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Chemical Characterization of Tissue Fractions from the Middle Lamella and Secondary Wall of Black Spruce Tracheids

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Summary. Elemental and functional group analyses were carried out on tissue fractions from the secondary wall and middle lamella of black spruce tracheids. The secondary wall lignin was found to contain 1.7 times as much methoxyl per C_9 as the middle lamella lignin, indicating a substantial proportion of unmethylated para-hydroxyphenylpropane residues in the middle lamella. The content of carbonyl groups was at least three times larger in middle lamella lignin than in secondary wall lignin. The carboxyl content of the middle lamella was found to be about three times as large as that of the secondary wall. Elemental analyses show a higher carbon and lower oxygen content in the middle lamella than in the secondary wall.

Introduction

The analysis of functional groups in wood and its components has been carried out for many years. Potentiometric, spectrophotometric, chemical, and chromatographic analyses have been used to determine the contents of various functional groups in wood (Browning 1967a). Only recently, however, has much work been done on the distribution of wood components as well as functional group distribution.

The methoxyl group (OCH_3) is a characteristic functional group of the lignin polymer. Methoxyl contents are usually determined by some modification of the original method of Zeisel (1885) in which the methoxyl group is converted to methyl iodide upon boiling the material with strong hydriodic acid. Methoxyl contents of the milled wood lignins from various softwoods have been found in the range of 12.5% for redwood (*Sequoia sempervirens*), to 16.2% for western larch (*Larix occidentalis*) sapwood (Sarkanen et al. 1967). These values correspond to 0.87 and 1.03 methoxyl groups per phenylpropane residue (OCH_3/C_9). The methoxyl content of a sample has sometimes been used as a method for determining lignin contents (Hardell et al. 1980a). As yet, no work has been reported on the topochemical distribution of methoxyl groups in softwood tracheids although, for birch, the methoxyl content of the lignin in the secondary wall of the fibre has been found to be larger than the methoxyl content of middle lamella lignin (Fergus, Goring 1970; Musha, Goring 1975; Hardell, et al. 1980b; Cho et al. 1980).

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Carbonyl groups are present in the lignin in wood and in isolated lignin preparations (Browning 1967b). Spruce milled wood lignin contains about 0.2 carbonyl groups per phenylpropane unit (Adler, Marton 1959). Considerable work has been done on quantitative measurement of the amounts of carbonyl groups in different chemical environments, such as α or β to the aromatic ring (Brauns, Brauns 1960a) but no work has been reported in which the topochemical distribution of carbonyl groups in the wood cells has been studied.

The distribution of phenolic hydroxyl groups across the wood cell wall has been measured (Yang, Goring 1980; Hardell et al. 1980a), and approximately twice as many PhOH groups per C_9 residue were found in secondary wall lignin as in middle lamella lignin.

The presence of carboxyl groups in lignin has been the subject of considerable controversy. It is generally believed that the lignin in wood has no carboxyl groups while some isolated lignins, such as lignosulfonic acid, may contain these groups (Brauns, Brauns 1960b). However, wood tissue is known to contain carboxyl groups as some of the hemicelluloses are acidic in nature and there is a small fraction of pectic acid (Meier 1961). Meier studied the distribution of carbohydrates across the cell wall and noted a higher relative content of pectic acid in the compound middle lamella of spruce and pine.

Elemental analyses have been reported for numerous lignin preparations (Sarkanen, Hergert 1971). However, most of the work has been done on milled wood lignin and the exact elemental composition of lignin in wood remains unknown.

The purpose of the present work was to determine the concentrations of methoxyl, carbonyl, and carboxyl groups in tissues from the middle lamella and secondary wall of black spruce tracheids. Elemental analyses were reported but phenolic hydroxyl contents were not considered. The results, where appropriate, were compared to previous data on isolated lignins.

Experimental

Fractions of wood tissue from the compound middle lamella and secondary wall of black spruce (*Picea mariana*) were prepared by the method of Whiting et al. (1981). Whole wood flour was also used. The fraction of middle lamella, secondary wall, and whole wood tissue had lignin contents of 60%, 22%, and 27%, respectively.

