

BIODEGRADATION OF GUAIACYL AND GUAIACYL-SYRINGYL LIGNINS  
IN WOOD BY PHANEROCHAETE CHRYSOSPORIUM

D. Tai\*, M. Terasawa\*\*, C.-L. Chen, H-m. Chang  
Department of Wood and Paper Science, North Carolina State University  
Raleigh, North Carolina 27650, USA  
and  
T. K. Kirk  
Forest Products Laboratory\*\*\*, Forest Service, USDA,  
Madison, Wisconsin 53705, USA

SUMMARY

Both high and low molecular weight lignin degradation products were isolated from spruce and birch woods decayed by Phanerochaete chrysosporium. The high molecular weight fractions were characterized by GPC and <sup>13</sup>C NMR spectroscopy. The data indicated that both lignins were degraded at the macromolecular level by the fungus. Birch lignin being more susceptible to degradation by the fungus than spruce lignin, and that syringylpropane units in birch lignin were preferentially degraded over guaiacylpropane units. Analysis of the <sup>13</sup>C NMR spectra resulted in elucidation of structural changes in the lignins caused by fungal degradation; both spruce and birch lignins appear to be degraded via similar modes and pathways, which are discussed in terms of sidechain, aromatic ring and alkyl-phenyl cleavages. The reactions involved include: (a) cleavage of β-O-4 linkages, (b) cleavage of C<sub>α</sub>-C<sub>β</sub> bonds with subsequent oxidation or reduction, (c) cleavage of C<sub>β</sub>-C<sub>γ</sub> bonds, (d) oxidative cleavage of aromatic rings and further degradation of the resulting intermediates, (e) oxidative cleavage of C<sub>7</sub>-C<sub>1</sub> bonds, and (f) oxidative decarboxylation of 5-aroxyvanillic acid moieties. From the low molecular weight fractions, 26 and 37 carbonylic acids were identified as fungal degradation products derived from spruce and birch lignins, respectively. The structures of these compounds support the involvement of the aforementioned reactions and suggest that in addition, an array of other reactions may also be involved in fungal degradation of the lignins.

I. INTRODUCTION

Although considerable research effort was made on the nature of lignin biodegradation, relatively little was known until very recently about the chemistry of lignin biodegradation. Constraints causing the slow progress in this field are traceable to the complex nature of lignin in terms of chemical

---

\* Present Address: Nanjing Technological College of Forest Products,  
Nanjing, China.

\*\* Present Address: Obihiro University, Obihiro, Hokkaido, Japan, 080.

\*\*\* Maintained in cooperation with the University of Wisconsin, Madison,  
Wisconsin, USA

structure, and to the resistance of lignin to intensive degradation by most of microorganisms in natural environments. However, considerable progress has been made in very recent years on the chemistry of lignin degradation by white-rot wood decaying fungi, particularly Phanerochaete chrysosporium, through two complementary approaches. One approach is characterization of the degradation products obtained from fungal degradation of lignin in woods (1-10), following earlier pioneering work (11-16). The other is investigation of the degradative pathways of model compounds related to lignin in ligninolytic culture discussed in other chapters in this volume.

In the first approach, extraction of wood decayed by white-rot fungus yields a small quantity of polymeric biodegraded lignin and a mixture of low molecular weight products. The polymeric biodegraded lignin has been characterized by a variety of chemical and spectroscopic methods (1-2, 11-13), including, very recently, <sup>13</sup>C NMR spectroscopy (3-7), providing considerable insight into the chemistry of lignin biodegradation. The low molecular weight fraction, however, have received little attention except for the identification of vanillin, syringaldehyde, vanillic acid and syringic acid (14-16). Very recently, we have analyzed the low molecular weight acidic fractions obtained from spruce (Picea glauca) and birch (Betula papyrifera) woods decayed by P. chrysosporium by high performance liquid chromatography (HPLC) as well as by gas chromatography/mass spectrometry (GC/MS), after fractionation and subsequent acetylation and methylation. Numerous compounds have thus been identified (5, 6, 14-16).

In this review, the chemistry involving biodegradation of guaiacyl and guaiacyl-syringyl lignins in woods will be discussed on the basis of the characterization of both high- and low-molecular-weight lignin degradation products obtained from spruce and birch woods decayed by P. chrysosporium.

## 11. FUNGAL DECAY OF WOODS, EXTRACTION OF DECAYED WOODS, AND ISOLATION OF BIODEGRADED LIGNINS

### A. Fungal Decay of Spruce and Birch Woods

Pre-extracted wood chips of spruce (Picea glauca) and birch (Betula papyrifera) were sterilized and partially decayed by Phanerochaete chrysosporium Burds. ME 446 (ATCC 35590) to an average total weight loss of about 8% per original dry weight of wood (3, 5, 8, 9). Loss in lignin based on the original lignin contents of the woods was 20% for spruce and 32% for birch (3,5). These correspond to dry weight losses of about 5.6 g and 6.4 g of the original lignin per 100 g of spruce and birch woods, respectively.

### B. Extraction of the Decayed Woods

The decayed wood chips, either spruce or birch, were air-dried and ground to pass a 40-mesh screen. The wood meal was successively extracted in a soxhlet apparatus with petroleum ether (b.p. 60-80°C) and chloroform, each continuously for 24 hours, respectively. Further extraction with methanol and 96% aqueous dioxane was carried out by steeping the air-dried wood meal in sufficient volume of the solvent for 24 hours at room temperature, followed by filtering and air-drying the decayed wood meal before the next extraction (3,5). Yields of

extracts from the decayed spruce and birch woods are given in Table 1. The methanol extracts were the major fractions among the extracts obtained from the decayed woods with yields of 0.58% and 2.2% for the spruce and birch, respectively.

Table 1. Yields of Extracts from Spruce and Birch Woods Decayed by *P. Chrysosporium*\*.

Solvent	Spruce	Birch
Petroleum Ether	2.5	1.6
Chloroform	2.0	2.9
Methanol	5.8	22.0
96% Aqueous Dioxane	5.0	8.7

\* Yield: g/kg Air-Dried Decayed Wood

Preliminary investigations indicated that constituents of the corresponding extract were similar in both cases:

- (a) Petroleum Ether-Extract: mostly wax-like materials, such as fatty acids, their esters, higher aliphatic acids, and even higher hydrocarbons.
- (b) Chloroform-Extract: mostly resinous materials, including various phenolics. Vanillin, pinosresinol and unknown phenols were detected in the extract from the decayed spruce wood by thin layer chromatography (tlc). Vanillin, syringaldehyde and syringresinol were similarly detected in the extract from the decayed birch.
- (c) Methanol-Extract: mostly lignin-like materials. Polymeric biodegraded lignins were isolated, in addition to low molecular weight acids and phenols derived from lignin.
- (d) 96% Aqueous Dioxane-Extract: mostly lignin-like materials and oligomeric carbohydrates in the form of lignin-carbohydrate complexes.

### C. Fractionation and Isolation of Polymeric Biodegraded Lignins from Methanol Extracts

The methanol extracts from the decayed spruce and birch woods were individually separated into acidic, phenolic and neutral fractions by means of successive treatment with dilute sodium bicarbonate and sodium hydroxide solutions at room temperature. The acidic fraction was the major fraction for both the spruce and birch methanol extracts, forming about 60% and 70% of the extractives, respectively. In terms of the yield on a decayed wood basis; however, the spruce acidic fraction accounts for only 0.355, whereas the birch acidic fraction is 1.6%; the latter is about 5 times more than the former. The phenolic fraction accounted for only about 14% of the spruce methanol extract, and about 10% of the birch methanol extract. The quantity of neutral fraction was insignificant in both cases.

The spruce fractions were further divided into high molecular weight acidic and phenolic fractions (S-M-HA and S-M-HP), and low molecular weight acidic and

phenolic fractions (S-M-LA and S-M-LP) by means of solubility in water, chloroform and tetrahydrofuran (3). The S-M-HA fraction was the major fraction of the spruce methanol-extract, forming about 45% (of the extract).

The birch acidic fraction was fractionated into high molecular weight acidic fractions 1, 2 and 3 (B-M-Ha-1, B-M-HA-2 and B-M-HA-3) and low molecular weight acidic fraction (B-M-LA) in a similar manner (5). The B-M-HA-2 and B-M-LA fractions were the major fractions of the birch methanol-extract, forming about 31% and 26% of the extract. The birch phenolic fraction consisted mostly of low molecular weight substances (B-M-LP).

