles exhibited a low birefrigence in polarized light but birefrignant material was present in some particles indicating that material from the secondary walls had been removed with some of the middle-lamella particles. The fraction has a lignin content of 40% according to the Klason technique and 41% by calculation from methoxyl analyses using a value of 15.6% methoxyl for the guaiacyl lignin. This is a considerably lower lignin concentration than the value of 60% (Klason) reported for the fraction obtained by the density fractionation by Whiting et al. (1981) and Saka et al. (1982). Assuming that the value of 60% lignin is the true lignin content of the compound middle lamella and assuming a value of 22% lignin for the secondary wall, the calculated contents of middle-lamella and secondary-wall material in our fraction are 47.4% and 52.6% respectively. This would mean that, of the total lignin in our sample, 71% would come from the "true compound middle lamella" and 29% from the secondary wall. The microscopic analysis of the fraction does not

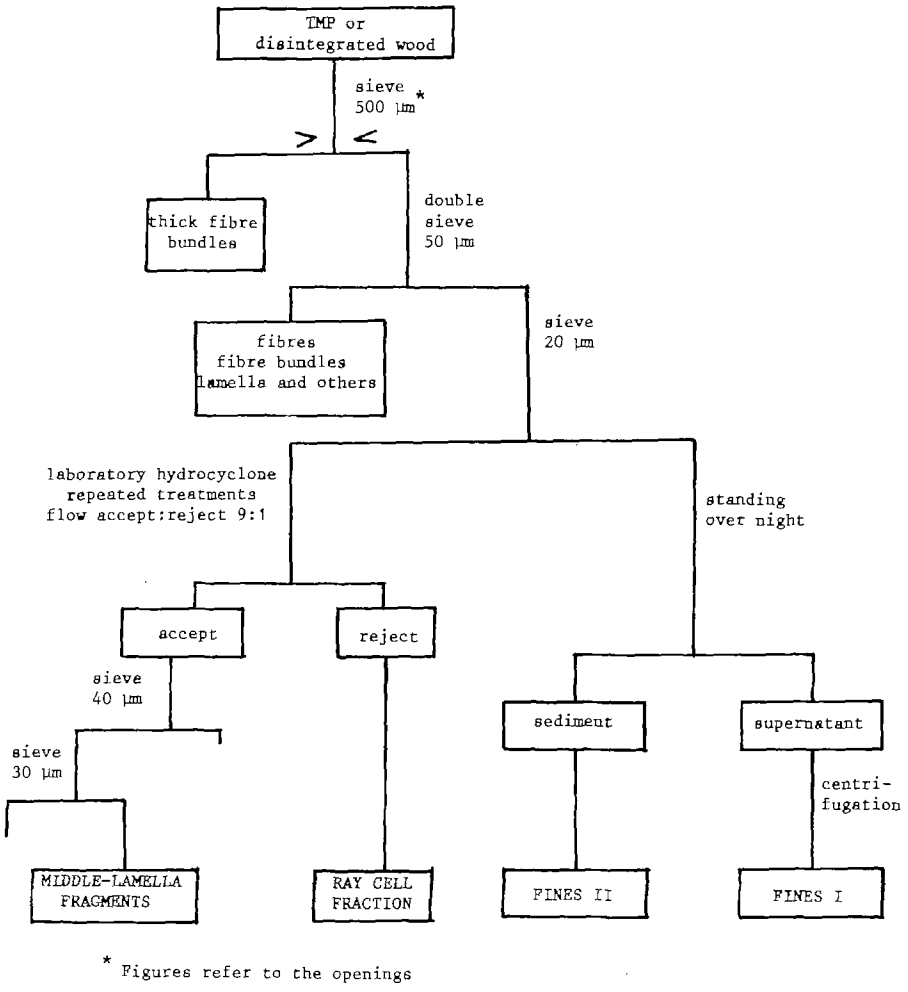

Fig. 1. Separation scheme for middle-lamella fragments

support such a large contamination by secondary-wall material but, even if this were the case, the portion of middle-lamella lignin in the sample would be large enough to give an extensive portion of p-hydroxyphenylpropane units in the lignin.

