PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Chemicals from Lignin

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Phil. Trans. R. Soc. Lond. A 1987 **321**, 485-494
doi: 10.1098/rsta.1987.0026

Phil. Trans. R. Soc. Lond. A **321**, 485–494 (1987)
Printed in Great Britain

Chemicals from lignin

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Prospects for processing the lignin component of lignocellulose by microbiological attack to give aromatic feedstock chemicals remain remote because so little is known about the biodegradative processes that lead to monomeric units. Additionally, a wide variety (over 100) of monomeric, dimeric and trimeric compounds have been identified as products from lignins exposed to white-rot fungi; most of these products appear in very low yields. The successions of ligninolytic communities of microorganisms, furthermore, possess enzyme systems to metabolize some of these products to carbon dioxide and water. The potential oxidative catabolic pathways for a few examples of these products are discussed, and compared with the general catabolic strategies that microbes have evolved for the utilization of benzenoid compounds. Anaerobic systems, although no less important, are not discussed here.

1. Introduction

After cellulose, lignins are the second most abundant renewable sources of reduced carbon compounds. They are synthesized in terrestrial plants by random polymerization reactions of 3,4-di- and 3,4,5-tri-substituted phenylpropenyl alcohols (coumaryl, coniferyl, sinapyl) in which the 4-position is hydroxyl, and the 3- and 5-positions are methoxyls (Crawford 1981). This process leads to a variety of carbon–carbon and carbon–oxygen bonds that characterize the biological, chemical and physical properties of lignins in their intimate relations with other cellular constituents, principally celluloses and hemicelluloses. Because of the near random and achiral nature of lignin structures, their insolubility and their associations with other biopolymers, and the dominance of non-hydrolysable bonds, progress towards understanding the chemistry and enzymology of lignin(s) biodegradation has until recently been slow (Crawford 1981). None the less, a remarkable body of information has been assembled about the natural history of successions and interactions of microbial communities responsible for the deterioration and degradation of wood. This naturally leads to potential solutions for the preservation of materials and attempts to exploit natural and modified lignocellulosic wastes, to provide useful products (Levy, this symposium). Recent studies of extracellular peroxidative enzymes produced by the white-rot fungus *Phanerochaete chrysosporium* have indicated several mechanisms that relate to the depolymerization of lignins. The putative radical reactions understandably lead to the multitude of compounds that have been identified as biodegradation products of lignin by pure cultures of the white-rot fungus *P. chrysosporium* (Eriksson, Farrell, Kirk, Palmer *et al.*, this symposium). The most-frequently discussed monomeric degradation products are vanillin and gallic acid, but neither can be considered as high-volume chemical feedstocks and it is doubtful that either could be produced from lignins by commercially viable processes (P. J. Senior, unpublished results).

The remit for this contribution was to discuss the metabolic processing of the products of

lignin biodegradation. Clearly, a topic that occupies volumes can only be addressed in a Discussion Meeting in generalities or by very specific selections of topics. I have attempted to do both by a consideration of what we know about the general strategies of oxidative biodegradations of benzenoid compounds, and of some specific reactions or reaction sequences known, or predictable, that can account for identified products and their further mineralization by white-rot fungi. A number of ether-cleavage reactions are discussed, and finally some questions are posed for potential discussion. References provided are almost random in selection.

2. General catabolic strategies

Aromatic compounds are mineralized by microorganisms in well-defined enzyme-catalysed reaction sequences. Aerobic degradation is initiated by oxygenases that incorporate molecular oxygen into the benzene ring. These enzymic reactions and the dioxygenative cleavage of catechols or quinols to yield aliphatic intermediates are widely distributed throughout the microbial world, both among bacteria and eukaryotes. The general rules are that 1,2- or 1,4-biphenols must be formed before the ring is suitable for fission (Chapman 1972). For the monomeric phenylpropane units present in lignins, the benzene nucleus is already highly substituted with oxygen functions, and with little exception catechol substitutions are already present, although in part protected by methylation. Thus catabolic processing of monomeric lignin degradation products to aliphatic intermediates is largely caused by demethylation and ring-fission reactions. Oxygenative processes assume prominence for this, but alternative enzyme catalysed reactions are also used in peroxidative (Kirk, Palmer, this symposium), reductive (Evans 1977; Schink & Pfennig, 1982; Kaiser & Hanselman 1982; Grbic-Galic & Young 1985) and hydrolytic (Eriksson, this symposium; Donnelly & Dagley 1981) reactions.