The compositions of methanol-extracts from the spruce and birch woods decayed P. chrysosporium are summarized in Table 2. It can be observed that the high molecular weight part of the birch methanol extract consists mainly of the acidic fraction B-M-HAs. No high molecular weight phenolic substances were found in the birch methanol extract. Furthermore, the birch M-HA fractions, particularly B-HA-2, had a considerably lower methoxyl content than milled wood lignin (MWL) of birch, which has a methoxyl content of about 22%.

**Table 2. Compositions of Methanol-Extracts from Spruce and Birch Woods Decayed by P. Chrysosporium.**

Fraction	Spruce			Birch		
	g/kg wood*	%/MeOH-Ext.	OCH <sub>3</sub> %	g/kg wood*	%/MeOH-Ext.	OCH <sub>3</sub> %
M-HA-1	2.6	44.8	10.83	2.2	10.0	12.52
M-HA-2				6.8	30.9	7.52
M-HA-3				1.0	4.5	11.31
M-HP	0.6	10.3	13.79			
M-LA	0.3	5.2		5.8	26.4	
M-LP	0.2	3.6		2.3	10.4	
M-Neut.	0.1	1.7		0.1	0.5	
Total	3.8	65.6		18.2	82.7	

\* Per Air-Dried Decayed Wood

From the results presented in the sub-sections A, B and C of this section, it appears that birch lignin (guaiacyl-syringyl lignin) is more susceptible to fungal degradation by P. chrysosporium than spruce lignin (guaiacyl lignin). The possible basis for this difference is discussed below.

### III. CHARACTERIZATION OF POLMERIC BIODEGRADED LIGNINS

#### A. Gel Permeation Chromatography

Purified polymeric biodegraded lignins were chromatographed on Sephadex G-15 and LH-60 columns, using N,N-dimethylformamide (DMF) containing 0.1 M lithium chloride as solvent. Chromatograms were obtained by monitoring effluents with UV detector at 280 nm.

Gel permeation chromatograms of the purified S-M-HA and S-M-HP fractions indicated that the molecular weights were greater than 1,500 but less than 20,000, since these fractions were excluded from the Sephadex G-15 column, but not from the Sephadex LH-60 column (3), as shown in Fig. 1.

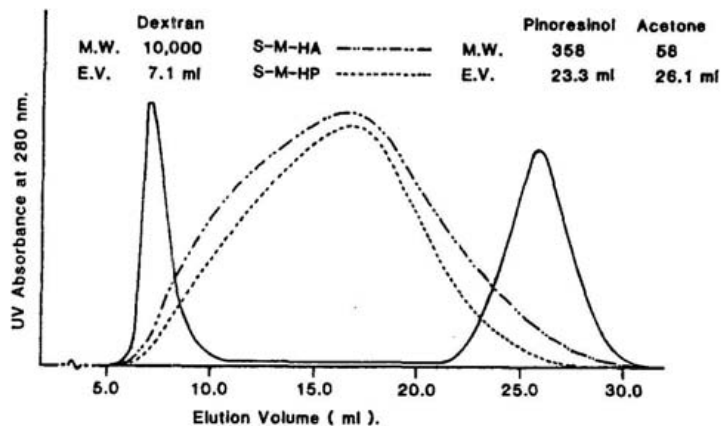


Figure 1. Gel Permeation Chromatograms of Biodegraded Spruce Lignin (S-M-HA and S-M-HP) Fractions  
 Column: Sephadex LH-60 (30 x 1 cm),  
 Solvent: DMF with 0.1 M LiCl

Similar results were obtained for the purified B-M-HA-1 and B-M-HA-2 fractions, as shown in Fig. 2. Gel permeation chromatograms of the purified

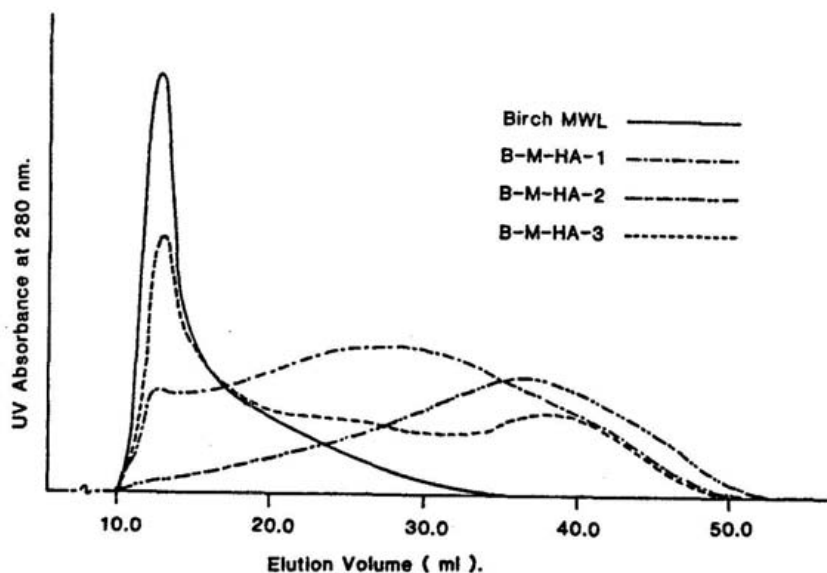


Figure 2. Gel Permeation Chromatograms of Birch MWL and Biodegraded Birch Lignin Fraction B-M-HA.  
 Column: Sephadex LH-60 (55 x 1 cm)  
 Solvent: DMF with 0.1 M LiCl

B-M-HA-3 fraction indicated that its molecular weight was also greater than 1,500. The fraction also contained components that were excluded from the Sephadex LH-60 column. A similar result was obtained for the purified birch MWL preparation (5).

The polymeric nature of these biodegraded lignins is of primary importance, since any change in structures observed in the lignins would indicate degradative reactions at the macromolecular level.

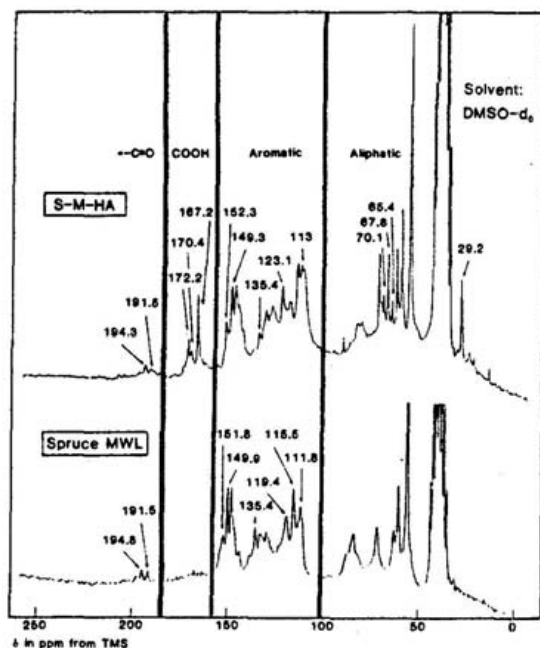


Figure 3.  $^{13}\text{C}$  NMR Spectra of Spruce MWL and Biodegraded Spruce Lignin Fraction S-M-HA.

## B. $^{13}\text{C}$ NMR Spectroscopy

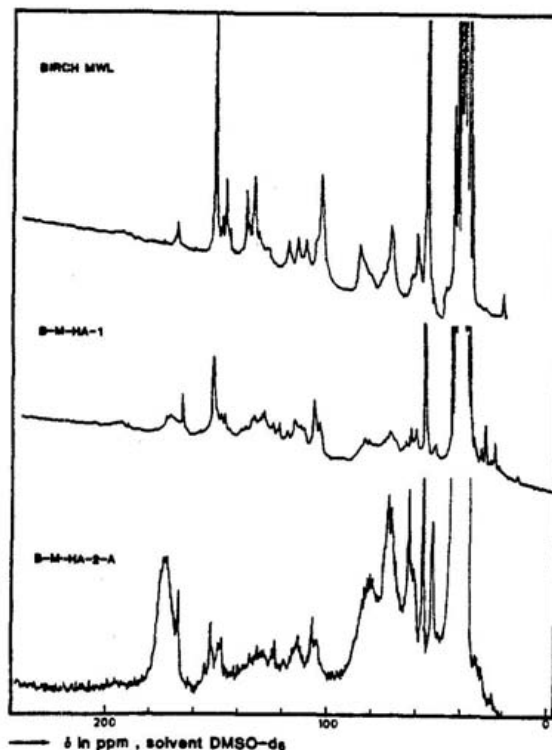
$^{13}\text{C}$  NMR spectra of the biodegraded lignins were obtained with a JEOL FX 60 Fourier transform spectrometer. Deuterated dimethylsulfoxide ( $\text{DMSO-}d_6$ ) was used as solvent. The signal for the  $\text{CD}_3$  group of  $\text{DMSO-}d_6$  was locked to the spectrometer. The signal had a  $\delta$  value of 39.6 ppm relative to tetramethylsilane (TMS) which was used as internal reference.

