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Metabolism of a Phenylcoumaran Substructure Lignin Model Compound in Ligninolytic Cultures of *Phanerochaete chrysosporium*

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Abstract. The degradation of the phenylcoumaran substructure model compound methyl dehydrodiconiferyl alcohol by the white-rot wood decay fungus *Phanerochaete chrysosporium* was investigated using culture conditions optimized for lignin oxidation. Initial attack was in the cinnamyl alcohol side chain, which was oxidized to a glycerol structure. This was subsequently converted by loss of the two terminal carbon atoms, $C_{\beta'}$ and $C_{\gamma'}$, to yield a $C_{\alpha'}$-aldehyde structure, which was further oxidized to the $C_{\alpha'}$-acid compound. The next detected intermediate, a phenylcoumarone, was produced by double bond formation between C_α and C_β, and oxidation of the C_γ-alcohol to an aldehyde group. Further oxidation of C_γ to an acid yielded the next intermediate. The final identified degradation product was veratric acid. No products from the 5-substituted aromatic ring, and no phenolic products, were found. The initial glycerol-containing intermediate was a mixture of the *threo* and *erythro* forms, and no optical activity could be found, suggesting that its formation might have involved non-stereospecific $C_{\alpha'}-C_{\beta'}$ epoxidation followed by non-enzymatic hydrolysis of the epoxide.

Key words: Lignin biodegradation – White-rot fungi – Fungus physiology – Wood decay – Aromatic metabolism – *Phanerochaete chrysosporium*

Although the biodegradation of lignin is of central importance to the earth's carbon cycle, the specific reactions involved are not yet known. Prominent among the microorganisms that degrade the complex phenylpropanoid polymer are the wood-decaying Basidiomycetes (Ander and Eriksson 1978; Crawford and Crawford 1980; Hovland 1980; Kirk et al. 1980). Characterization of polymeric lignin after attack by these organisms, and identification of low molecular weight lignin degradation products from decaying wood, have provided considerable insight into the chemistry of degradation. However, the specific reactions and the biochemistry will probably have to be elucidated through investigations of the metabolism of lignin substructure models, lignin itself being too heterogeneous. Substructure model compounds have been the key to answering many questions about lignin structure and reactions, and new methods for their synthesis (Nakatsubo and Higuchi 1979, 1980) have now made their use even more attractive.

The ligninolytic enzyme system in the wood decay fungus *Phanerochaete chrysosporium*, and presumably in other ligninolytic Basidiomycetes, is most unusual in that it is a manifestation of secondary metabolism and is not induced by lignin (Keyser et al. 1978; Kirk and Fenn 1980), Cultures become ligninolytic following growth limitation caused by nitrogen – (Keyser et al. 1978) or carbon – (Jeffries et al. unpublished) starvation. The simple culture conditions favoring appearance and maintenance of ligninolytic activity have been described for this fungus (Keyser et al. 1978; Kirk et al. 1978), making it possible to study the fate of lignin-related compounds in the presence of the ligninolytic system, in the absence of lignin, and in the absence of primary growth.

Initial reactions in the metabolism of a phenylcoumaran substructure model compound in ligninolytic cultures of *P. chrysosporium* are described here. This important substructure type accounts for 6% and 9–12% of the intermonomer linkages in birch and spruce wood lignins, respectively (Adler 1977). Previous research has defined the initial chemical reactions comprising the metabolism of a phenylcoumaran model compound by the soil fungus *Fusarium solani* (Ohta et al. 1979). It is shown here that the pathways in *Phanerochaete* and *Fusarium* differ in several respects.

Materials and Methods

Organisms and Culture Conditions

Phanerochaete chrysosporium ME-446 (ATCC 35540) was maintained at 30°C on 2% malt agar slants. Copious conidiation occurred within one week, and 1.5–3.5-week-old slants provided spore inoculum (Kirk et al. 1978) for the cultures here.

