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Lignin degradation during softwood decaying by the ascomycete *Chrysonilia sitophila*

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Abstract

Softwood *Pinus radiata* was degraded by the ascomycete *Chrysonilia sitophila* during 3 months. The total weight loss of the wood was 20% and the carbohydrate and lignin losses were 18% and 25%, respectively. Decayed wood was extracted with solvents of increasing polarity. Methanol and dioxane yielded extracts containing representative low molecular weight degraded lignins. The overall structure of the degraded lignins, as shown by U.V./visible, I.R., ^{1}H and ^{13}C NMR spectroscopy, GPC, functional group and elemental analyses, was compared with the structure of milled wood lignin extracted from undecayed *P. radiata*. The compilation of the data allows us to suggest oxidative $C\alpha$-$C\beta$ and β-O-aryl cleavages for the mechanism of lignin degradation by this ascomycete. New saturated carbons on the side chain of the degraded lignins were detected. Based on these data a reductive ability of this microorganism was also suggested.

Introduction

The lignin biodegradation process has been exhaustively studied through several approaches such as degradation of low molecular weight lignin model compounds, isolated lignins, ^{14}C labelled lignins and lignocellulosic materials. Typical reactions caused by basidiomycetes during lignin biodegradation, namely depolymerization including carbon-carbon and β-O-aryl cleavages, oxidative degradation of the side chain of the polymer and oxidative ring opening were demonstrated (Eriksson et al. 1990; Higuchi et al. 1990).

Studies on lignin biodegradation are concerned with basidiomycetes. However, other microorganisms are also involved in the lignocellulosic decaying processes. Nilsson et al. (1989) demonstrated that some higher ascomycetes, particularly *Daldinia concentrica*, degraded Aspen wood with the same intensity as *Trametes versicolor*, a basidiomycete typically classified as white-rot fungus. Some bacteria of the actinomycetes class have also been investigated for their role in lignin biodegradation (Vicuna 1988).

In the light of the current knowledge about lignin biodegradation, it is important to find out if microorganisms other than basidiomycetes degrade lignin in the same way.

The ascomycete *Chrysonilia sitophila* (Campos et al. 1986) could degrade several lignocellulosic materials, their isolated components (Durán et al. 1988; Ferraz & Durán 1989; Rodriguez & Durán 1991; Ferraz et al. 1991), and low molecular weight lignin model compounds (Nogueira et al. 1992). Also, ligninolytic enzymes isolated from the culture broth of *C. sitophila* showed similar characteristics to those of *Phanerochaete chrysosporium* (Durán et al. 1987, 1987b).

The aim of this work is to demonstrate whether the ascomycete *Chrysonilia sitophila* degrades lignin during wood decaying in a pattern similar to basidiomycetes.

Materials and methods

Chemicals

Polystyrene standards were supplied by Sigma Co. Lignin model compounds with molecular weight in the range of 168 to 392 Da were previously synthesized in our laboratory (Nogueira et al. 1992). Gossypol (M.W. 518 Da) was supplied by Merck Co. All the other reagents were of analytical grade.

Microorganism and inoculum

Chrysonilia sitophila was isolated from a xylophagous insect (Campos et al. 1986), and maintained at 5° C in 85 mmol/l NH_4^+ Fries-1.0% glucose agar plates (Ferraz & Durán 1989). The composition of the Fries modified medium was: KH_2PO_4, 1.0 g/l; $MgSO_4$, 0.5 g/l; $CaCl_2$, 0.1 g/l; NaCl, 0.1 g/l; $FeSO_4 \cdot 7H_2O$, 0.2 mg/l; $CuSO_4 \cdot 5H_2O$, 0.1 mg/l; $MnSO_4$ 0.02 mg/l; $ZnCl_2$, 0.15 mg/l; $(NH_4)_2SO_4$, 5.6 g/l, buffered with 50 mmol/l potassium biphthalate at pH 6.0. The inoculum was grown in 1% glucose-Fries modified medium during 5 days. Afterward the culture medium of the inoculum was changed for fresh medium and shaken. The resulting suspension was added to the sterilized wood. Wood sterilization was done in dry conditions at 121° C for 3 hours.

Biodegradation

Sawdust from a 30-year old *Pinus radiata* D. Don was biodegraded in 1000 ml Erlenmeyer flasks containing 5 g of wood and 20 ml of culture medium at 28–30° C, for 3 months. 1% glucose-Fries modified medium was added to the culture during the first month of degradation. In the last 2 months the moisture was maintained by addition of 1% glucose solution. The mycelium was separated from the residual wood by continuous washing with water. The decayed wood was filtered by filter paper and dried overnight at 80° C. The experiments were carried out with 6 replicate cultures and one uninoculated control.