The  $^{13}\text{C}$  NMR spectra of the spruce and birch biodegraded lignins, some of which were shown in Figs. 3 and 4, were analyzed in detail, using those of spruce and birch MWLs as well as those of model compounds synthesized as references (3-7). The results are summarized as follows.

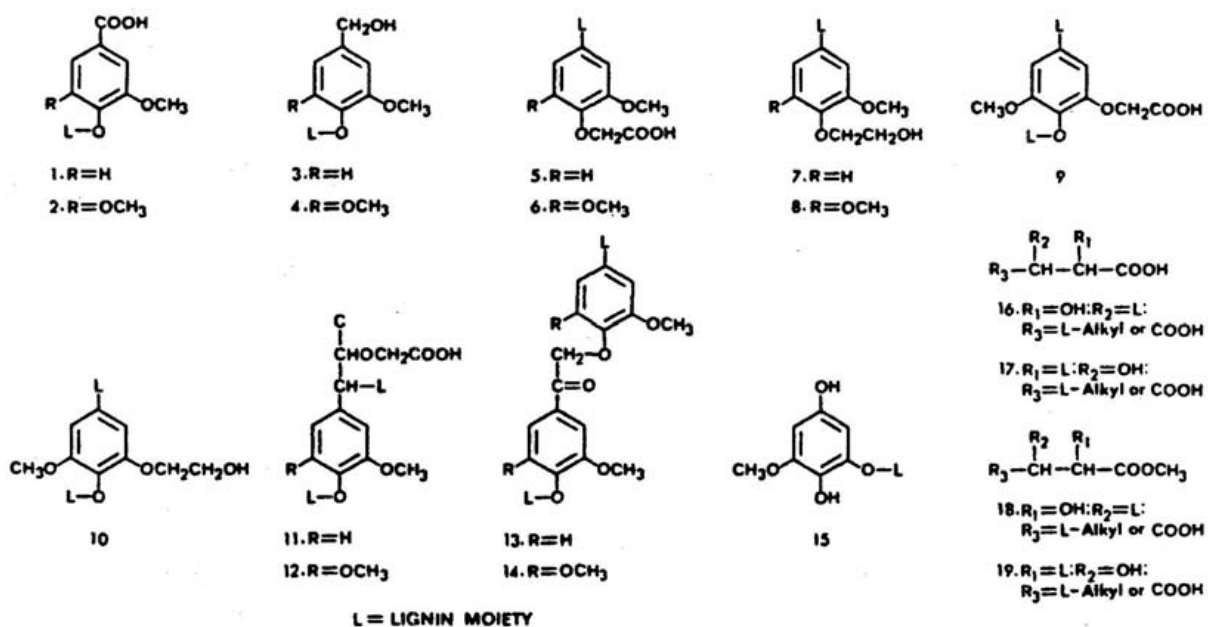
### 1. Biodegraded Spruce Lignins

The spectrum of S-M-HA fraction indicated the presence of newly formed sub-structures, namely, 4-O-alkylated vanillic acid (1), 4-O-alkylated vanillyl

alcohol (3), aroxyacetic acid (5), 2-aroxyethanol (7), alkoxyacetic acid (11) and saturated aliphatic hydrocarbon moieties (14,15), shown in Fig. 5.



**Figure 4.**  $^{13}\text{C}$  NMR Spectra of Birch MWL and Biodegraded Birch Lignin Fractions B-M-HA-1 and B-M-HA-2-A.



**Figure 5.** New Structures in Biodegraded Spruce and Birch Lignin (M-HAs) Elucidated by  $^{13}\text{C}$  NMR Spectroscopy.

The spectrum of S-M-HP fraction was similar to that of spruce MWL, except for the presence of signals corresponding to saturated aliphatic hydrocarbons (3).

## 2. Biodegraded Birch Lignins

The B-M-HA-2 fraction was fractionated into several sub-fractions by molecular sieve chromatography using Sephadex LH-20 column and methanol as solvent. The B-M-HA-1 and B-M-HA-3 fractions, and the major fractions from the B-M-HA-2 fraction were characterized by <sup>13</sup>C NMR spectroscopy. The spectra of these fractions indicated that the biodegraded birch lignins (B-M-HAs) contained newly formed sub-structures of the types 4-O-alkylated vanillic and syringic acids (1 and 2), 4-O-alkylated vanillyl and syringyl alcohols (3 and 4), aroxyacetic acids (5, 6 and 9), alkoxyacetic acids (11 and 12), 2-aroxyethanols (7, 8 and 10), 4-O-alkylated α-aroxyacetosyringones (13 and 14), methoxyhydroquinone ether (15), α- and β-hydroxy-acids (16 and 17), α- and β-hydroxy-acid methyl esters (18 and 19) and saturated hydrocarbons (5-7) (Fig. 5). The spectra indicated that the lignin in birch wood underwent intensive modification in both aromatic rings and side chains by the fungus.

## IV. CHARACTERIZATION OF LOW MOLECULAR WEIGHT ACIDIC FRACTIONS

Gel permeation chromatograms of the spruce and birch M-LA fractions indicated that these fractions contained components that were excluded from the Sephadex G-15 column. In order to remove these components having molecular weight greater than 1,500, the S-M-LA and B-M-LA fractions, dissolved in a minimal amount of tetrahydrofuran and chloroform, respectively, were added to ten volumes of diethyl ether to precipitate the higher molecular weight components. The precipitates were centrifuged off, and the ether-soluble parts were used for further analysis (6, 8-10).

### 1. Ether-Soluble Part of S-M-LA Fraction

The ether-soluble part was first analyzed directly by high performance liquid chromatography (HPLC), then by gas chromatography/mass spectrometry (GC/MS) after acetylation and methylation.

A total of 28 compounds were identified as the constituents of the ether-soluble part of the S-M-LA fraction (Fig. 6). These compounds included: (a) ten aromatic carboxylic acids -- 4-hydroxybenzoic acid (20), isovanillic acid (22), vanillic acid (23), veratric acid (24), 4-hydroxy-5-methoxyphthalic acid (30), *meta*-hemipinic acid (31), 4-hydroxy-5-methoxyisophthalic acid (32), isohemipinic acid (33), dehydrodivanillic acid (34), and 2'-hydroxy-2,3'-dimethoxydiphenyl-ether-4,5'-dicarboxylic acid (35); three carboxylic acids derived from degradation of guaiacylglycerol-β-aryl ether (β-O-4) units -- 3-methoxy-4-(4-hydroxy-3-methoxy-β-oxophenethoxy) benzoic acid (3), 3-hydroxy-4-(4-hydroxy-3-methoxy-β-oxophenethoxy) benzoic acid (38), and 2-O-(α-formyl-β,4-dihydroxy-3-methoxystryl)-2-hydroxy-4-oxo-2-butenic acid (39); (c) six carboxylic acids derived from degradation of 2,3-bisguaiacyl-1,3-propanediol (β-1) units -- 3-formyl-4-hydroxy-4-(4-hydroxy-3-methoxyphenyl) butanoic acid (40), 4-formyl-5-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-4-pentenoic acid (41), α-hydroxy-methyl-β-hydroxyferulic acid (42), 4-carboxy-5-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-2-

oxo-4-pentenoic acid (43),  $\alpha$ -(2-hydroxyethyl)- $\beta$ -hydroxyferulic acid (44), and 2-( $\alpha$ , 4-dihydroxy-3-methoxybenzylidene)-4-carboxy- $\beta$ -butenolide (45); (d) seven carboxylic acids derived from degradation of biphenyl (5-5) units -- 2-(2-hydroxy-3-methoxy-5-carboxyphenyl)-4-formyl-4-methyl- $\alpha$ -butenolide (46), 2-(2-hydroxy-3-methoxy-5-carboxyphenyl)-4-acetyl-4-methyl- $\alpha$ -butenolide (47), 2-(2-hydroxy-3-methoxy-5-carboxyphenyl)-4-carboxy- $\beta$ -butenolide (48), 5-(2-oxoethyl)vanillic acid (49), 5-(3-oxopropyl)vanillic acid (50),  $\beta$ ,2-dihydroxy-3-methoxy-5-carboxycinnamic acid (51), and 5-(1-hydroxy-3-oxo-1-propenyl)vanillic acid (52); (e) two carboxylic acid of unknown origin -- ferulic acid, and 2-(2-methylpropyl)-3-phenylpropanoic acid. Vanillic acid (23) was the major component (20%) of the ether-soluble part of S-M-LA fraction. Compounds 22, 24, 31, 33, 48 and 49 were moderately abundant. Other compounds were minor components (8, 9).