From the extensive data base of catabolic pathways for aromatic compound catabolism, it is clear that a large number of catechols and quinols are substances for *o*- or *m*-ring-cleavage enzymes to give *cis,cis*-muconic acids or 2-hydroxymuconic-6-oxohexadienoic acids, e.g. 2-hydroxymuconic semialdehyde from catechol, respectively (Dagley *et al.* 1960; Chapman 1972; Gibson 1984; Kieslich, 1984; table 1). The *m*-cleavage pathways are noted for their relaxed substrate specificity allowing the metabolic processing of a variety of analogous substrates. These *m*-fission activities appear to be restricted to bacteria. Eukaryotes, in particular fungi and yeasts, seem to have evolved only *ortho*-ring cleavage pathways (scheme 1) and this might suggest that they possess less catabolic potential. However, it is now apparent

Table 1. Examples of frequently used ring-cleavage substrates

substrate	known ring-cleavage sites	
	prokaryotes	eukaryotes
catechol	1,2; 2,3	1,2
protocatechuate	2,3; 3,4; 4,5	3,4
2,3-dihydroxybenzoate	2,3; 3,4	2,3
3,4-dihydroxyphenylacetate	2,3	
2,3-dihydroxyphenypropionate	1,2	
3-phenylcatechol	2,3	
quinol	1,2	
hydroxyquinol	1,2; 2,3	1,2
gentisate	1,2	

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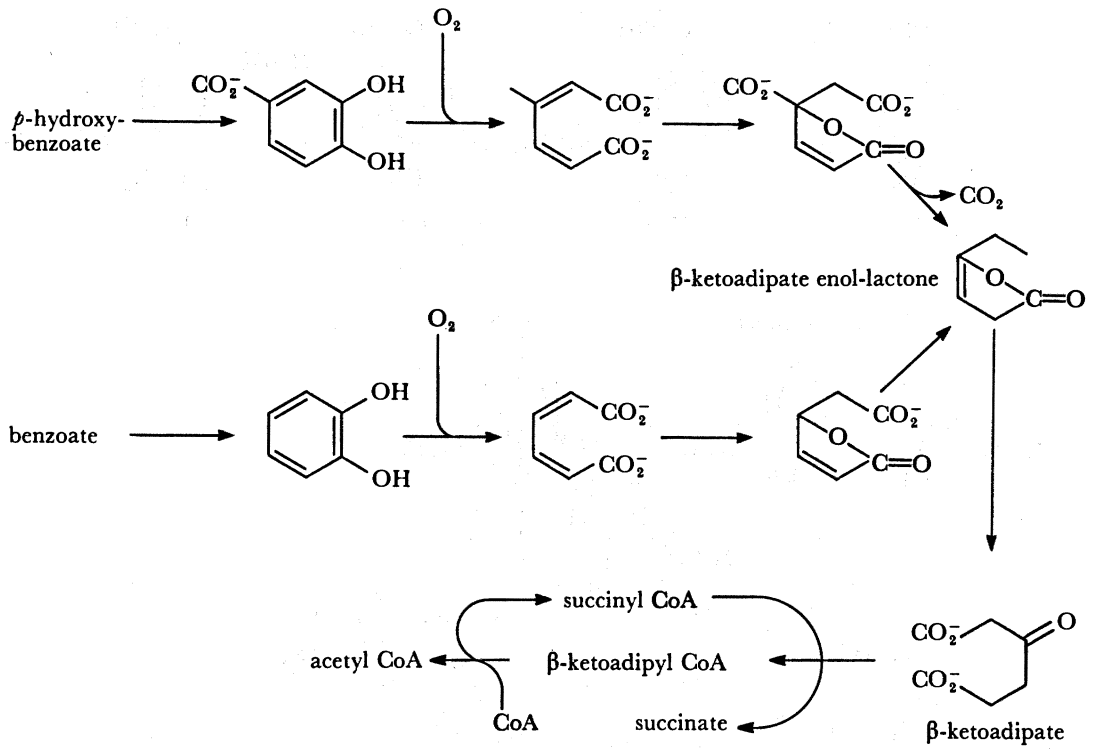
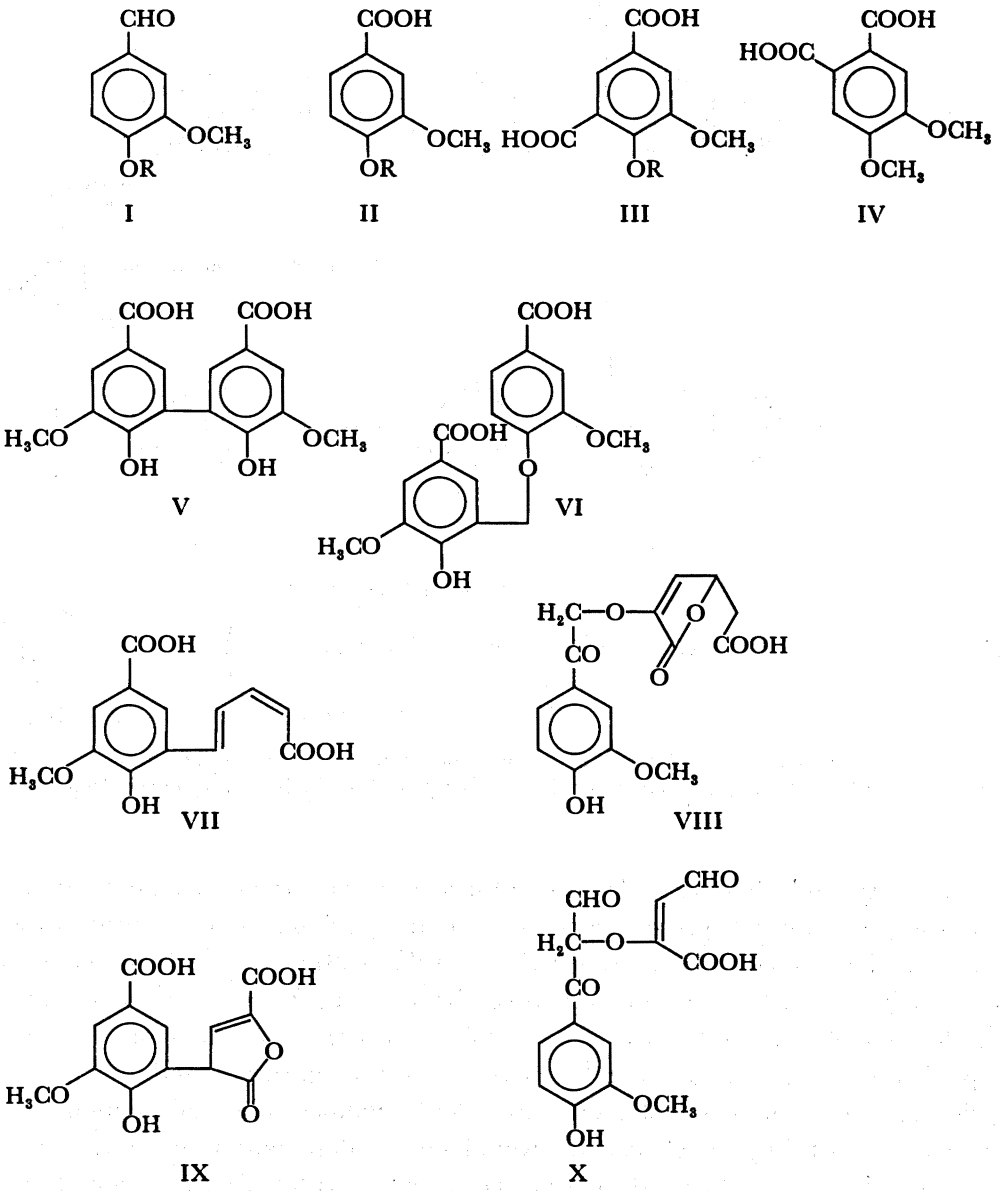