Determination of lignin and carbohydrates in the wood samples

Wood samples were extracted successively with diethyl ether and 95% ethanol. The extracted wood sample was analysed for its Klason lignin content by the ASTM method (1966). The soluble fraction of Klason hydrolysis was analysed for its total sugar content using the Dinitrosalycilic acid method (Mandels et al. 1986).

Extraction of Milled Wood Lignin (MWL)

MWLs were extracted from sound wood and from the wood sample decayed during 3 months. Wood samples were extracted successively with diethyl ether and 95% ethanol. The extracted wood sample was milled in a steel ball mill for 17 days in the presence of toluene. Afterwards the MWLs were extracted from the milled wood with a 90% dioxane aqueous solution during 24 hours at 25–30° C. Another extraction was performed in the residual wood using a fresh 90% dioxane solution during 144 hours. The extracts were combined and evaporated at a reduced pressure. The crude MWLs were purified as follows: 1. dissolution in 90% acetic acid and precipitation in water followed by washing with water and drying over P_2O_5; 2. the dry material was dissolved in 1,2-dichlorethane/ethanol (2:1), centrifuged and the soluble fraction precipitated in diethyl ether at 4° C. The final precipitate was washed with diethyl ether and dried over P_2O_5.

Recovery of biodegraded lignins from decayed wood

Decayed wood samples were combined and continuously extracted in a soxhlet apparatus successively with petroleum ether, chloroform, acetone, methanol and a 96% dioxane aqueous solution, each one for 8 hours. The extracts were evaporated at a reduced pressure and dried over P_2O_5.

Chemical and physical characterization of lignins

Gel Permeation Chromatography of the methanol and 96% dioxane extracts were performed on a 66 × 1.1 cm Sephadex LH-20 column using N,N-dimethylformamide at 0.3 ml/min as eluent. Detection of the extracts was achieved in a LKB-UVCORD S2138 detector at 275 nm. Lignin model compounds with molecular weight in the range of 168 to 518 Da were used as molecular mass markers for the column system. The exclusion volume of the column was determined by elution of a 12.5 kDa polystyrene standard. MWLs were analysed on a 60 × 1.0 cm Sephadex LH-60 column using N,N-dimethylformamide containing 0.1 M LiCl at 0.1 ml/min as eluent. MWLs were detected in each of the 1 ml eluted fraction at 280 nm. Standard polystyrenes of 4.0, 12.5, 25.0, 28.0 and 47.5 kDa

were used as molecular mass markers in this column system. The polystyrene standards were detected at 205 nm. All the gel permeation chromatography experiments were performed by injection of 0.8–1.0 mg of lignin samples.

U.V. and visible spectra were recorded by an Intralab DMS-100 spectrometer. U.V. spectra were obtained from 50 mg/l in 54.6% dioxane aqueous solutions. Visible spectra were obtained from 500 mg/l in 96% dioxane aqueous solutions. I.R. spectra were recorded from KBr dishes containing 1% of lignin samples in a Perkin Elmer 1430 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained from acetylated samples dissolved in $CDCl_3$ with tetramethylsilane as an internal standard. The acetylation was performed with acetic anhydride in pyridine as described by Lenz (1968). Acetylated MWLs were recovered by pouring the reaction mixture into diethyl ether. As acetylated biodegraded lignins do not precipitate in diethyl ether, the excess of acetic anhydride was degraded by the addition of methanol. The solvent mixture was evaporated by azeotropic distillation with toluene and ethanol at a reduced pressure (Morck & Kringstad 1985). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded by a 300 MHz Varian Gemini 300 spectrometer. The ^{13}C-NMR was recorded using a routine technique with continuous proton decoupling, 45° pulse width and a 0.8 sec acquisition time.

Elemental Analyses were performed by a Perkin Elmer CHN-2400 analyser. Oxygen was determined as 100% - (%C + %H). Methoxyl content in the MWLs was determined by the Browing procedure (Browing 1967). For the degraded lignins the methoxyl content was estimated by ^{1}H-NMR spectroscopy (Chen & Robert 1988). Carbonyl and phenoxy hydroxyl groups were determined by U.V. spectroscopy as described before (Adler & Marton 1959; Wexler 1964).

Results

Wood degradation and recovery of the degraded lignins

The weight loss of *Pinus radiata* decayed by *Chrysonilia sitophila* during 3 months was 20 ± 4%. Carbohydrate and lignin losses were 18 ± 5% and 25 ± 7%, respectively.