## 2. Ether-Soluble Part of B-M-LA Fraction

The ether-soluble part was first analyzed in the same manner described for the S-M-LA fraction (5). However, the analysis, by GC with a capillary column (HP Silicone SE-54, 12mx 0.2mm i.d.) revealed more than 150 components.

To identify constituents of the ether-solubles efficiently, the sample was acetylated, and the resulting acetylated mixture was divided into ether-soluble, and ether-insoluble but chloroform-soluble parts at room temperature. The ether-soluble part of the acetylated sample was then methylated and fractionated into 25-sub-fractions by combination of GPC (Sephadex LG-20) and HPLC (silica gel). The first ten sub-fractions were analyzed by GC/MS, respectively, resulting in identification of about 100 compounds (6, 10). These compounds included aromatic acids, aliphatic acids with or without a terminal p-quinone moiety, phenols, and even higher hydrocarbons (10).

Among the carboxylic acids identified, 37 contained either a guaiacyl or syringyl moiety (Fig. 6), and must have been produced by fungal degradation of the birch lignin. These carboxylic acids included: (a) seventeen aromatic carboxylic acids -- protocatechuic acid (21), 5-O-methylgallic acid (25), 4,5-O-dimethylgallic acid (26), syringic acid (27), 3,4,5-O-trimethylgallic acid (28), 4,5-dihydroxy-phthalic acid (29), and 2'-hydroxy-2,6,3'-trimethoxybiphenylether-4,5'-dicarboxylic acid (36) in addition 60 compounds 19, 22-24 and 30-35, detected also in the S-M-LA fraction; (b) seven carboxylic acids derived from C-2 substituted arylpropane ( $\alpha$ -2 or  $\beta$ -2) units -- 2-carboxy-4-hydroxy-5-methoxybenzoylformic acid (59), 2-carboxy-4,5-dihydroxy-5-methoxybenzoylformic acid (60), 2-carboxy-4,5-dihydroxy-3-methoxybenzoylformic acid (61), 3-hydroxy-6-acetylbenzoic acid (62), 6-(1-hydroxyethyl)protocatechuic acid (63), 6-(1-hydroxy-ethyl)isovanillic acid (64), and 6-(1-hydroxyethyl)veratriracid (65); (c) two carboxylic acids derived from degradation of arylglycerol- $\beta$ -arylether ( $\beta$ -O-4) units -- 2-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-3-oxopropanoic acid (66), and 2-hydroxy-3-(2-hydroxy-3,5-dimethoxyphenyl)-3-oxopropanoic acid (67); (d) eight carboxylic acids derived from degradation of biphenyl (5-5) units -- 3-(2-hydroxy-3-methoxy-5-carboxyphenyl)propanoic acid (53), 2-hydroxy-3-methoxy-5-carboxymandelic acid (54), 5-acetylvanillic acid (55), 5-(1-hydroxyethyl)-vanillic acid (56), 5-(4-hydroxy-1-oxobutyl)vanillic acid (57), 4-(2-hydroxy-3-methoxy-5-carboxyphenyl)-2-oxo-butanoic acid (58), and 5-(1,4,5-trioxopentyl)-protocatechuic acid (68) in addition to compounds 49 and 51 detected in the S-M-LA fraction; (3) one carboxylic acid derived from degradation of unusual C-2

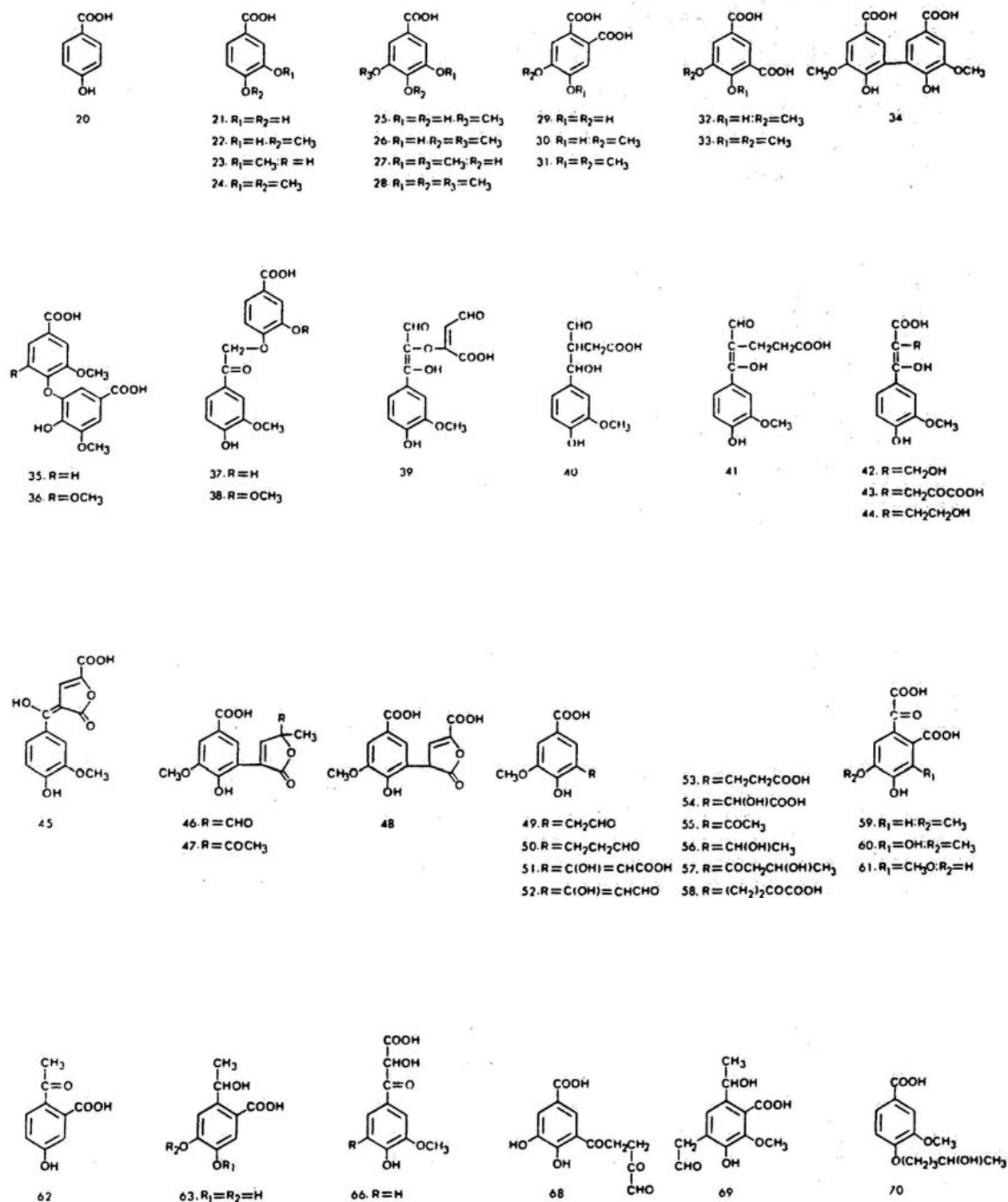


Figure 6. Compounds Identified in Spruce and Birch M-LA Fractions by CG/MS.

substituted biphenyl or phenylcoumaran (5-5 or  $\beta$ -5) units -- 3-hydroxy-2-methoxy-4-(2-oxoethyl)-5-(1-hydroxyethyl)benzoic acid (69); and (f) one carboxylic acid derived from degradation of biphenyl ether (4-O-5') unit -- 3-methoxy-4-(4-hydroxypentanoxy)benzoic acid. Syringic acid (27) and vanillic acid (23) were the major components of the ether-soluble part of B-M-LA fraction, about 20% and 10%, respectively. Compounds 22, 25, 26, 28, 59, 61 and 67 were moderately abundant components, each forming about 5% of the part. Other compounds were minor components (5, 10).

