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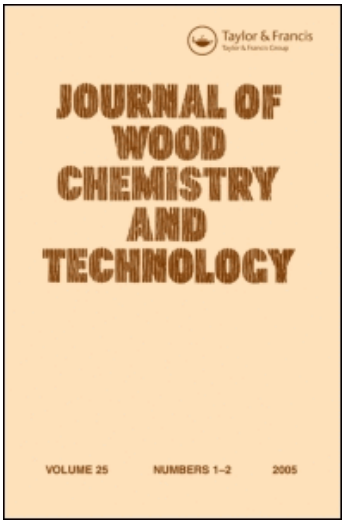

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Structural Characteristics of Compound Middle Lamella Lignin

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STRUCTURAL CHARACTERISTICS OF COMPOUND MIDDLE LAMELLA LIGNIN

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ABSTRACT

One of the most important targets of current lignin chemistry is to visualize the inhomogeneities of lignin in the wood cell wall. The soft xylem fraction was separated from birch wood at the end of May, 1983. Cell walls of the soft xylem fraction were extremely thin and were mainly composed of compound middle lamella. Degradation products from the soft xylem by alkaline nitrobenzene oxidation and permanganate oxidation strongly indicated that compound middle lamella lignin in hardwood must be rich in guaiacyl units and highly condensed.

INTRODUCTION

It is generally believed that structures of lignin are not homogeneous in the cell wall. For example, Goring et al.[1] measured the UV spectra of ultra-thin transverse sections of birch wood and determined the lignin content and the ratio between guaiacyl and syringyl units in the lignin. This information obtained by UV-microscopic analysis was very useful to indicate the structural inhomogeneity of lignin in the cell wall.

However, in order to have definite information about the chemical structures of lignins in the different morphological regions, it is extremely important to separate cell wall fractions such as vessel, secondary wall

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and compound middle lamella of wood fiber.

The authors[2] have developed a method for the separation of a lignin rich film-like substance from high yield pulp. This method basically depends on fractionation of pulp by a combination of sieving and gravitational sedimentation. Because of the high lignin content (43.8%), this substance is attributed to compound middle lamella separated from the fiber surface by mechanical defibration.

Hardell et al.[3,4] also have fractionated wood powder and high yield pulp into wood fiber, ray cell and fine fractions by a similar method, and attributed the fine fraction to compound middle lamella.

It is very interesting to note that lignin in both of the cell wall fractions attributed to compound middle lamella were shown to be guaiacyl rich by alkaline nitrobenzene oxidation. However, it is still not clear whether these fractions really represent compound middle lamella because of their extremely low yields.

In addition, Goring et al.[5,6] have also developed a new method for the separation of cell wall fractions by centrifugal sedimentation. But as far as we know, it has not been applied to hardwoods.

In the earlier paper,[7] the authors discussed the origin of Björkman's milled wood lignin (MWL) and concluded that the compound middle lamella lignin is preferentially extracted from hardwood meals as MWL at the initial stage of vibratory ball milling. So, MWL might provide some information about the chemical nature of compound middle lamella lignin. However, MWL is, in any case, a mixture of lignin fractions from every morphological region of the cell wall.

In order to have direct information about the nature of the compound middle lamella, the authors, in this paper, tried to characterize the soft xylem lignin. The basic idea of this study is as follows. Lignification of hardwood cell wall initiates at the cell corner and

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compound middle lamella regions. Therefore, if we can look at the lignin in the freshly lignified soft xylem, it will give us a clear idea of the chemical nature of compound middle lamella lignin.

EXPERIMENTAL

Separation of Soft Xylem from Birch Wood

A 12 year old birch wood (Betula maximowiczii Regel) was felled at the end of May, 1983 at the Univ. Forest, Chichibu, Japan. After peeling off the outer and inner barks, the soft xylem was carefully collected by scratching the surface with a dull blade. This is basically according to the techniques reported previously.[8] Soft xylem was kept in ethanol and heated to 70°C for 10 min to deactivate the enzymes which might be present in the soft xylem. Then, it was sequentially extracted with ethanol-benzene (1:2), acetone-water (1:1) and methanol to completely remove the extractives. From 77kg of birch wood (dry weight 43kg), about 5g of extractive-free soft xylem was obtained.