Experimental cultures were grown in a dilute mineral salts-thiamine medium containing 1.2 mMolar nitrogen (equimolar NH_4NO_3 and L-asparagine), and 1% glucose as growth substrate (Kirk et al. 1978). The medium was buffered with 0.01 M poly (acrylic acid), pH 4.5. This buffer was prepared from high molecular weight poly (acrylic acid) (Aldrich, mol. wt. = 250,000); a solution was dialyzed overnight against tap water before being adjusted to pH 4.5, diluted to the desired concentration, and sterilized at 121°C for 20 min. The polymeric buffer was found to have about 70% of the buffering capacity of 2,2-dimethylsuccinic acid in the desired range of pH 4–5, and was used to preclude extracting buffer together with degradation products of model compounds. Ligninolytic activity and growth are similar in cultures buffered with 2,2-dimethylsuccinic acid (Fenn and Kirk 1979) and with poly (acrylic acid).

Abbreviations: TLC = thin layer chromatography; LDA = lithium diisopropyl amide; DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone; MS = mass spectrometry; UV = ultraviolet spectroscopy

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All experiments were conducted with 20 ml cultures grown in 300 ml Erlenmeyer flasks, maintained at 37–39° C. The flasks were flushed with 100% O_2 3–4 days after inoculation and maintained under this atmosphere. Elevated O_2 enhances ligninolytic activity (Kirk et al. 1978; Reid 1979; Yang et al. 1980) and lignin dimer metabolism (Weinstein et al. 1980), both by increasing the titer of the ligninolytic system and by increasing the rate of its action (Bar-Lev and Kirk unpublished).

Compounds were added to 5–6-day-old cultures as aqueous solutions or suspensions, 1–10 mg/culture. All compounds were first dissolved in N,N-dimethylformamide (DMF) and added to stirred water to give suspensions (or with compound II, a solution), such that 1–2 ml/culture provided the desired amount of substrate. [The final amount of DMF was < 100 µl/culture, at which concentration it has no effect on lignin metabolism (Kirk et al. 1978)]. Substrates containing carboxyl groups (V, VI, VIII) were dissolved by addition of 1 M NaOH to pH 4.5 prior to addition to the cultures. No attempt was made to add the substrates aseptically, and no problems with contamination were encountered. Following addition of the compounds, all cultures were flushed again with 100% O_2, and incubated in that atmosphere.

The progress of degradation of substrates was followed by extracting one of the replicate cultures each day and examining the extract by thin layer chromatography (TLC). Degradation could not be followed by UV spectrometric examination of the culture filtrates as with *Fusarium* (Ohta et al. 1979), because the substrates were rapidly adsorbed or taken up by the mycelium. Extractions followed acidification of whole cultures to pH 2 with 1 M HCl, and were done with a 1:1 (v:v) mixture of $HCCl_3$:acetone, 3×, total 60 ml/culture, and finally with 20 ml/culture of $HCCl_3$. Combined extracts were washed with brine, dried over Na_2SO_4, and evaporated. DMF was removed under high vacuum. Heat-killed (control) cultures, incubated at 39 C under 100% O_2, did not catalyze the reactions described here.

Isolation of Metabolic Products

Degradation intermediates were separated and purified by preparative TLC using silica gel (Merck 60 F_{254}). The following solvent systems were used: I → II + III and II → III, 5% CH_3OH in CH_2Cl_2; III → IV + V (product mixture methylated with diazomethane in diethyl ether), ethyl acetate/*n*-hexane, 1:1 (v:v); V → VI + veratric acid (product mixture methylated), CH_2Cl_2; VI′a → VI′ (product mixture methylated), ethyl acetate/*n*-hexane, 1:2 (v:v); VI′ → VII′ + VIII′ (product mixture methylated), ethyl acetate/*n*-hexane, 1:2 (v:v); VII′ dimethyl ester further purified by TLC using CH_2Cl_2.

Syntheses

Compound I: Methyl Dehydrodiconiferyl Alcohol. Dehydrodiconiferyl alcohol was synthesized by the method reported earlier (Nakatsubo and Higuchi 1979) and converted to its methyl derivative I by treatment with diazomethane in ether, or methyl iodide/sodium hydride in DMF, at room temperature. ^{1}H NMR($CDCl_3$)δ: 3.40–3.80 (IH,m, β-CH–). 3.81(3 H,s), 3.82(3 H,s), 3.87(3 H,s), 4.23(2 H, broad d, J = 5.5 γ-CH_2–), 5.53(IH,d,J = 6.5,α-CH–), 6.13(IH,dt,J = 15.5, 5.5, β' = CH–), 6.48(IH,d,J = 15.5,α'-CH=), 6.65–7.00 (5 H,m).