Methoxyl contents were measured in two ways, infrared spectroscopy and hydriodic acid cleavage. The hydriodic acid method was the one described by Hardell et al. (1980a). The sample (0.1 – 0.8 mg) was treated with 57% HI at 140 °C for 18 h. The methoxyl groups were cleaved and the methyl iodide produced was analyzed by gas chromatography. The infrared method involved measurement of the absorption band at 1430 cm^{-1} (Sarkanen et al. 1967; Musha, Goring 1975). This band arises from C–H vibrations in methoxyl groups and can be used as a measure of the relative methoxyl contents of the samples. A sample of known methoxyl content, milled

wood lignin, was used as a reference. The absorbance at 1430 cm^{-1} was divided by the absorbance at 1510 cm^{-1}, the aromatic C–C band, so that an absorptivity based on the aromatic content resulted. From this the methoxyl content was calculated. Methoxyl analyses obtained by pyrolysis gas chromatography (Whiting, Goring 1981 b) were also used.

Carbonyl analyses were performed by Schwarzkopf Microanalytical Laboratories, New York. Carbon, hydrogen, and oxygen analyses were performed by Guelph Chemical Laboratories Ltd., Guelph, Ontario, Canada.

Ultraviolet spectra of the samples were obtained by suspending the tissue in a 25 % sucrose solution and recording the spectra by using a turbid sample holder in the spectrometer. This holder keeps the sample very close to the photodetector so that noise resulting from light scattering is reduced.

The carboxyl group content of the whole wood sample was determined by titration. A known amount of wood tissue, about 2 g, was washed with 0.1 N HCl to remove bound metal ions. The acid-treated tissue was washed with water until the pH of the eluant was neutral. The sample was then placed in 100 ml of 0.1 NaCl solution and kept under a stream of nitrogen to prevent the absorption of CO_2. After 1 h, the sample was titrated with 0.001 N NaOH and the endpoint determined potentiometrically. The neutralized wood tissue was allowed to stand for 1 h to allow the last trace of acid to be released by the sample. The endpoint was then re-established. The carboxyl contents of the middle lamella and secondary wall samples were determined by infrared spectrophotometry. The peak at 1715 cm^{-1} is known to arise from carboxyl groups (Hergert 1971). The carboxyl content of the tissue fractions was obtained by comparing the 17.5 cm^{-1} peak area with that for the whole wood sample of known carboxyl content.

Results and Discussion

As shown in Table 1, agreement between the three methods of methoxyl analysis was good. The uncertainty in the average ratio of methoxyl content of secondary wall lignin to methoxyl content of middle lamella lignin was ± 5 %. The methoxyl content of whole wood lignin can be calculated from the data for middle lamella lignin using the values of 77 % and 23 %, respectively, for the contents of lignin in the secondary wall and middle lamella in whole wood (Fergus et al. 1969). The calculated values, in brackets in Table 1, are in excellent agreement with the observed values.

The results show clearly that the middle lamella lignin is low in methoxyl content. It appears that only about 60 % of the C_9 residues are methoxylated. These data are supported by UV-visible spectroscopy. Musha and Goring (1975) predicted that lignins containing large proportions of para-hydroxyphenylpropane groups (low methoxyl content) should show a flattening of the ultraviolet absorption peak at 280 nm. Such behavior was observed in the spectrum of the middle lamella tissue fraction, as shown in Fig. 1. The ratio of the absorbance at 280 nm to that at 260 nm (Table 1) decreases as the methoxyl content decreases.