Deproteinization of Soft Xylem

Nitrogen content of the extractive-free soft xylem was 2.2% which indicates that protein content in this sample should be higher than 13%.[9] For the further analysis of the soft xylem fraction, it seemed to be essential to remove protein without any chemical change of lignin. For this purpose, the soft xylem fraction was treated three times with pepsin (Diagnostic System Inc., 3600u/mg) under the following conditions : (soft xylem fraction 2.5g + pepsin 0.5g) / 100ml 0.1N-HCl, 39±1 °C, 24hr, with shaking. After every pepsin treatment, nitrogen content of the soft xylem was determined. It was found that after three sequential treatments of pepsin,

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nitrogen content decreased to 0.66%. Although the nitrogen content was still not negligible, it seemed to be low enough to proceed with further analysis.

Concerning possible acid catalyzed condensation of lignin during pepsin treatment, it was confirmed in the preliminary experiment to not be significant based on the alkaline nitrobenzene oxidation of soft xylem before and after the pepsin treatment.

Alkaline Nitrobenzene Oxidation

A 40mg sample was sealed in a 10ml volume stainless steel autoclave with 4ml 2N-NaOH and 0.24ml nitrobenzene and heated at 160 °C for 2 hr. The mixture was treated according to the procedure reported previously,[10] and the aromatic aldehydes formed by the reaction were determined by G.L.C. as trimethylsilyl ethers. The conditions for G.L.C. were as follows. Column; OV-1, column temperature; 180 °C, carrier gas; He 15ml/min, H_2; 0.8 atm, air; 1L/min, detector; FID, internal standard; acetoguaiacone.

Potassium Permanganate Oxidation

A 400mg sample and 20ml of kraft cooking liquor (NaOH 3.5g + $Na_2S \cdot 9H_2O$ 3.1g/ 100ml) were sealed in a 50ml volume stainless steel autoclave after bubbling N_2 gas for 1 min and heated for 2hr at 170 °C. The mixture was acidified and saturated with NaCl and then extracted with acetone-chloroform (2:1). The extract was evaporated to dryness and methylated with $(CH_3)_2SO_4$.

Potassium permanganate oxidation of the methylated sample was performed by the procedure reported previously,[11] and the aromatic acids formed by the reaction were determined by G.L.C. and GC-MS (JEOL DX-300) as methyl esters. G.L.C. conditions were as follows. Column; SP 2100, column temperature; 100-240 °C, 4°C/min, carrier gas; N_2 22.5ml/min, H_2; 0.8 atm, air; 1L/min, detector; FID, internal standard; pyromellitic acid methyl ester.

Sugar Analysis

Neutral sugar components in the samples were analyzed by the alditol acetate method.[12] Acidic sugar

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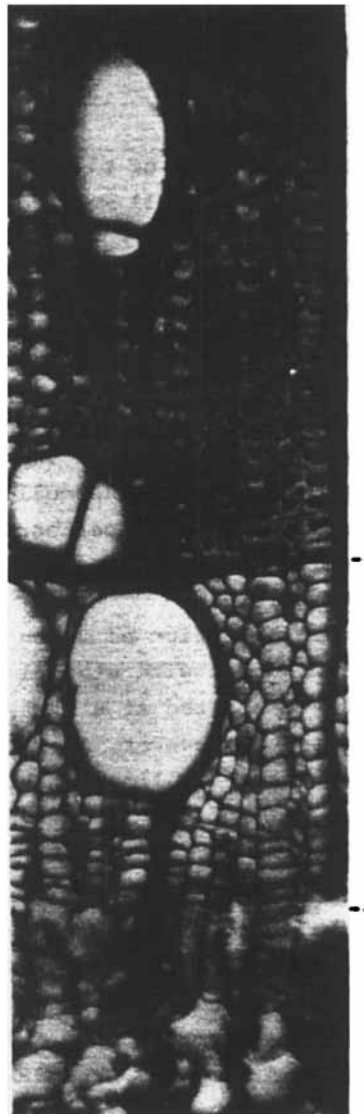

Photo 1

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components were determined by the method developed by Blumenkrantz et al.[13]

RESULT AND DISCUSSION

Preparation of Soft Xylem

Photo 1 shows the cross section of birch wood after peeling off the outer bark. Cell walls in the soft xylem are extremely thinner than those in the old tissue and are at the beginning of the secondary wall formation. In other words, those cell walls are presumably composed mainly of compound middle lamella. Cross sections of birch wood after peeling off the inner bark and then after scratching off the soft xylem are shown in Photos 2 and 3, respectively. These photos show that the soft xylem tissue was collected successfully without contamination of inner bark and old tissue.