### 3. Identity of Compounds Detected in Ether-Soluble Parts of S-M-LA and B-M-LA Fractions

The identities of compounds 20-38 were established by comparison of their HPLC and GC retention times, including spiking, as well as by comparison of their mass spectra with those of authentic samples (5, 8-10). The structures of other compounds are tentative, since they were elucidated solely on the basis of the mass spectra obtained by the GC/MS analyses.

## V. MODES OF FUNGAL DEGRADATION OF LIGNINS IN WOODS

As wood is decayed by white-rot fungi, all of the components are degraded progressively from the cell lumens toward the middle lamella (17,18). The hyphae of the fungi are normally localized in the cell lumens, and penetrate from one cell to another through openings, or by producing bore holes in the cell walls (19,20). Degradation of the wood components is concentrated at the surfaces exposed to the lumens and contiguous capillary openings (19). The relative amounts of lignin and carbohydrates degraded by white-rot fungi differ considerably, depending on the nature of the fungus species, as does the order of preferential attack. In the present study, *P. chrysosporium* preferentially degraded the lignin in both spruce and birch woods. The modes of lignin degradation by this fungus are discussed below on the basis of the results described in the previous sections.

### A. Biodegradation of Lignin at Macromolecular Level

The GPC studies of the S-M-HA, S-M-HP and B-M-HAs indicated that the spruce and birch biodegraded lignin fractions had molecular weights in the range of 1,500-20,000. These facts, together with the  $^{13}\text{C}$ NMR characteristics of these fractions shows that the spruce birch lignins underwent fungal degradation in part at the macromolecular level via cleavage of side chains as well as oxidative cleavage of aromatic moieties.

### B. Preferential Degradation of Syringylpropane Units

BirchMWL was estimated to have aromatic ring uncondensed syringylpropane and guaiacylpropane units in a molar ratio (US/UG molar ratio) of about 1.6 from integration of signals in the aromatic tertiary C-atom region ( $\delta$  100-130 ppm) of the  $^{13}\text{C}$ NMR spectrum, as shown in Fig. 7 (6,7). This ratio is in good agreement with the US/UG molar ratio of about 1.7 for MWL of birch (*Betula verrucosa*) estimated from potassium permanganate oxidation of methylated MWL by Larsson and Miksche (21). The US/UG ratios for the birch biodegraded lignins (B-M-HAs) were estimated to be in the range of 0.8-1.0 from the corresponding  $^{13}\text{C}$  NMR

spectra. An example is given in Fig.7. The results indicate that the aromatic rings of uncondensed syringylpropane units were preferentially degraded over those of uncondensed guaiacylpropane units by the fungus. Kirk et al (29) found earlier that syringylpropane units were degraded preferentially during the initial phase of the decay of birch wood by the white-rot fungus, Coriolus versicolor.

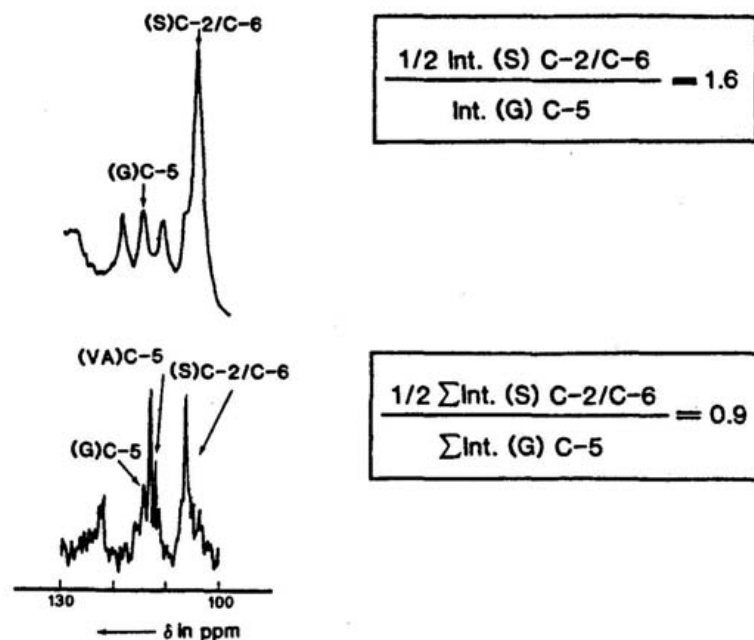


Figure 7. US/UG Molar Ratios of BirchMWL (A) and a Biodegraded Birch Lignin Fraction (B) Estimated by <sup>13</sup>C NMR Spectroscopy.

Two possible causes for the preferential degradation of the syringylpropane units over the guaiacylpropane units in birch lignin can be considered: (a) the fungus attacks from the lumens and encounters first a lignin rich in syringylpropane units, as postulated by Kirk *et al* (29), and (b) syringylpropane units in the birch lignin are more labile to degradation by the fungus than guaiacylpropane units because of the nature of chemical bondings associated with the syringylpropane units. The first possibility is plausible, because Fergus and Goring (23) found that lignin in the vessel secondary walls and middle lamellae of birch xylem consisted mostly of guaiacylpropane units, whereas that in the major part of the wood – the fiber secondary walls -- was composed mainly of syringylpropane units. In addition, lignin in the middle lamella around fibers and ray cells contained both guaiacylpropane and syringylpropane units. However, the first possibility cannot explain the fact that spruce lignin is less susceptible to degradation by the fungus than is birch lignin, as found in this investigation. Thus, the second possibility must also be involved in the preferential degradation of syringylpropane units.

### C. Cleavage of Side Chains

Recently, degradation of lignin-related model compounds by white-rot fungi

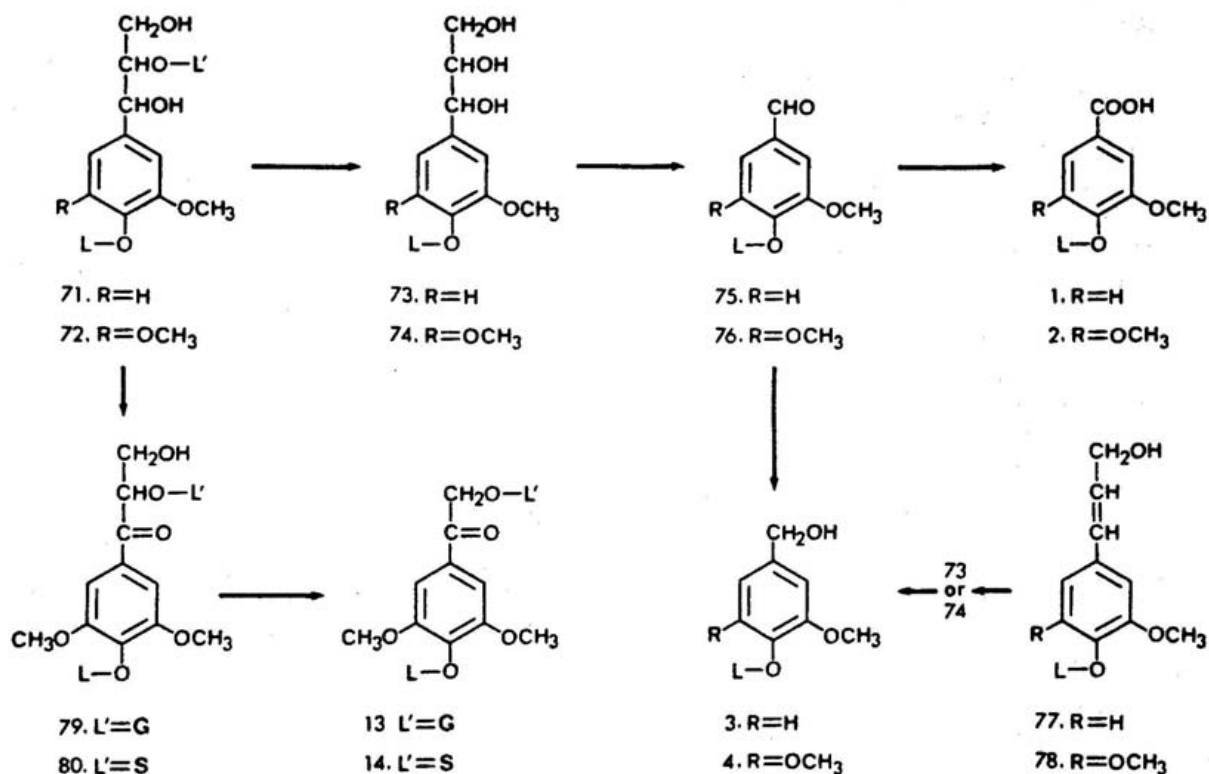
P. chrysosporium has been intensively investigated in the laboratories of Higuchi (24-26), Gold (27-33) and Kirk (34,35). In the cases of arylglycerol-aryl ether ( $\beta$ -O-4) models (25, 27-31), dehydrodiconiferyl alcohol ( $\beta$ -5) models (24, 27) and 2,3-diarylpropane-1,3-diol( $\beta$ -1) models (32-35), non-phenolic compounds or non-phenolic moieties are generally degraded via cleavage of the  $C_\alpha$ - $C_\beta$  bonds to produce 4-O-alkylated vanillic acid and/or 4-O-alkylated vanillyl alcohol derivatives. In contrast, phenolic compounds undergo oxidative coupling/polymerization, and to some extent, degradation via alkyl-phenyl cleavage ( $C_a$ - $C_c$  cleavage) to produce methoxyquinone and/or related compounds.