To recover biodegraded lignins decayed wood was successively extracted with solvents of increasing polarity. Kirk & Chang (1974, 1975) and later Chua

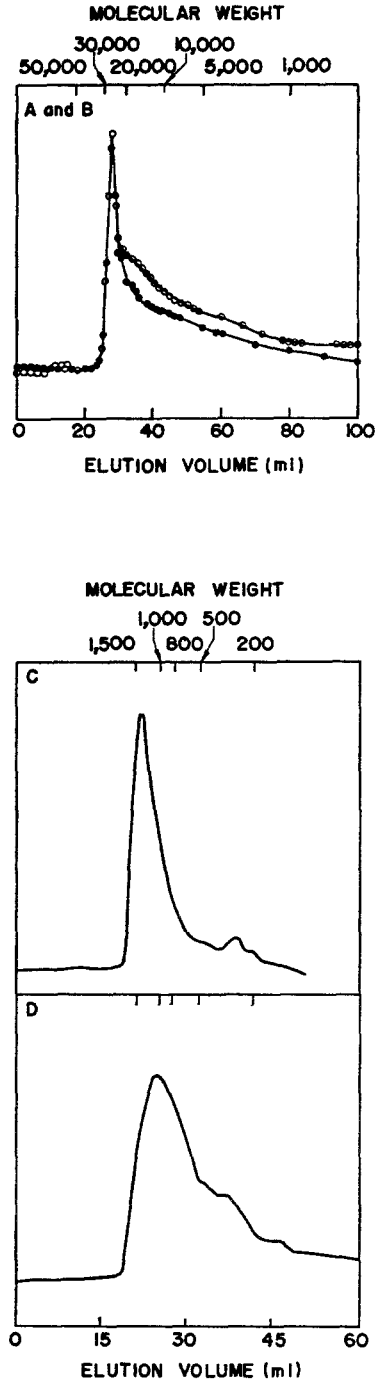

Fig. 1. Gel permeation chromatography of lignins. A and B were eluted on a 60.0 × 1.0 cm Sephadex LH-60 column using 0.1 M LiCl/N,N-dimethylformamide at 0.1 ml/min as eluent (-o-) Milled wood lignin from sound *Pinus radiata* wood and (-•-) Milled wood lignin from three months decayed *Pinus radiata* wood by *Chrysonilia sitophila*. C and D were eluted on a 66.0 × 1.1 cm Sephadex LH-20 column using N,N-dimethylformamide at 0.3 ml/min as eluent. (C) Degraded lignin dioxan and (D) Degraded lignin methanol.

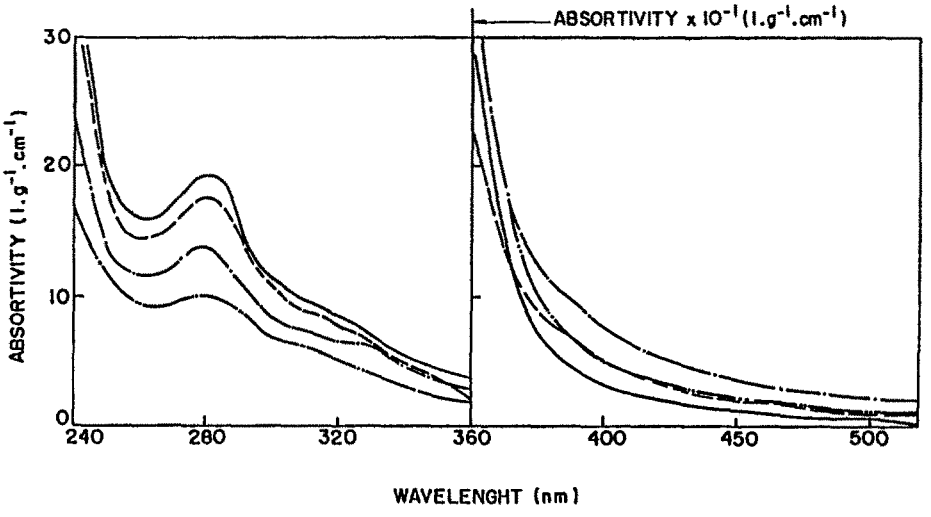

Fig. 2. U.V./Visible spectra of lignins. (——) Milled wood lignin from sound *Pinus radiata* wood; (- - -) Milled wood lignin from three months decayed *Pinus radiata* wood by *Chrysonilia sitophila*; (-•-•-) Degraded lignin dioxan and (-•••-) Degraded lignin methanol.

et al. (1982), Robert & Chen (1989) and Tai et al. (1990) have shown that methanol and dioxane/water extracts obtained from decayed wood contain representative biodegraded lignins resulting from the decaying process. The yields of the extracts in g/100 g of dry wood were 0.19%, 0.35%, 0.35%, 0.60% and 0.90% for the solvents petroleum ether, chloroform, acetone, methanol and 96% dioxane, respectively. The first three extracts were not studied since they mainly contain extractives from the decayed wood as described by Chua et al. (1982). Methanol and dioxane extracts were named Degraded Lignin Methanol (DLM) and Degraded Lignin Dioxane (DLD).