Glycerol Derivative II, Threo-Form. To a stirred solution of I (32.7 mg, 0.1 mM) in 6 ml of dioxane/pyridine (1:1, w/w), 25.4 mg of osmium tetroxide was added. After stirring for 12 h under nitrogen, the reaction mixture was evaporated in vacuo and dissolved in 10 ml of ethanol/water (1:1, w/w) which contained 126 mg of Na_2SO_3 (1 mM), and then refluxed for 1 h. The precipitate was filtered off and washed with acetone. The combined filtrate and washings was evaporated in vacuo to about 3 ml. The product was partitioned between ethyl acetate and brine. The ethyl acetate solution was dried over Na_2SO_4, evaporated in vacuo to give a brown oil which was purified by TLC (6% methanol/CH_2Cl_2) to afford a colorless glass (25 mg, 61.6%). ^{1}H NMR($CDCl_3$) (acetate) δ: 1.99, 2.01, 2.03 and 2.06 (12 H, four s), 3.84(6 H,s), 3.88(3 H,s), 3.60–3.95 (1 H,m, β-CH–), 4.10–4.46(4 H,m,γ,γ'-CH_2–), 5.43(1 H,d,J = 7.5, α-CH–), 5.30–5.45(1 H,m, β'-CH–), 5.84(1 H,d,J = 7.5, α-CH–), 6.70–6.90(5 H,m) (Fig. 1). MS(*m/e*): 574(M^+, 24.3), 514(85.7), 327(95.7), 151(35.7), 43(100).

Compound III. This compound was synthesized by methylation of the appropriate intermediate from synthesis (Nakatsubo and Higuchi 1979) of dehydrodiconiferyl alcohol, by using methyl iodide/sodium hydride in DMF at room temperature. ^{1}H-NMR($CDCl_3$)δ: 3.50–4.10(3 H,m, β-CH– and γ-CH_2–), 3.83(3 H,s), 3.84(3 H,s), 3.93(3 H,s), 5.86(1H,d,J = 7.0, α-CH–), 6.70–7.00(3 H,m), 7.25–7.50(2 H,m), 9.69(IH,s,α'-CHO) MS(m/e): 344(M^+, 80), 326(100), 314(72.5), 311(62.5), 151(35).

Compound IV. To a stirred solution of compound III (172 mg, 0.5 mM) in methanol (5 ml), 9.5 mg of $NaBH_4$ (0.25 mM) was added at 0° C under nitrogen, and stirring continued 10 min. The product was partitioned between ethyl acetate and brine, the ethyl acetate solution dried over Na_2SO_4 and evaporated in vacuo to give a colorless oil which was crystallized from CH_2Cl_2/*n*-hexane to afford colorless crystals (155 mg, 89.5%). ^{1}H NMR($CDCl_3$): 3.50–4.00(3 H,m, β-CH– and γ-CH_2–), 3.76(3 H,s), 3.78(3 H,s), 3.82(3 H,s), 4.48(2 H, broad s, α'-CH_2–), 5.43(IH,d, J = 6.5, α-CH–), 6.60–6.90(5 H,m). MS(*m/e*): 346(M^+, 54.7), 328(100), 316(55.8), 313(58.5), 297(34), 151(31.3).

Compound V. To a vigorously stirred solution of compound III (103 mg, 0.3 mM) dissolved in 5 ml of ethanol and 0.5 ml of NaOH solution (3 N), $AgNO_3$ (510 mg, 3 mM) in a minimum amount of water (about 0.5 ml) was added dropwise to give a black precipitate. Stirring was continued overnight, and the precipitate filtered off and washed with hot water. Combined filtrate and washings were evaporated to about 8 ml in vacuo. This was washed twice with methylene chloride, acidified with 1 M HCl and extracted with $CHCl_3$/acetone (1:1). The extract was washed with brine, dried over Na_2SO_4, and evaporated in vacuo to give white crystals (90 mg, 83.3%). MS(*m/e*) (methyl ester): 374(M^+, 68.9), 356(100), 344(72.2), 341(53.3), 151(35.6).