Nitrobenzene oxidation

The yields of the major degradation products from the nitrobenzene oxidation of whole wood, middle lamella and compression wood are summarized in Table 1. The yields of p-hydroxybenzaldehyde from whole wood and from the middle lamella are very small whereas the presence of p-hydroxybenzaldehyde in the oxidation products of compression wood is quite clearly significant.

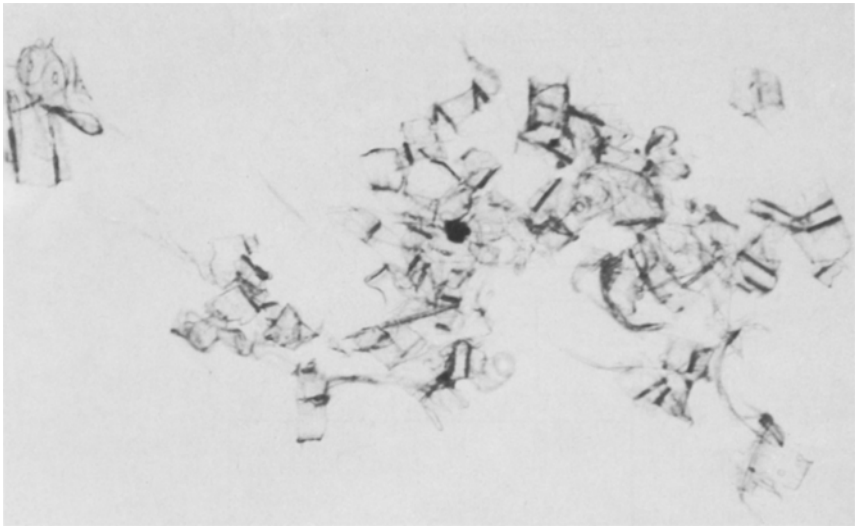

Fig. 2. The appearance of the separated middle-lamella fragments. The middle-lamella cellcorners are visible as the darker parts of the fragments. Light microscopy

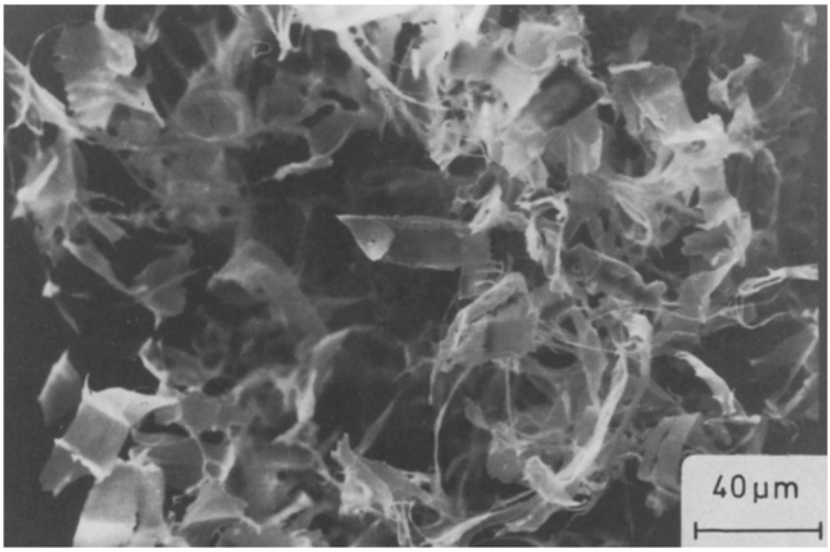

Fig. 3. The appearance of the middle lamella fragments. Scanning electron microscopy