Gel permeation chromatography analyses

The GPC of DLM, DLD, MWL from sound (reference lignin) and decayed wood are given in Fig. 1. As MWLs eluted in the exclusion volume of the Sephadex LH-20 column, these lignins were chromatographed in a Sephadex LH-60 column. DLM and DLD showed significantly lower molecular weight values than both MWLs. These results show that these extracts contain low molecular weight lignins released as fragments from the lignin matrix by the decaying process.

U.V./Visible and I.R. spectroscopic analyses

The U.V./Visible spectra of lignins are given in Fig. 2. The absorptivity of degraded lignins at 280 nm was lower than that of MWL from sound wood (18.9 $L.g^{-1}.cm^{-1}$ for MWL from undecayed wood and 13.8 $L.g^{-1}.cm^{-1}$ and 10.2 $L.g^{-1}.cm^{-1}$ for DLD and DLM, respectively). Also, the ratio between absorptivities at 280, 310 and 405 nm changed in degraded lignins. The ratios for $a_{280/310}$ and $a_{280/405}$ were 1.9 and 59.6 for MWL from undecayed wood and 1.9 and 20.1 for DLD and 1.6 and 20.9 for DLM. Low absorptivities at 280 nm observed in DLM and DLD can indicate a lowering of aromaticity of these degraded lignins. Also, the presence of shoulders at 405 nm in the spectra of DLM and DLD and the decrease in the ratio of absorptivities at 280 and 405 nm can indicate the presence of carbonyl groups linked to aromatic rings (Vanucci et al. 1988) in DLM and DLD.

I.R. spectra of lignins are showed in Fig. 3. The absorbance ratio of bands at 1730, 1660 and 1600 cm-1 (assigned to carboxyl, carbonyl and aromatic ring, respectively) with the reference band at 1510 cm-1, assigned to aromatic ring, were calculated for each spectrum. The values observed for MWL from sound wood were 0.24, 0.37 and 0.55, respectively; for DLD, 0.34, 0.51 and 0.58; and for DLM, 0.76, 0.78 and 0.82. The increases observed in the values of 1730/1510 and 1660/1510 absorbance ratios for DLM and DLD could indicate that these lignins have higher ratios between carboxyl and carnonyl groups, and aromatic moieties, than MWL from undecayed wood.

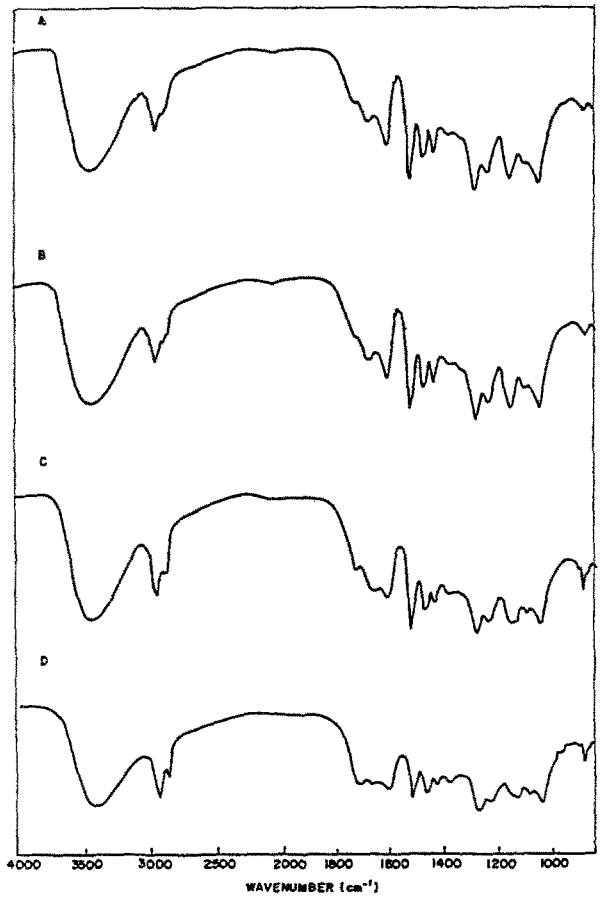

Fig. 3. Infrared spectra of lignins. (A) Milled wood lignin from sound *Pinus radiata* wood; (B) Milled wood lignin from three months decayed *Pinus radiata* wood by *Chrysonilia sitophila*; (C) Degraded lignin dioxan and (D) Degraded lignin methanol.