Compound VI (Methyl Ester). This compound was obtained from dehydrodiisoeugenol (Leopold 1950) via eight steps: 1) MeI/NaH in DMF, room temperature (over 90% yield). 2) Metachloroperbenzoic acid in CH_2Cl_2, 0° C (80%). 3) NaOMe in 20% MeOH/CH_2Cl, r.t. (90%). 4) $Pb(OAc)_4$ in refluxing benzene (80%). 5) Ag_2O in ethanol, r.t. (90%). 6) DDQ in refluxing benzene (70%). 7) SeO_2 in refluxing xylene (70%). 8) Diazomethane in 20% MeOH/CH_2Cl_2. ^{1}H NMR($CDCl_3$)δ: 3.97(3 H,s), 3.99(3 H,s), 4.01(3 H,s), 4.08(3 H,s), 6.99(IH,d,J = 8.0), 7.39(IH, broad s), 7.43(IH,dd,J = 8.0, 2.0), 7.57(IH,d,J = 2.0), 8.50(IH,d,J = 2.0), 10.29(IH,s). MS(*m/e*): 370(M^+, 100), 355(12.3), 339(18.5),327(8). Uv $\lambda_{max}^{MeOH/CH_2Cl_2}$ nm: 246, 290, 333.

Compound VII, Dimethyl Ester. This compound was prepared by a modification of the method reported earlier (Nakatsubo and Higuchi 1979). The β-hydroxyester intermediate, prepared by the aldol condensation, in the presence of lithium diisopropyl amide (LDA), of 5-carbomethoxymethyl benzylvanillin dimethylacetal and ethyl vanillin, was converted to the dimethylester of compound VII′ by the following five steps: 1) H_2/10% Pd-C in MeOH, r.t. (95%); 2) BF_3-ET_2O in CH_2Cl_2, r.t. (60%); 3) $KMnO_4$ in dioxane/H_2O, r.t. (90%); 4) CH_2N_2 in 20% MeOH/CH_2Cl_2 (95%); 5) DDQ in refluxing benzene (90%). ^{1}H NMR ($CDCl_3$): 1.52(3 H,t,J = 7.0), 4.00(9 H,s), 4.07(3 H,s), 4.21(2 H, q,J = 7.0), 7.01(1 H,d,J = 8.0), 7.57(1 H,d,J = 1.5), 7.76(1 H,dd,J = 8.0,2.0), 7.80(1 H, broad s), 8.38(1 H,d,J = 1.5). MS(*m/e*): 414(M^+, 100), 386(35), 355(12.5), 325(11.5).

Instrumentation. A JASCO J-20 Spectropolarimeter was used with a 1 mm cell for the CD spectrum. ^{1}H NMR spectra were taken with a Hitachi R-22 high resolution NMR spectrometer (90 MHz), with TMS as internal standard. Chemical shifts and coupling constants are given in δ-values and Hz, respectively. Mass spectra were taken with a Shimadzu-LKB 9000 gas chromatograph-mass spectrometer; the relative abundance of each peak is designated in parentheses.

Results

Additon of synthetic ^{14}C-lignin to 5- or 6-day-old cultures resulted in immediate release of $^{14}CO_2$, demonstrating the presence of the ligninolytic system (*cf.* Keyser et al. 1978). Subsequent addition of model compounds resulted in their metabolism.

Methyl dehydrodiconiferyl alcohol (I, 90 mg in 9 cultures) had nearly completely disappeared (86%) 24 h after addition to