The spruce biodegraded lignin, S-M-HA fraction, contained 4-O-alkylated vanillic acid and vanillyl alcohol moieties 1 and 3. The birch biodegraded lignins, B-M-HA fractions, contained 4-O-alkylated syringic acid and syringyl alcohol moieties 2 and 4 in addition to the moieties 1 and 2. Thus, it is evident that oxidative cleavage of the  $C_\alpha$ - $C_\beta$  bond in side chains occurred at the macromolecular level during the fungal degradation of lignins in spruce and birch woods.

Since non-phenolic arylglycerol- $\beta$ -arylether units are the major sub-structures in both spruce and birch lignins (21, 36), 4-O-alkylated vanillic and syringic acid moieties 1 and 2 were mostly produced from the non-phenolic  $\beta$ -O-4 units 71 and 72 as shown in Scheme 1, involving cleavage of the  $\beta$ -O-4 bonds to the arylglycerol intermediates 73 and 74 (30, 31), subsequent oxidative cleavage of the  $C_\alpha$ - $C_\beta$  bonds to the corresponding benzaldehyde intermediates 75 and 76, and further oxidation of the aldehyde groups, respectively (25). Alternatively, reduction of the aldehyde intermediates 75 and 76 led to formation of 4-O-alkylated vanillyl and syringyl alcohol moieties 3 and 4, respectively (25, 27, 28, 30, 31). Moiety 1 could also be produced from non-phenolic  $\beta$ -5 units, which are moderately abundant sub-structures in spruce lignin but minor ones in birch lignin (22, 37), via oxidative cleavage of the  $C_\alpha$ - $C_\beta$  bond in the phenylcoumaran( $\beta$ -5) substructures (24). Moieties 3 and 4 could be also formed from the corresponding non-phenolic  $\beta$ -1 units, which are minor sub-structures both in spruce and birch lignins, via oxidative cleavage of the  $C_\alpha$ - $C_\beta$  bonds, respectively (32-35). Nakatsubo *et al.* (24) found that the terminal cinnamyl alcohol moiety in a phenylcoumaran model dimer is degraded by P. chrysosporium via a phenylglycerol and  $C_\alpha$ - $C_\beta$  cleavage. Similarly, Shimada *et al.* (38) found that veratryl alcohol, the major biosynthetic metabolite of the fungus; was actually a degradation product of the true secondary metabolite, 3,4-dimethoxycinnamyl alcohol. Thus, terminal cinnamyl alcohol moieties of the types 77 and 78 in spruce and birch lignins could be degraded to moieties 3 and 4 by the fungus via the same pathway.

The  $C_\alpha$ - $C_\beta$  cleavages in the lignins have been found very recently to be catalyzed by an extracellular enzyme (39). Moieties of the type  $\alpha$ -aroxyacetosyringone 13 and 14 were present in the biodegraded birch lignins, indicating that the birch lignin also underwent cleavage of  $C_\beta$ - $C_\gamma$  bonds in side chains during the fungal degradation at the macromolecular level. The identification of compounds 37 and 38 and the S-M-LA fraction indicated further that cleavage of the  $C_\beta$ - $C_\gamma$  bond also occurred during the fungal degradation of spruce lignin. In this case, however, it is not known whether the cleavage occurred at the macromolecular level or in small fragments released from the spruce lignin.

The 4-O-alkylated  $\alpha$ -arylacetosyringonemoieties 13 and 14 were probably produced from non-phenolic  $\beta$ -O-4 units of the type 72 via oxidation of the  $\alpha$ -hydroxyl groups to the correspondig  $\alpha$ -carbonyl intermediates 79 and 80, and subsequent cleavage of the C<sub>β</sub>C<sub>γ</sub> bonds in the side chains involving a reverse aldol addition with elimination of formaldehyde, respectively.



Scheme 1. Possible Pathways for Degradation of Side Chains in Non-Phenolic  $\beta$ -O-4 units by P. chrysosporium.

L = L' = Lignin Moieties; G = Guaiacylpropane Units;  
S = Syringylpropane Unit.

#### D. Cleavage of Aromatic Rings

In contrast to the situation with other microorganisms, the modes of aromatic ring cleavage in lignin-degrading fungi are not well known (37). Buswell et al (40, 41) found that vanillic acid was converted into methoxyhydroquinone via oxidative decarboxylation by a partially purified enzyme from P. chrysosporium. However, no further degradation of the product was observed in cell-free systems. Instead, hydroxyguinol was found to undergo intra-diolcleavage of the aromatic ring by a dioxygenase to produce 4-oxo-2-hexene-1,6-dioic acid which was then reduced to 3-oxohexane-1,6-dioic acid. The results imply that cleavage of aromatic rings by the fungus requires a catechol structure, and that extracellular enzymes are involved in the process.

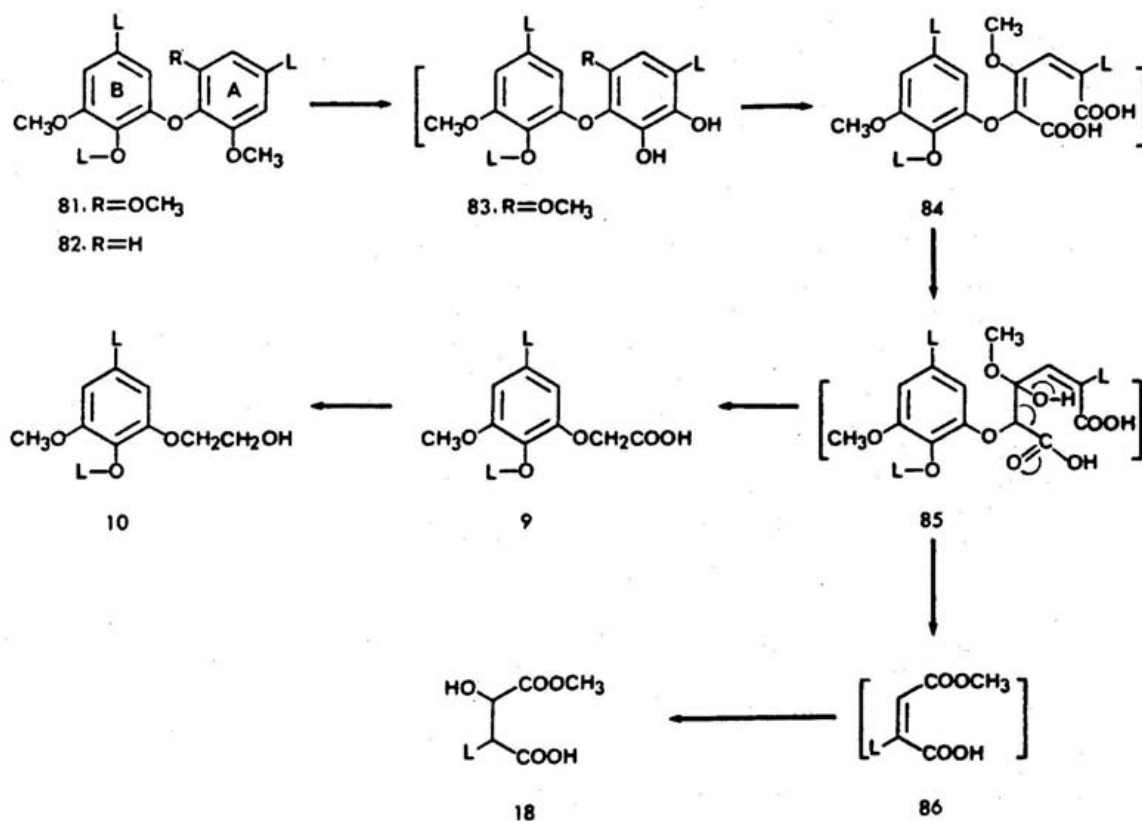
Aroxy- and alkoxyacetic acid moieties 5 and 11, and 2-aroxyethanol moiety 7 were present in the spruce biodegraded lignin, S-M-HA fraction. In addition to these moieties, the birch biodegraded lignins, B-M-HA fraction, also contained syringyl type aroxy- and alkoxyacetic acid moieties 6 and 12, 2-aroxyethanol moiety 8 as well as guaiacyl type aroxyacetic acid 9 and 2-aroxyethanol moiety 10. The latter two were probably derived from fungal degradation of biphenyl ether (4-O-5') units, which are moderately abundant sub-structures in birch lignin. Moreover,  $\alpha$ - and  $\beta$ -hydroxyacid moieties 16 and 17, and corresponding methyl ester moieties 18 and 19 found in the birch biodegraded lignins were not detected in the spruce biodegraded lignin. This fact suggests that the formation of moieties 16-19 was probably associated with oxidative cleavage of aromatic rings, particularly in syringyl groups.