Table 1. Analyses of the lignin in secondary wall, whole wood and middle lamella tissue fractions

Tissue fraction	Percent lignin	Infrared methoxyl (OCH_3/C_9)	HI methoxyl (OCH_3/C_9)	Pyrolysis methoxyl[a] (OCH_3/C_9)	Average methoxyl (OCH_3/C_9)	A_{280}/A_{260}	Percent carbonyl
Secondary wall	22	1.02	1.06	1.00	1.03 ± 0.03	1.16	N.D.[b]
Whole wood	27	0.91 (0.93)[c]	0.96 (0.98)	0.91 (0.90)	0.93 ± 0.03 (0.94)	1.14	N.D.
Middle lamella	60	0.64	0.65	0.57	0.62 ± 0.04	1.00	2.4
Ratio SW/ML	–	1.59	1.63	1.75	1.66	–	⩾ 3.4

[a] Results from Whiting and Goring (1981b)
[b] N.D. Not detectable
[c] Values in brackets calculated from middle lamella and secondary wall data

Such a large difference in the methoxyl contents of the lignin in the secondary wall and middle lamella may help to explain some of the differences in the chemical reactivity of the two lignins. Lignins with para-hydroxyphenylpropane groups will have a higher probability of containing carbon-carbon bonds originating at the 3- or 5-position of the aromatic ring. Such bonds would be difficult to break, and the middle lamella lignin would accordingly be expected to be less readily dissolved in pulping. This has been found to be the case, particularly in kraft and acid sulphite pulping (Procter et al. 1967; Kerr, Goring 1976; Whiting, Goring 1981a).

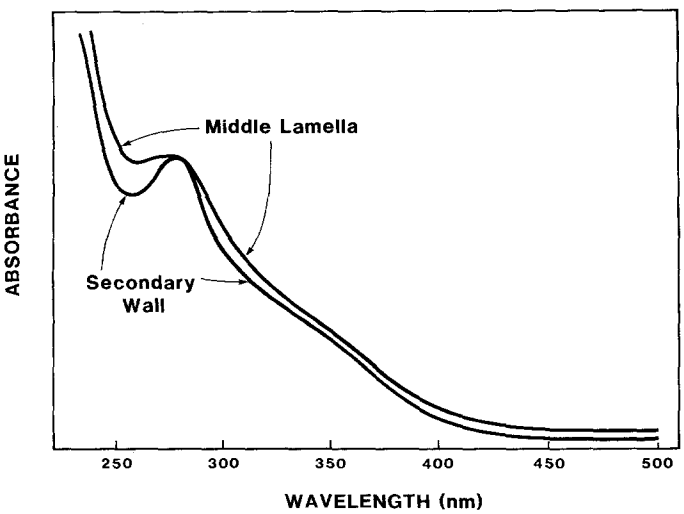

Fig. 1. UV-visible spectra of middle lamella and secondary wall tissue fractions. Wood falls between the two

It is interesting to note that, for hardwoods, the middle lamella contains an excess of guaiacyl lignin while the lignin in the secondary wall of the fibres is rich in syringyl residues (Fergus, Goring 1970; Musha, Goring 1975; Hardell et al. 1980b). The trend of low methoxyl contents in the middle lamella lignin is therefore true also in hardwoods although their overall methoxyl content is higher than that of the softwoods.

The results of the carbonyl analyses are reported in Table 1 as percent carbonyl of the lignin. Only the middle lamella tissue sample had a carbonyl content large enough to measure. The carbonyl content of the whole wood and secondary wall tissue was below the limit of detection of 0.3 %. If we assume that the whole wood tissue had a carbonyl content of 0.3 %, exactly at the limit of detectability, and that all the carbonyl resides in the lignin, a carbonyl content of 1.1 % can be calculated for the whole wood lignin. The carbonyl content of secondary wall lignin can then be calculated to be 0.7 %, using the proportions of middle lamella and secondary wall lignin reported earlier. This is, then, the largest value of carbonyl content possible for secondary wall lignin, as any higher value would have caused the carbonyl content of the whole wood to be detected. The ratio of middle lamella lignin carbonyl content to that of the secondary wall must, therefore, be greater than 3.

Whiting and Goring (1981 c) have shown that milled wood lignin originates predominantly in the secondary wall of the tracheid. Therefore, the value of 0.2 carbonyl groups per phenylpropane unit, reported by Adler and Marton (1959), should represent the carbonyl content of secondary wall lignin. However, this value is much higher than the value of 0.05 carbonyl groups per phenylpropane unit, the maximum possible value based on the present results. The reason for this anomaly is not known.