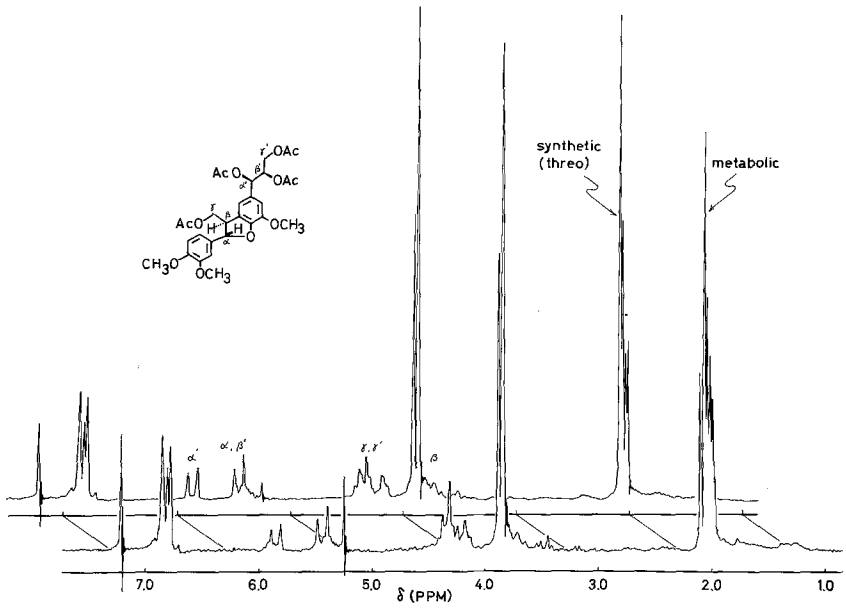

Fig. 1
^{1}H-NMR spectra of synthetic and metabolic glycerol derivatives

cultures. Extraction yielded two major products, which were identified, and several minor ones which were not pursued. One of the major metabolites, II (9%), resulted from oxidation of the cinnamyl alcohol side chain to form a novel glycerol structure. This product was characterized by ^{1}H NMR and mass spectrometry (MS), and its identity was confirmed by MS and ^{1}H NMR comparison with the synthetic compound. No optical activity was detected using CD spectrometry and comparison of the ^{1}H NMR spectra of the acetates of the synthetic (*threo*) and metabolic products (Fig. 1) indicated that the latter was a mixture of *erythro* and *threo* isomers. The second major product from I was compound III (17%), formed by removal of $C_{\beta'}$ and $C_{\gamma'}$. As with compound II, this product was characterized spectroscopically and its identity confirmed by synthesis.

Addition of compound II to ligninolytic cultures (14 mg, 7 mg/culture) resulted in 86% conversion in 15 h to III (20%; TLC, MS), and other products (TLC), establishing the pathway I → II → III.

Twenty hours after being added to ligninolytic cultures, compound III (10 mg, 5 mg/culture) had been both reduced and oxidized at $C_{\alpha'}$, producing compounds IV and V. Yields were not determined, but TLC indicated approximately 20% and 30%, respectively, plus approximately 30% starting material. Structures of these products were confirmed by comparisons with the synthetic samples by TLC and MS. Other products from III were seen on TLC plates but were present in only minor amounts. Compound V was the major product from III; addition of IV to cultures resulted in its oxidation to III and V, but reductions of V to III and IV were only minor reactions. This indicated the sequence I → II → III → V.

The active cultures converted compound V (85 mg, 5 mg/culture) into a number of products. Conversion was slower than with the preceeding reactions, and cultures were not extracted until 5 days' incubation. One of the metabolites exhibited strong sky-blue fluorescence under long wave UV light, and was identified as the phenylcoumarone VI (< 1%), formed by oxidation at C_γ and dehydrogenation across $C_\alpha - C_\beta$. The product was characterized by UV and MS, and its identity confirmed by comparisons with the synthesized compound (MS, UV, TLC). Compound VI was present in about the same amount throughout the 5-day incubation, demonstrating that it was being metabolized further. Thus the sequence I → II → III → V → VI was established.

Also identified in trace amounts in cultures to which compound V had been added was veratric acid (comparison with the authentic compound by MS). However, because this compound can arise via de novo synthesis (C.-L. Chen, H.-M. Chang, and T. K. Kirk, unpublished results), probably by oxidation of veratryl alcohol (Lundquist and Kirk 1978), it could not be concluded to be a product from V. To avoid the issue of de novo synthesis, therefore, further studies were conducted with 4-*O*-ethyl- instead of 4-*O*-methyl-derivatives. Similar strategy was used recently by Enoki et al. (1980).

Preliminary experiments with the ethyl analog of compound VI (VI′, 3 mg, 1 mg/culture) confirmed that it was readily metabolized in ligninolytic cultures. It was also found that the C_γ-alcohol analog of VI′(VI′a) was oxidized to the C_γ-aldehyde compound VI′. Consequently, compound VI′a, which is much more soluble in the culture medium than VI′ and more easily synthesized, was used in the next experiment. When added to cultures, VI′a (45 mg, 5 mg/culture) was oxidized not only to the aldehyde (TLC), which did not accumulate, but also to the dicarboxylic acid VII′ (< 0.5 mg, 3 days' incubation). In addition, 4-ethoxy-3-methoxybenzoic acid (VIII′, trace) was detected in the same extract. Both VII′ and VIII′ were identified by comparisons with synthetic samples using MS. These results indicated that compound VI′a was converted to VI′, and VI′ to VII′ and VIII′.