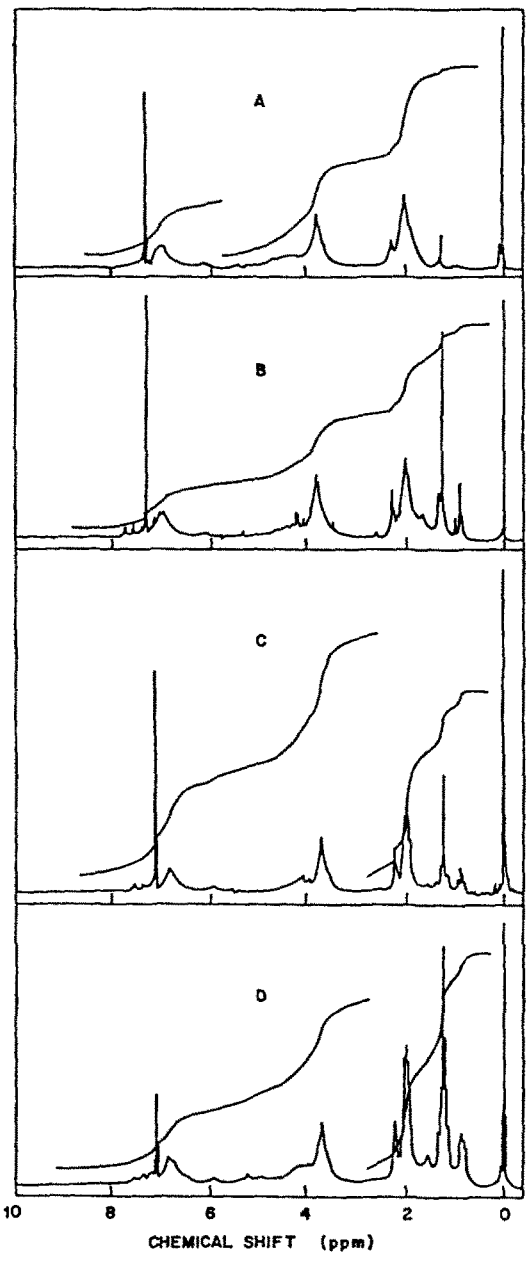

Fig. 4. ^{1}H-NMR spectra of lignins. (A) Milled wood lignin from sound *Pinus radiata* wood; (B) Milled wood lignin from three months decayed *Pinus radiata* wood by *Chrysonilia sitophila*; (C) Degraded lignin dioxan and (D) Degraded lignin methanol.

Functional group and elemental analysis

The C_9 formula for lignins are shown in Table 1. Comparison of the C_9 formula of MWL from sound wood *P. radiata* and biodegraded lignins showed significant differences. An increase of 1.2 hydrogens/C_9 in DLD and 3.0 hydrogens/C_9 and 1 oxygen/C_9 in DLM was observed. In DLM a decrease of 0.3 OCH_3/C_9 was also detected. The phenolic hydroxyl and the carbonyl groups content were significantly lower only in DLM.

^{1}H-NMR and ^{13}C-NMR analyses of acetylated lignin samples

The ^{1}H-NMR and the ^{13}C-NMR spectra are given in Figs 4 and 5 and the signal intensity and assignment are shown in Tables 2 and 3, respectively. The ^{1}H-NMR spectra were integrated in defined chemical shift ranges. The total integral value was correlated with the amount of hydrogens per C_9 determined in each