Moiety 9 could have been produced from oxidative cleavage of ring A in the non-phenolic 4-O-5' unit 81 as shown in Scheme 2, involving 3-O-demethylation, subsequent hydroxylation at C-2 and further intra-diol cleavage of the resulting catechol intermediate 83 to produce 2,4-hexadiene-1,6-dioic acid intermediate 84. The intermediate 84 could undergo hydration on the double bond at C-2 of the  $\alpha,\beta$ -unsaturated acid moiety to give  $\beta$ -hemi-ketal-acid intermediate 85. A reverse aldol addition involving the 6-hemi-ketal-acid moiety would result in formation of aroxyacetic acid moiety 9 and maleic acid monoethyl ester moiety 86. Reduction of the former would produce 2-aroxyethanol moiety 10, while hydration of the latter would give  $\alpha$ -hydroxy-acid methyl ester moiety 18. The guaiacyl type non-phenolic 4-O-5' unit 82 could also undergo oxidative cleavage of ring B, and further degradation of the resulting 2,4-hexadiene-1,6-dioic acid intermediate via pathways similar to those for the degradation of 81 to produce moiety 9 and  $\alpha$ -hydroxy-acid moiety 16.

Moiety 5 could be formed from oxidative cleavage of ring B in the phenolic 4-O-5' unit 87, as shown in Scheme 3, via 3-O-demethylation and subsequent intra-diol cleavage of the resulting catechol intermediate 89 to give 2,4-hexadiene-1,6-dioic acid intermediate 90. The intermediate 90 would undergo further degradation via hydration of the double bond at C-2 of the  $\alpha,\beta$ -unsaturated acid moiety, and subsequent reversed aldol addition to produce aroxyacetic acid moiety 5 and *cis*-4-oxo-2-butenoic acid intermediate 92. The former would give 2-aroxyethanol moiety 7 on reduction, whereas the latter would produce  $\alpha$ -hydroxyacid moiety 16 via hydration of the double bond and oxidation of the aldehyde group. Degradation of the syringyl type phenolic 4-O-5' unit 88 via the same pathways would produce aroxyacetic acid moiety 6, 2-aroxyethanol moiety 8 and  $\alpha$ -hydroxy-acid moiety 16.

Alkoxyacetic acid moieties 11 and 12 could be produced from the non-phenolic 6-O-4 units 71 and 72 (L' = guaiacylpropane or syringylpropane unit) by degradation of aromatic rings in the L' moiety via similar pathways for the degradation of aromatic rings in the non-phenolic 4-O-5' units 82 and 81, respectively.

The 2-aroxyethanol moieties 7 and 8 could also result from cleavage of the C<sub>a</sub>-C<sub>b</sub> bond in side chains of corresponding non-phenolic  $\beta$ -O-4 units, as demonstrated by Enoki et al (28) in model compound studies. Oxidation of the moieties 7 and 8 could also lead to formation of aroxyacetic acid moieties 5 and 6, respectively. Recently, Ellwardt et al (42) investigated the modes of lignin degradation by white-rot and soft-rot fungi, using DHPs prepared from

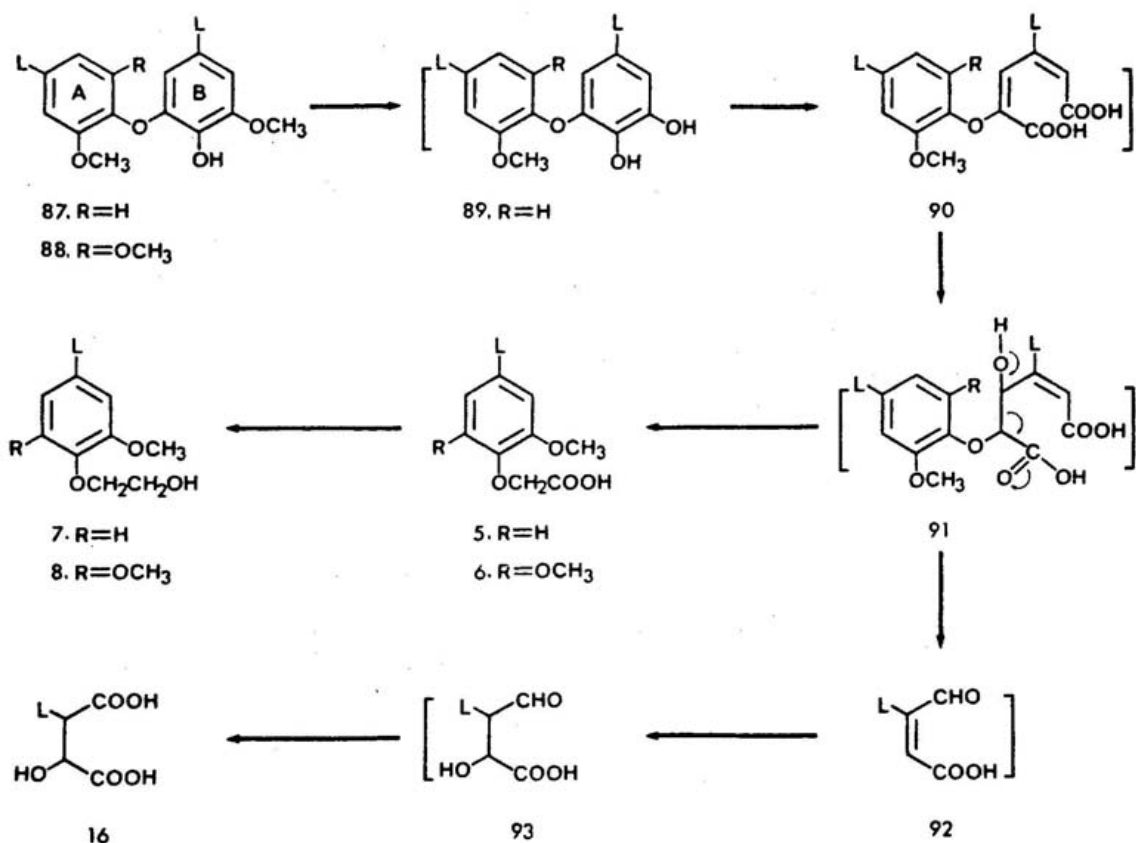


Scheme 2. Possible Pathways for Degradation of Aromatic Rings in Non-Phenolic 4-Q-5' Units by *P. chrysosporium*.  
 L = Lignin Moieties.

specifically <sup>13</sup>C-enriched coniferyl alcohols at C- $\alpha$ , C- $\beta$  and C-4, respectively, as substrates. The biodegraded DHPs were characterized by <sup>13</sup>C NMR spectroscopy. The results indicated that aroxy- and alkoxyacetic acid moieties 5 and 11 were produced via oxidative cleavage of aromatic rings, whereas 2'-aroxyethanol moiety 7 was formed via both cleavage of the C $\alpha$ -C $\beta$  bond in side chains and oxidative cleavage of aromatic rings. Thus, it is likely that aroxyacetic acid moieties 5, 6 and 9, alkoxyacetic acid moieties 11 and 12, and 2'-aroxyethanol moiety 10 were produced via oxidative cleavage of aromatic rings and further degradation of the resulting intermediates, whereas 2'-aroxyethanol moieties 7 and 8 were formed via both cleavage of C $\alpha$ -C $\beta$  bonds and degradation of aromatic rings.