Small amounts of several other compounds, the majority of which were more polar than VII′, were seen by TLC. None of these were phenolic, and 5-carboxyvanillic acid specifically was not among them.

Discussion

The pathway indicated by these results is depicted in Fig. 2. The phenylcoumaran substructure model I was first oxidized in the cinnamyl alcohol moiety to give II, containing a glycerol side chain. The fact that II was optically inactive suggests strongly that oxidation at the double bond was not stereoselective (or that subsequent reactions caused racemization). Although optically inactive, the product II could still have been *threo* or *erythro*, rather than the mixture that was found. The fact that

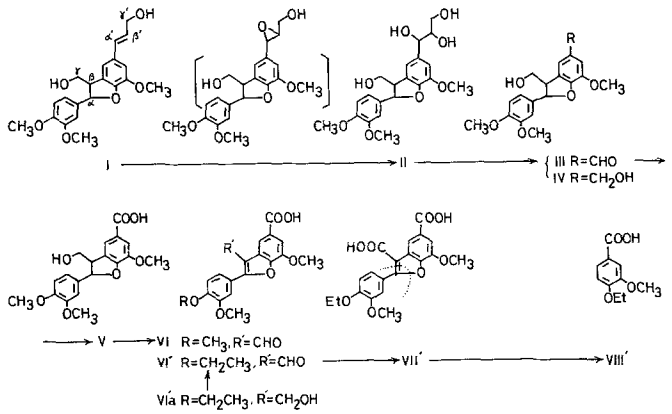

Fig. 2. Proposed pathway for the metabolism of dehydrodiconiferyl alcohol derivatives by *Phanerochaete chrysosporium* ME-446

both forms were produced by the fungal degradation suggests that the oxygen insertions at both $C_{\alpha'}$ and $C_{\beta'}$ were probably not enzymatically controlled. Alternatively, the starting material could have been a mixture of *cis* and *trans* forms, or further reactions could have caused racemization, resulting in the *erythro/threo* mixture. Neither of the last two possibilities is considered likely, however. Several possible explanations for these sterochemical results can be advanced; one plausible one is that non-stereoselective $C_{\alpha'}$-$C_{\beta'}$ epoxidation (enzyme-controlled or not) was followed by non-enzymatic hydrolysis of the epoxide. Sakai (1977) reported that a non-conjugated side chain double bond in the plant flavonoid phellamurin is converted into a glycol moiety during metabolism by *Aspergillus niger*. An epoxide intermediate was suspected but not established, and the stereochemistry of the glycol-containing product was not investigated. In any case, the observations here support the conclusions reached earlier that lignin degradation involves non-specific reactions (Keyser et al. 1978) which are primarily oxidative (Kirk and Chang 1975).

Removal of the two terminal carbons from the glycerol side chain (i.e. $C_\alpha - C_\beta$ cleavage) yielded the aldehyde III. Perhaps this was an oxidation similar to the side chain cleavage catalyzed in 20α, 22ζ-dihydroxycholesterol by an enzyme preparation from adrenyl glands; that reaction required molecular oxygen and NADPH (Shimizu 1968). Alternatively, Gold and coworkers (Enoki et al. 1980; Weinstein et al. 1980) have reported $C_\alpha - C_\beta$ cleavage in arylglycerol-β-aryl ether substructure models in ligninolytic cultures of *Phanerochaete chrysosporium*, and suggested an aldolase-type reaction following C_α-oxidation. C_α-oxidation was not observed, however.

Oxidation of III to V presumably was a straightforward reaction coupled with reduction of an electron acceptor. Although oxidations of aromatic aldehydes have been described in lignin-degrading basidiomycetes, reductions of aldehydes and acids have been far more commonly reported. In the present work, too, reductions of both III and V were seen. The significance of such reductions is not yet known, but they may well serve as internal electron transfer processes, generating oxidized electron acceptors for other oxidations. In any event, V was the intermediate on the degradation pathway here.

Conversion of V to VI involved both C_γ-oxidation and dehydrogenation across $C_\alpha - C_\beta$. It is not clear which came first; on chemical grounds, either reaction as the first would be expected to facilitate the second. Compound VI'a was shown to be readily oxidized in cultures to VI', and perhaps double bond formation occurred first. Importantly, identification of VI as a product of V established that a free phenolic group was not required for either reaction.