Structure of Lignin in Soft Xylem

Phloroglucinol-HCl color reaction of birch wood cross section thoroughly extracted with ethanol showed that some lignin is already deposited at almost all parts of the soft xylem tissue. However, it is very important to note that the degree of lignification is not homogeneous in the soft xylem tissue. Basically, lignin concentration in soft xylem cell wall increased gradually from outer cell to inner cell, and vessel cell wall and wood fiber cell wall around the vessel element showed higher lignin concentration than the other wood fiber cell walls in the soft xylem.

Lignin content in the pre-extracted and deproteinized soft xylem was determined to be 28.1% and 24.9% by the Klason and acetyl bromide methods, respectively. Nitrogen (0.89%) was also found in the Klason lignin of

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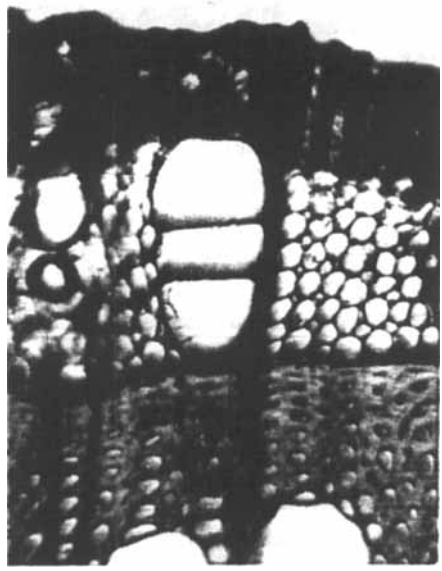

Photo 2

Photo 3

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the soft xylem. Thus, condensation of the nitrogen containing compound with lignin during the lignin determination might be one reason for the higher lignin content by the Klason method.

Since lignin content for the whole wood was 19.6% by the acetyl bromide method, the soft xylem has a higher lignin content than whole wood. Here, if the soft xylem tissue is composed of compound middle lamella only, it might be one half or less of the final lignification, because lignin content in the middle lamella of matured wood has been reported to be 34-40% at the area between the cell corners and 72-85% at cell corners.[1]

Table 1 shows the alkaline nitrobenzene oxidation products from the soft xylem and whole wood. First of all, total aldehyde yield from the soft xylem was only about one tenth of the whole wood. This clearly indicates the highly condensed structure of lignin in the

Table 1 Alkaline Nitrobenzene Oxidation Products from Soft Xylem and Whole Wood of Birch Wood

Products	Soft Xylem	Whole Wood
Total aldehyde	4.0%*	42.7%*
Vanillin	1.6	8.0
Syring-aldehyde	1.4	34.7
p-Hydroxy-benzaldehyde	1.0	-
S/V	0.69	3.62
P/V	0.79	-

* based on lignin content by acetyl bromide method
V: vanillin, S: syringaldehyde, P: p-hydroxy-benzaldehyde, S/V, P/V: molar ratio

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soft xylem. The S/V molar ratio for the soft xylem was also quite different from whole wood. That is, only 0.69 for soft xylem and 3.62 for whole wood. This means that of the non-condensed type structural units, the number of guaiacyl units is higher than that of syringyl units. This is quite contrary to the syringyl rich nature of the whole lignin. Since the highly condensed nature of soft xylem lignin is presumably attributed to condensed type guaiacyl units, contribution of guaiacyl units to the soft xylem lignin ought to be much higher than that of syringyl units. Lastly, it must also be pointed out that p-hydroxy benzaldehyde was confirmed only in the case of the soft xylem.