Carboxyl analyses were made on the secondary wall, whole wood, and middle lamella tissue samples, as well as some other samples with various lignin contents. As shown in Fig. 2, there was a linear increase in carboxyl content as the lignin content increased. It is important to note that this trend does not necessarily mean that the carboxyl groups are part of the chemical structure of the lignin. The increase in lignin content is due to an increasing proportion of middle lamella tissue in the fraction.

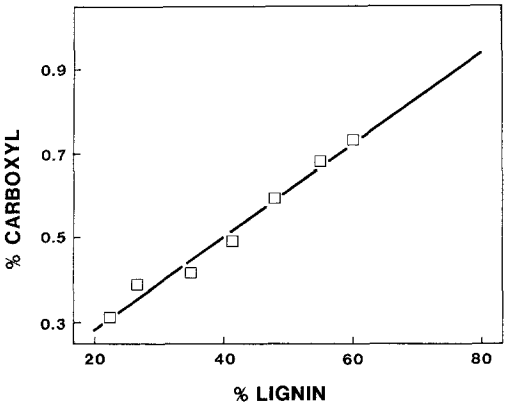

Fig. 2. Plot of percent carboxyl groups against percent lignin in tissue fractions

Table 2. Elemental analyses of tissue fractions

Tissue fraction	Percent lignin	Carbon (percent)	Hydrogen (percent)	Oxygen (percent)
Secondary wall	22	47.1	6.1	46.8
		(48.6)[a]	(6.2)	(45.2)
Whole wood	27	47.6	6.2	46.2
		(49.7)	(6.2)	(44.1)
Middle lamella	60	53.2	6.5	40.3
		(56.1)	(6.3)	(37.6)

[a] Values in brackets were calculated from elemental analyses for milled wood lignin and polysaccharide

Thus, the trend in Fig. 2 shows only that a high carboxyl content is associated with middle lamella tissue. If the lignin content is extrapolated to 80%, approximately the lignin content of the true middle lamella (Fergus et al. 1969), the carboxyl content is found to be about 0.95%. This is three times the value of 0.31% measured for the carboxyl content of the secondary wall tissue. The higher carboxyl content of the middle lamella could be due to the large pectic acid content in this region (Meier 1961).

Elemental analyses for the three tissue samples are given in Table 2. As expected, the middle lamella tissue had the highest carbon and lowest oxygen contents. If we assume that the samples contain only polysaccharide and lignin, we can calculate the elemental composition expected for each tissue fraction. The empirical formula of polysaccharide is $C_6H_{10}O_5$, which corresponds to 44.4% C, 6.2% H, and 49.4% O. Elemental analyses of black spruce milled wood lignin give 64.1% C, 6.4% H, and 29.5% O (Fleming, Bolker 1981). By means of these values, and the lignin contents of each fraction (Table 1), the elemental compositions given in brackets in Table 2 were calculated. In all cases, the calculated values of carbon content were higher than the observed values. The reason for these discrepancies is not known. Part of the anomaly may arise from the fact that $C_6H_{10}O_5$ is not the exact empirical formula for the total non-lignin fraction in wood. Or again, some of the lignin in the cell wall and middle lamella may contain more oxygen and less carbon than the milled wood lignin. Further research on carefully prepared fractions is necessary to resolve this anomaly.

Concluding Remarks

Significant differences in the chemical constitutions of middle lamella and secondary wall tissue of spruce wood have been found. The most important variation was the small value of the methoxyl content of the middle lamella lignin compared with that of the secondary wall lignin. This difference may help to explain observed differences in the way in which the two lignins react chemically. It also suggests that there must be two lignin precursors present during the biosynthesis of lignin in softwoods, namely coniferyl alcohol and para-coumaryl alcohol. Apparently, the latter is present during,

or takes part in, the stage when the cell is producing its middle lamella. It is interesting to note that milled wood lignin prepared from compression wood in *Pinus radiata* also shows a high para-hydroxyphenyl content (Bland 1961).

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