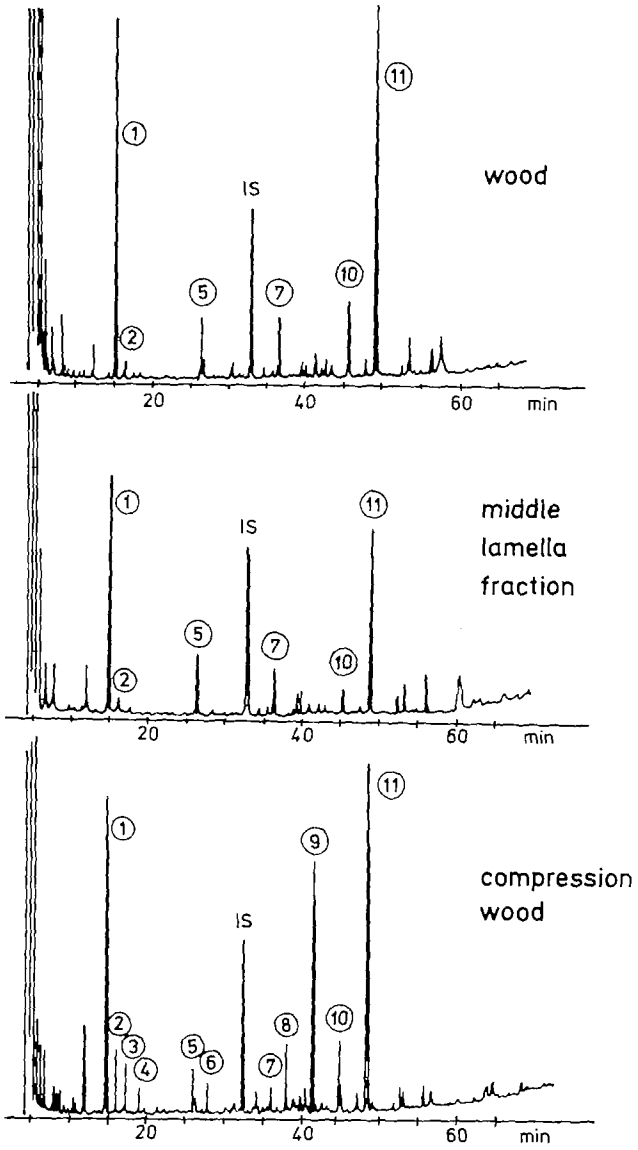

Fig. 4. Gas chromatograms of the products from acidolysis of whole-wood, middle-lamella and compression-wood lignin. The compounds which have been identified from the reaction mixture are shown in Fig. 5

Acidolysis

Fig. 4 shows gas chromatograms of the trimethylsilyl ethers of the acidolysis mixtures from whole wood, from the middle-lamella fraction and from compression wood. The amounts of the samples for the acidolyses were chosen to contain equal amounts of lignin so that the peak hights in the chromatogram would be directly

Table 1. Yield of degradation products from nitrobenzene oxidation of different wood fractions. The yields are expressed as mg degradation product per mg of lignin

	Vanillin	p-Hydroxy-benzaldehyde	Vanillic acid	p-Hydroxy-benzoic acid	Syring-aldehyde
Whole wood	20±1	0.1±0.1	1.7±0.2	0.15	<0.001
Middle-lamella fragments	15±2	0.1±0.1	1.6±0.3	<0.001	<0.001
Compression wood	16±3	2.6±0.5	1.4±0.5	<0.001	0.001