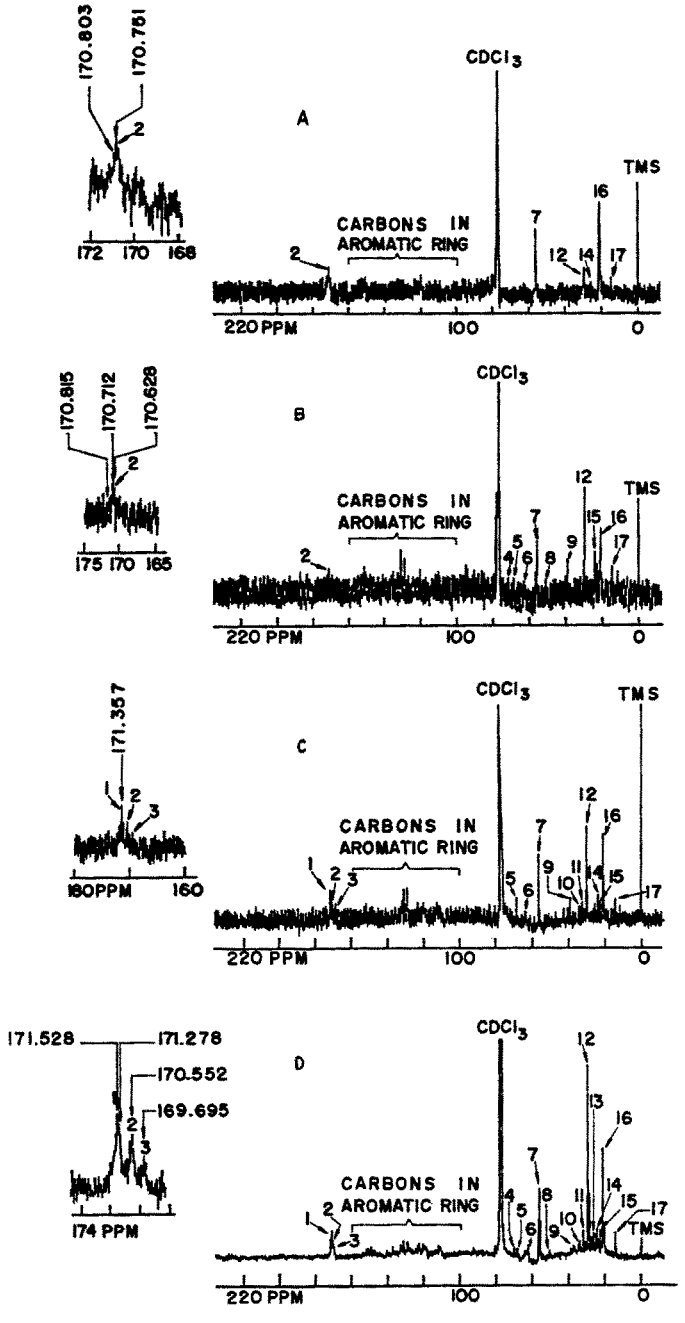

Fig. 5. ^{13}C-NMR spectra of lignins. (A) Milled wood lignin from sound *Pinus radiata* wood; (B) Milled wood lignin from three months decayed *Pinus radiata* wood by *Chrysonilia sitophila*; (C) Degraded lignin dioxan and (D) Degraded lignin methanol.

lignin (Chen & Robert 1988). In the acetoxyl region the integral value was divided by three to correlate with hydroxyl groups in unacetylated samples. Then for each chemical shift range the corresponding amount of hydrogen per C_9 was calculated.

In spite of the low signal to noise ratio the ^{13}C-NMR spectra from acetylated lignins showed some new sig-

Table 1. Functional group and elemental analysis of lignins from sound and decayed *Pinus radiata* wood by *Chrysonilia sitophila.*

	MWL from sound wood	MWL from decayed wood	Degraded lignin extracted with methanol (DLM)	Degraded lignin extracted with dioxane (DLD)
C_9 formula	$C_9H_{9.0}O_{3.4}(OCH_3)_{0.8}$	$C_9H_{7.9}O_{3.3}(OCH_3)_{0.7}$	$C_9H_{12.0}O_{4.4}(OCH_3)_{0.5}$	$C_9H_{10.2}O_{3.3}(OCH_3)_{0.7}$
Phenolic hydroxyl/C9	0.24	0.22	0.16	0.24
α-carbonyl in p-hydroxy etherified structures/C_9	0.051	0.050	0.025	0.041
γ-carbonyl, α-β unsaturated in p-hydroxy etherified structures/C_9	0.017	0.017	0.007	0.018

nals in biodegraded lignins and different intensities of the same signal in each spectrum. Table 3 shows the relative intensity and assignment of the main signals. The assignments of the observed ^{13}C-NMR signals were based on published papers (Chua et al. 1982; Hemmingson 1983; Morck & Kringstad 1985; Robert & Chen 1989; Tai et al. 1990). Comparison of the ^{1}H-NMR and ^{13}C-NMR spectra of the biodegraded lignins with MWL from sound *P. radiata* showed the following significant differences:

^{1}H-NMR spectra

- Decreases of hydrogens in aromatic and α-β unsaturated structures (6.29–7.17 ppm) in DLM and in MWL from decayed wood.
- Increases of Hα in β-5/α-O-4 structures (5.20–5.75 ppm) in DLM and DLD.
- Increases of Hγ in β-O-4, β-5, β-1 and equatorial Hγ in β-β structures (3.95–4.50 ppm) in DLM and DLD.
- Decreases of hydrogens in Ar-OCH_3 and H_β in β-5 and axial Hγ in β-β structures (3.55–3.95 ppm) in all biodegraded lignins.
- Increases of hydrogens in saturated structures (0.0–1.10 ppm) in all biodegraded lignins.