It is assumed that the phenylcoumarone VII' was the substrate for subsequent degradation, although it is possible that VI' was also degraded via products other than VII'.

It was considered likely that cleavage between C_α and C_β in the phenylcoumarone would occur, producing 4-ethoxy-3-methoxy-benzoic acid (VIII'), which was in fact found, and a 5-substituted vanillic acid structure in a reaction analogous to that involved in the oxidation of I to III. However, atempts to find a 5-substituted phenolic product were not successful; 5-carboxyvanillic acid was sought specifically on the assumption that the cleavage fragment at the 5-position would be oxidized further, as by *Fusarium* (Ohta et al. 1979). The fact that V and VII' were converted in ligninolytic cultures to a number of very polar, non-phenolic products suggests that cleavage of the 5-substituted aromatic moiety occurred as an alternate pathway of degradation, or perhaps as the major pathway producing VIII'. This would be consistent with the suspected mode of lignin degradation based on characterization of the polymer after fungal attack (Kirk and Chang 1975), and on the structures of low molecular weight products of fungal degradation of spruce lignin (Chen, Chang and Kirk, unpublished).

The pathway seen here differs in several respects from that of the degradation of dehydrodiconiferyl alcohol by *Fusarium solani* (Ohta et al. 1979). This phenolic substrate was oxidized by *Fusarium* at $C_{\gamma'}$ to give the corresponding cinnamyl aldehyde and cinnamic acid structures. The side chain in the latter was then oxidized with loss of two carbon atmos to yield the $C_{\alpha'}$-aldehyde, which is analogous to compound III seen here with *P. chrysosporium*. The aldehyde was then oxidized to the $C_{\alpha'}$-acid, just as with *Phanerochaete*. The next product found was 5-acetylvanillyl alcohol, obviously formed from the 5-substituted aryl moiety; no product from the other aromatic ring was detected, in contrast to the results of the present study. To explain the 5-acetylvanillyl alcohol, it was suggested (Ohta et al. 1979) that dehydration across $C_\beta - C_\gamma$ and subsequent double bond migration produced the C_γ-methyl phenylcoumarone which was oxidatively cleaved between C_α and C_β. At some stage, $C_{\alpha'}$ was reduced. 5-Acetylvanillyl alcohol was further converted to 5-carboxyvanillyl alcohol, the final degradation product detected in the case of *Fusarium*.

In addition to the degradation products of dehydrodiconiferyl alcohol seen with *Fusarium*, the tetrameric 5,5'-biphenyl-dehydrodimer, produced by an oxidative phenol coupling reaction, was identified (Ohta et al. 1979). Such reactions also are commonly seen with ligninolytic Basidiomycetes (e.g. Kirk et al. 1968); it was partly to avoid phenol oxidation that the methyl derivative was used here.

Also noteworthy is the fact that *Phanerochaete* did not demethylate the 4-*O*-methyl group of I. Such dealkylations are commonly observed in ligninolytic Basidiomycetes (e.g. Ishikawa et al. 1963; Kirk and Lorenz 1974). However, the results of Gold and coworkers (Enoki et al. 1980; Weinstein et al. 1980) suggest that non-phenolic (4-*O*-alkyl) substructure model compounds of the β-aryl ether type are not degraded via 4-*O*-dealkylation to more than a minor extent in ligninolytic cultures of *P. chrysosporium*. These workers observed further that culture conditions favoring lignin metabolism (Kirk et al. 1978) also favored metabolism of the dimeric model compounds.

All of the transformations described here with *P. chrysosporium* occurred in cultures which contained the full complement of enzymes for oxidizing lignin to CO_2, and we presume

that the ligninolytic enzyme system was responsible. Physiological support for this contention has been obtained through study of the initial reactions, converting the cinnamyl alcohol side chain to the glycerol side chain and subsequent loss of the two terminal carbons. By the use of the simple substrate 3,4-dimethoxycinnamyl alcohol, these reactions have been found to be catalyzed by a non-inducible, secondary metabolic system, just as is oxidation of lignin to CO_2. Furthermore, L-glutamate strongly represses responsible catalyst, as it does the ligninolytic system, probably through a general repression of secondary metabolism. Nevertheless, it is recognized that final proof of the relevance of the reactions seen here to lignin metabolism rests on studies with the polymer. Having specific reactions identified makes such investigations feasible.

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