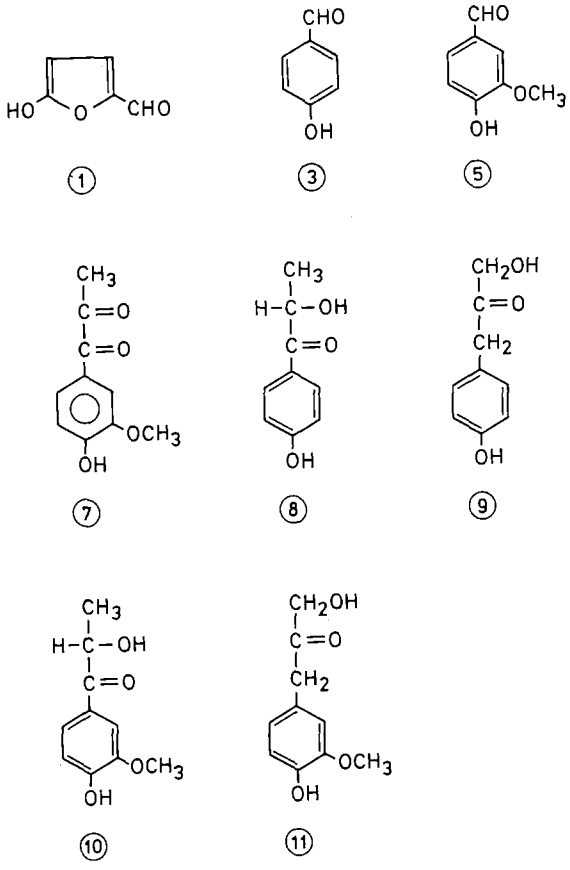

Fig. 5. Compounds found in the acidolysis mixture. The numbers refer to peak numbers in the chromatograms (Fig. 4)

comparable. Fig. 5 gives the compounds that have been identified from the reaction mixtures. Compounds 1, 3, 5, 9, 11 have been identified by comparison of mass spectra and retention times with authentic compounds as references. Compound 8 has been tentatively identified by comparison with published mass spectra data (Higuchi et al. 1972). Compounds 7 and 10 have been tentatively identified by

mass spectra from authentic compounds obtained from Dr. Knut Lundquist. From the chromatograms it is obvious that lignin in the compression wood has a very different structure from the lignin in the normal wood and in the middle lamella. In the chromatograms from compression wood, five new peaks (3, 4, 6, 8, 9) appear. Three of these peaks were identified to be p-hydroxybenzaldehyde (3), 3-hydroxy-1-(4-hydroxyphenyl)-2-propanone (9) and 2-hydroxy-1-(4-hydroxyphenyl)-1-propanone (8). All these come from p-hydroxyphenylpropane units in the lignin. These compounds were not detectable in either the whole wood or the middle lamella fraction.

The chromatogram of the lignin from the middle lamella shows the same degradation pattern as the lignin from the whole wood. The yields of 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone (11), 1-(4-hydroxy-3-methoxyphenyl)-1,2-propandione (7) and 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (10), all of which are degradation products from guaiacylglycerol-β-ether structures, are lower from the middle-lamella lignin than from the whole wood lignin.

Discussion

Both the very low yield of p-hydroxybenzaldehyde following nitrobenzene oxidation and the absence of p-hydroxyphenyl compounds in the acidolysis mixture point to the fact that the lignin in our middle-lamella sample is of guaiacyl type and has a negligible content of p-hydroxyphenyl-propane units. This, together with data on bromination of different wood fractions (Westermark 1985) suggests that the low methoxyl content and the suggested high content of p-hydroxyphenyl-propane units in the middle-lamella fraction isolated by Whiting and Goring (1982a) can be due to contamination by compression wood. It is also noticeable that Sorvari et al. (1983) find no differences in methoxyl content between milled wood lignin (MWL) made from their middle lamella fraction and the MWL from whole wood. This indicates that their middle-lamella fraction is also of guaiacyl type. Since compression wood is sometimes very difficult to detect even under the microscope, great care must be taken in selecting the wood when the density gradient technique is used to isolate the middle lamella. It is also noticeable that examination by polarized light microscopy to investigate the birefringence of the sample cannot be used to differentiate between compression wood and the middle lamella since he S_2(L) in compression wood is also isotropic towards polarized light. Even if no differences in building units can be detected between the middle-lamella and the whole-wood lignin, there seems to be a clear difference in chemical structure between the two lignins. The considerably lower yields of the degradation products of uncondensed β-aryl ether structures in the middle-lamella lignin is an indication of this.

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