The soft xylem was also analyzed by permanganate oxidation after the heating with kraft cooking liquor and the methylation of free phenolic hydroxyl groups with $(CH_3)_2SO_4$. As shown in Table 2, again the soft

Table 2 Permanganate Oxidation Products of Soft Xylem*1 and Whole Wood*1 of Birch Wood

Methyl Ester	Relative amounts, molar ratio	
	Soft Xylem	Whole Wood
Anisic acid	0.03(0.02-0.03)	-
Veratric acid	1.00	1.00
Trimethyl-gallic acid	0.24(0.18-0.33)	2.17(1.74-2.67)
Isohemipinic acid	0.05(0.04-0.06)	0.02(0.01-0.02)
Metahemipinic acid	0.04(0.02-0.05)	0.01
TA/VA	0.24	2.17
Yield, %*2	11.8	27.1

*1 preheated with kraft cooking liquor and methylated with $(CH_3)_2SO_4$

*2 based on lignin content by acetyl bromide method

TA: trimethylgallic acid methylester

VA: veratric acid methylester

TA/VA: molar ratio

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xylem lignin provides quite different results from those of the whole wood lignin. Total yield of aromatic acids from soft xylem lignin was less than one half of that from whole wood lignin, based on the lignin contents determined by the acetyl bromide method. The relative yield of tri-methylgallic acid to veratric acid was about one ninth of that for whole wood lignin. This means that the soft xylem lignin is rich in guaiacyl units rather than syringyl units. On the other hand, isohemipinic and metahemipinic acids which correspond to the condensed type guaiacyl units in lignin, were obtained in higher relative quantities to veratric acid from the soft xylem than whole wood. Besides these acids, trace amounts of 6-carboxy-tri-

methylgallic acid and 5-carboxyanisic acid which were attributable to the condensed type syringyl units and the condensed type p-hydroxyphenyl units, respectively, were confirmed by GC-MS in the case of the soft xylem. These results are in very good accordance with those of alkaline nitrobenzene oxidation discussed earlier.

Based on this information, it would be possible to discuss the structural characteristics of compound middle lamella lignin, if the soft xylem was only composed of wood fibers. However, this is not the case. Some amount of vessel elements were also observed in the soft xylem as shown in Photo 1, and lignin in the vessels has been reported to be guaiacyl rich, too. Therefore, it is extremely important to prepare a vessel-free wood fiber fraction for further analysis and discussion of the lignin structure in the compound middle lamella between fibers.

Only 15mg of wood fiber fraction was separated from the mechanically defibrated soft xylem using a Stereo Scopic Microscope. Phloroglucinol-HCl color reaction of some fibers gave a light reddish-violet color, but others showed only a reddish-pink color. This must be due to different degrees of lignification in the soft xylem tissue as mentioned before, and the former fraction of wood fibers seemed to originate from the tissue around the vessel elements. Table 3 shows the yields of the alkaline nitrobenzene oxidation products from the wood fiber fraction thus obtained and the original soft xylem. Here, it is very interesting that again very low total aldehyde yield and S/V molar ratio were obtained from the wood fiber fraction of the soft xylem. It strongly suggests that lignin in the wood fiber fraction of the soft xylem, in other words, compound middle lamella between fibers, must have a guaiacyl-rich and highly condensed nature.

Sugar compositions of the soft xylem and whole wood are shown in Table 4. Rhamnose and arabinose

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Table 3 Nitrobenzene Oxidation Products of Fiber Fraction from Soft Xylem

Sample		Soft Xylem	Fiber Fraction
Aldehyde* (%)	Vanillin	5.0	7.3
	Syring-aldehyde	4.0	2.2
	Total	9.0	9.5
S/V		0.67	0.25

* based on lignin content by acetyl bromide method
S/V: molar ratio

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Table 4 Sugar Compositions of Soft Xylem and Whole Wood

Sample	Neutral Sugar,%						Acidic Sugar,%
	Rha	Ara	Xyl	Man	Gal	Glu	
Soft Xylem	1.04 (2.52)	2.70 (6.55)	7.32 (17.77)	+	++	30.13 (73.14)	23.1
Whole Wood	-	-	17.20 (31.45)	++	+	47.0 (68.55)	12.6

( ): relative composition of neutral sugar

residues were found only in the case of the soft xylem. The soft xylem fraction also showed a much higher acidic sugar content compared with the whole wood which has been known as a typical characteristic of the sugar composition of the primary wall.

CONCLUSION

1. The soft xylem fraction separated from birch wood at the end of May, 1983, in the middle of Japan, was a good material for analysis of the compound middle lamella lignin.
2. Lignin in compound middle lamella is rich in guaiacyl units rather than syringyl units. It is quite different from the syringyl rich nature of whole wood lignin.
3. Lignin in compound middle lamella is highly condensed.

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