#### E. Alkyl-Phenyl Cleavage

Recently, Goldsby et al (29) found that phenolic  $\beta$ -Q-4 model compounds were metabolized by *P. chrysosporium* to produce methoxyhydroquinone. The compound was also produced from vanillic acid via oxidative decarboxylation by an enzyme isolated from the fungus (40) and by hydrogen peroxide in the presence of peroxidase (43, 44).



Scheme 3. Possible Pathways for Degradation of Aromatic Rings in Phenolic 4-O-5' Units by *P. chrysosporium*.  
 L = Lignin Moieties.

Methoxyhydroquinone ether moiety 15 was found in the birch biodegraded lignins, but was not detected in the spruce biodegraded lignins. This suggests that the moiety was produced from 4-O-5' units containing a syringylpropane moiety, such as units of the types 88 shown in Scheme 4, via oxidative alkyl-phenyl (C<sub>α</sub>-C<sub>i</sub>) cleavage or oxidative cleavage of the C<sub>α</sub>-C<sub>β</sub> bond in the guaiacylpropane moiety, and subsequent oxidative decarboxylation of the resulting 5-aroxyvanillic acid intermediate 94.

#### F. Reactions Involved in Formation of Identified Carboxylic Acids

Structures for carboxylic acids identified in the S-M-LA and B-M-LA fractions indicate that fungal degradation of Both spruce and birch lignins involves an array of reactions in addition to those discussed in the previous sub-sections.

Since about two-thirds of the structures shown in Fig. 7 are tentative, as mentioned earlier, it is not appropriate to postulate possible degradative pathways leading to formation of these compounds without further verification of



3. Chua, M. G. S., C.-L. Chen, H-m. Chang and T. K. Kirk, *Holzforschung*: 1982. 36, 165-172.
4. Chen, C.-L., M. G. S. Chua, J. Evans and H-m. Chang: 1982. *Holzforschung*, 36, 239-247.
5. Tai, D., M. Terazawa, C.-L. Chen, H-m. Chang and T. K. Kirk: 1982. Proc. 1982 TAPPI Research and Development Division Conference, 263-272.
6. Chen, C.-L., D. Tai, M. Terazawa, H-m. Chang and T. K. Kirk: 1983. Proc. 1983 International Symposium on Wood and Pulping Chemistry, 3, 13-18.
7. Tai, D., M. Terazawa, C.-L. Chen, H-m. Chang and T. K. Kirk: 1983. Proc. 1983 International Symposium on Wood and Pulping Chemistry, 4, 144-149.
8. Chen, C.-L., H-m. Chang and T. K. Kirk: 1982. *Holzforschung*, 36, 3-9.
9. Chen, C.-L., H-m. Chang and T. K. Kirk: 1983. *J. Wood Chem. and Technol.* 3(1), 35-59.
10. Terazawa, M., D. Tai, C.-L. Chen, H-m. Chang and T. K. Kirk: 1983. Proc. 1983 International Symposium on Wood and Pulping Chemistry, 4, 150-155.
11. Hata, K.: 1966. *Holzforschung*, 20, 142-147.
12. Kirk, T. K., and K. Lundquist: 1970. *Sevensk Papperstidn.*, 73, 294-306.
13. Doi, S., N. Morohoshi and T. Haraguchi, *Mokuzai Gakkaishi*: 1974. 20, 230-237.
14. Henderson, M. E. K.: 1955. *Nature (London)*, 175, 634-635.
15. Higuchi, T., and I. Kawamura: 1955. *J. Jap. For. Soc.*, 37, 298-302.
16. Higuchi, T., I. Kawamura and H. Kawamura: 1956. *Mokuzai Gakkaishi*, 2, 31-35.
17. Cowling, E. B.: 1961. *U. S. Dept. Agri. Tech. Bull. No. 1258*, 1-79.
18. Schmid, R., and W. Liese: 1964. *Fr. ARch. Mikrobiol.*, 47, 260-276.
19. Wilcox, W. W. : 1970. *Bot. Rev.*, 36, 1-28.
20. Eriksson, K.-E.: 1980. *Pure and Appl. Chem.*, 53, 33-43.
21. Larsson, S. and G. E. Miksche: 1971. *Acta Chem. Scand.*, 25, 647-662.
22. Kirk, T. K., H-m. Chang and L. F. Lorenz: 1975. *Wood Sci. Technol.*, 9, 81-86.
23. Fergus, B. J. and D. A. I. Goring: 1970. *Holzforschung*, 24, 113-117.
24. Nakatsubo, F., T. K. Kirk, M. Shimada and T. Higuchi: 1980. *Arch. Microbiol.*, 128, 416-420.
25. Higuchi, T.: 1981. Proc. The Ekman-Days 1981, 3, 16-24.
26. Umezawa, T., F. Nakatsubo and T. Higuchi: 1982. *Arch. Microbiol.*, 131, 124-128.
27. Weinstein, D. A., K. Krisnangkura, M. B., Mayfield and M. H. Gold: 1980. *App. Env. Microbiol.*, 39, 535-540.
28. Enoki, A., G. P. Goldsby and M. H. Gold: 1980. *Arch. Microbiol.*, 125, 227-232.

29. Goldsby, G. P., A. Enoki and M. H. Gold: 1980. Arch. Microbiol., 128, 190-195.
30. Enoki, A., G. P. Goldsby and M. H. Gold: 1981. Arch. Microbiol., 129, 141-145.
31. Enoki, A., G. P. Goldsby, K. Krisnangkura and M. H. Gold: 1981. FEMS Microbiol. Lett., 10, 373-377.
32. Enoki, A. and M. H. Gold: 1982. Arch. Microbiol., 132, 123-130.
33. Kutsuki, H., A. Enoki and M. H. Gold: 1983. Photochem, Photobiol., 37, 1-7.
34. Kirk, T. K., F. Nakatsubo and I. P. Reid: 1983. Biochem. Biophys. Research Comm., 37, 200-204.
35. Kirk, T.K. and F. Nakatsabo: 1983. Biochim. Biophys. Acta 756, 376-384.
36. Erickson, M. and G. E. Miksche: 1973. Acta. Chem. Scand., 27, 903-914.
37. Crawford, R. C.: 1981. Lignin Biodegradation and Transformation, Chapt. 6, Wiley-Interscience, New York.
38. Shimada, M., F. Nakatsubo, T. K. Kirk and T. Higuchi: 1981. Arch. Microbiol., 129, 321-324.
39. Tien, M. and T. K. Kirk: 1983. Science (in press).
40. Buswell, J. A., P. Ander, B. Pettersson and K.-E. Eriksson: 1979. FEBS Lett., 103, 98-101.
41. Buswell, J. A. and K.-E. Eriksson: 1979. FEBS Lett., 104, 258-260.
42. Ellwardt, P.-Chr., K. Haider and L. Ernst: 1982. Holzforschung 35, 103-109.
43. Krisnangkura, K. and M. H. Gold: 1979. Phytochemistry, 18, 2019-2021.
44. Ander, P., A. Hatakka and K.-E. Eriksson: 1980. Arch. Microbiol., 125, 189-202.

Tai, D.; Terasawa, M.; Chen, C.-L.; Chang, H-m.; Kirk, T. K. Biodegradation of guaiacyl and guaiacyl-syringyllignins in wood by Phanerochaete chrysosporium. In: Higuchi, T.; Chang, Hou-min.; Kirk, T. Kent, eds. Recent advances in lignin biodegradation: Proceedings of an international seminar, organized under the auspices of the U.S. - Japan cooperative science program; 1983 May 31-June 2; Kyoto, Japan. Toyko, Japan: Uni Publishers Co., Ltd.; 1983: 44-63.