^{13}C-NMR spectra

- Signal '1' at 171.4 ppm assigned to carbonyl in aliphatic carboxylic acids was not observed in MWL from sound wood *P. radiata* and appeared with a medium intensity in DLM and DLD.
- Signal '5' at 68.3 ppm assigned to OCH_2 in -O-CH_2-COOH structures showed medium intensity in DLD and was weak or very weak in the other degraded lignins. This signal in MWL from sound wood was not observed.
- Signals '9', '11', '14', '15' and '17' at 38.9, 32,2, 24,6, 23,1 and 14,1 ppm, respectively, assigned to saturated carbons in the n-propyl side chain, appeared or increased in intensity in the biodegraded lignins.
- Signal '12' at 29.7 ppm assigned to α-CH_2 in -CH_2CH_2COOH structures showed medium and very strong intensities in MWL from sound wood and in the biodegraded lignins, respectively.

Discussion

Our results allowed us to concentrate efforts on studying the possible chemical changes which occurred in the low molecular weight degraded lignins. MWL

Table 2. ^{1}H-NMR data of lignins from sound and decayed *Pinus radiata* wood by *Chrysonilia sitophila*

Chemical shift (ppm)	Number of hydrogens/C_9				Assignment
	MWL from sound wood	MWL from decayed wood	Degraded lignin with methanol (DLM)	Degraded lignin with dioxane (DLD)	
7.23–7.90	0.5	0.5	0.6	0.7	Ar-H in Ar-COR
6.25–7.17	1.6	1.4	1.4	1.9	Ar-H in Ar-R Hα in Ar-CH=CH-CHO Hβ in Ar-CH=CH-CHO Hα in Ar-CH=CH-CH_2OAc
5.75–6.25	0.2	0.4	0.3	0.3	Hα with αOAc in β-O-4 and β-1 structures Hβ in Ar-CH=CHCH_2OAc
5.20–5.75	0.2	0.2	0.5	0.4	Hα with αOAc in β-5, β-O-4 and β-1
4.50–5.20	0.7	0.5	0.7	0.9	Hβ in β-O-4 Hγ in Ar-CH=Ch-CH_2OAc Hα in β-β structures
3.95–4.50	1.1	1.2	1.6	1.5	Hγ in β-O-4, β-5, β-1 and β-β structures
3.55–3.95	2.2	1.9	1.4	2.0	Ar-O-CH_3 (major), Hβ in β-5 and Hγ in β-β structures
2.50–3.55	0.7	0.7	0.8	0.6	Hβ in β-1, β-β and others
2.20–2.50	0.3	0.3	0.3	0.3	H in Ar-OAc except for 5-5 units
1.50–2.20	1.1	0.8	1.2	1.2	H in Aliphatic OAc and Ar-OAc in 5-5 units
1.10–1.50	0.2	1.6	3.6	1.5	H in nonoxygenated saturated carbons
0.00–1.10	0.3	0.5	1.0	0.7	H in nonoxygenated saturated carbons

extracted from decayed wood showed few changes in structure compared with MWL from sound wood.

After compiling the results we suggested the occurrence of some new substractures in low molecular weight degraded lignins (DLM and DLD) which includes aliphatic carboxylic acids and saturated carbons in the side chain of biodegraded lignins. The last substructure could be also attributed to fatty acids (Chen & Robert 1988), but the previous extraction of decayed wood with apolar solvents invalidates this possibility.

The occurrence of substructures such as **Ar-OCH_2COOH** can be suggested in DLM as supported by the following results: 1. presence of signals '1' and '5' in the ^{13}C-NMR spectra; 2. increase of oxygen and hydrogen per C_9; 3. decrease in the phenolic hydroxyl content and 4. increase in the ratio carboxyl/aromatic moieties revealed by the IR data. Also, new substructures such as **Ar-CH_2COOH** could be suggested in both DLM and DLD. These types of substructures were suggested based on: 1. presence of signal '1' and the increases in the intensity of signal '12' in the ^{13}C-NMR spectra of DLM and DLD; 2. increases of hydrogen per C_9 in DLD and hydrogen and oxygen per C_9 in DLM; 3. increases of hydrogen content linked to saturated carbons observed in ^{1}H-NMR spectra; 4. presence of new saturated carbons in ^{13}C-NMR spectra and 5. increases in the ratio carboxyl/aromatic moieties revealed by the IR data.

Robert & Chen (1989) studied the structure of lignin extracted from decayed spruce wood (Chen et al. 1982) using ^{13}C-NMR quantitative analysis. The authors reviewing the current knowledge in lignin biodegradation have suggested that the major reaction

Table 3. ^{13}C-NMR data of lignins from sound and decayed *Pinus radiata* wood by *Chrysonilia sitophila.*

Peak number	Chemical shift (ppm)	Relative intensities in lignins spectra (*)				Assignment (**)
		MWL from sound wood	MWL from decayed wood	Degraded lignin extracted with methanol (DLM)	Degraded lignin extracted with dioxane (DLD)	
1	171.4	–	–	m	m	C=0 in -O-CH_2COOH or -CH_2CH_2COOH (1,2)
2	170.8	w	w	w	w	C=O in primary acetyl aliphatic (3)
3	169.7	–	w	vw	vw	C=O in secondary acetyl aliphatic (3)
4	70.1	–	w	vw	–	-OCH_2- in Ar-OCH_2CH_2OH (1,2)
5	68.3	–	w	vw	m	-OCH_2- in -OCH_2COOH (1.2)
6	62.3	–	w	w	vw	γ-CH_2 in β-aryl ethers (4)
7	56.1	S	S	S	S	CH_3 in Ar-OCH_3 (4)
8	50.7	–	w	vw	–	Cβ in phenylcumaran and β-1 structures
9	38.9	–	m	vw	m	α-CH_2 in saturated n-propyl side chain (3,4)
10	34.1	–	–	vw	w	α-CH_2 in saturated n-propyl side chain (3,4)
11	32.2	–	–	m	w	α-CH_2 and β-CH_2 in Ar-$CH_2CH_2CH_2$OAc or α-CH_2 in -CH_2CH_2COOH (3,5)
12	29.7	m	VS	VS	VS	α-CH_2 and β-CH_2 in Ar-$CH_2CH_2CH_2$OAc or α-CH_2 in -CH_2CH_2COOH (3,5)
13	27.5	–	w	–	–	Cβ in Ar-C-CH_2-C (1)
14	24.6	w	–	w	m	C in saturated n-alkyl groups (1,3,5)
15	23.1	–	m	m	w	γ-CH_3 in Ar-COH-CH_3 (4)
16	21.1	VS	VS	VS	VS	CH_3 in acetyl group (3)
17	14.1	w	m	m	m	γ-CH3 in Ar-$CH^2CH^2CH^3$ (2,3,4,5)

(*) Relative intensities: vw = very weak; w = weak; m = medium, S = strong; VS = very strong.

(**) References: (1) Robert & Chen 1989; (2) Chua et al. 1982; (3) Morck & Kringstad 1985; (4) Hemmingsson 1983; (5) Tai et al. 1990.

started by the fungal enzymes during wood decaying was the Cα-Cβ cleavage followed by Cβ centered radical formation. Then the degradation of the Cβ centered radical can occur by several side reactions.

The formation of substructures such as **Ar-OCH_2COOH** suggested in this work could be supported by a pathway similar to the one described by Robert & Chen (1989) where one Cα-Cβ cleavage could produce the substructure **Ar-OCH_2CH_2OH** that could be further oxidized to **Ar-OCH_2COOH**. However, the presence of substructures such as **Ar-CH_2CH_2COOH** only can be explained by a Cβ-O-aryl cleavage instead of a Cα-Cβ cleavage. However, the presence of substructures containing nonoxygenated saturated aliphatic carbons, such as α-CH_2, can not be explained in a direct way. The presence of this type of substructures in biodegraded lignins was also observed by Chua et al. (1982) and by Tai et al. (1990). These substructures clearly show an ability of microorganisms that degrades lignin to produce reductive reactions on the side chain of the polymer, besides the well known oxidative reactions.

In a previous work we have demonstrated the degradation pathway of low molecular weight lignin model compounds by *C. sitophila* (Nogueira et al. 1992). We were able to demonstrate the ability of this ascomycete to produce β-O-4 cleavage in a similar way as previously reported for *P. chrysosporium.* This previous work corroborates the present results since β-O-4 cleavage could explain the formation of the suggested substructures such as **Ar-CH_2CH_2COOH.**

Other reactions such as the oxidative ring opening and the formation of quinone like substructures could be also suggested by U.V./Visible analyses. Also, demethylation reactions were suggested in DLM since a decrease of 0.3 OCH_3/C_9 was observed. Resistence of β-5/α-O-4 structures to biodegradation was suggested since the Hα content in this kind of structure increased in DLM and DLD as shown in the ^{1}H-NMR spectra (Fig. 4 and Table 3). Nevertheless, the last suggested reactions were not evidenced by several analytical techniques in the same manner as the first ones.

Conclusion

The lignin biodegradation during softwood decay by the ascomycete *Chrysonilia sitophila* was evaluated. Low molecular weight degraded lignins were obtained by methanol and dioxane/water extraction of decayed wood. Evidences for the occurrence of oxidative reactions such as Cα-Cβ and Cβ-O-aryl cleavages of lignin followed by the formation of aliphatic carboxylic acids in the side chain of the polymer were obtained. The biodegraded lignins also exhibited new substructures containing nonoxygenated saturated aliphatic carbons in the side chain.

The major conclusion of this paper is the indication that one microorganism of the class of the ascomycetes produces lignin degradation reactions similar to those produced by basidiomycetes.

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