SCHEME 1. The *ortho*-ring-cleavage pathways for catechol and protocatechuate leading to β-ketoadipyl CoA in bacteria. In eukaryotes, the γ-carboxymuconolactone is replaced by the β-carboxymuconolactone isomer.

that several compounds that are *m*-cleavage substrates for bacteria are metabolized further by eukaryotes to give catechol, protocatechuate or hydroxyquinol, suitable substrates for their *o*-cleavage reaction sequences (Anderson & Dagley 1980, 1981; table 1). Because the white-rot fungi are widely regarded as the main agents of delignification, and *P. chrysosporium* is the species of choice for experiment, further discussion will focus on some of its metabolic activities. One might predict from the 3,4-di- and 3,4,5-tri-substitution patterns, and the predominant C_α–C_β-cleavage reaction in depolymerization (Kirk, Palmer *et al.* this symposium), that protocatechuate (3,4-dihydroxybenzoate) would be the dominant ring-cleavage substrate; *P. chrysosporium*, however, replaces the carboxyl of vanillate with hydroxyl, and hydroxyquinol formed becomes the substrate for ring fission.

3. EXAMPLES OF LIGNIN BIODEGRADATION PRODUCTS

The selection of compounds identified as products (over 100) of lignin biodegradation by *P. chrysosporium* for further discussion is somewhat arbitrary but is related to (*a*) the known metabolism of their demethylated analogues, and (*b*) the prediction of catabolic pathways for some dimeric products. In scheme 2, the structures of ten monomeric and dimeric lignin-biodegradation products are shown. They were selected to show their origins from the lignin polymer, as well as their probable catabolic reaction paths. Only four of these are considered further, namely compounds III, IV, IX and X, representative of monomer substitution patterns, and dimer linkages (Nakatsuba *et al.* 1982; Higuchi 1982).

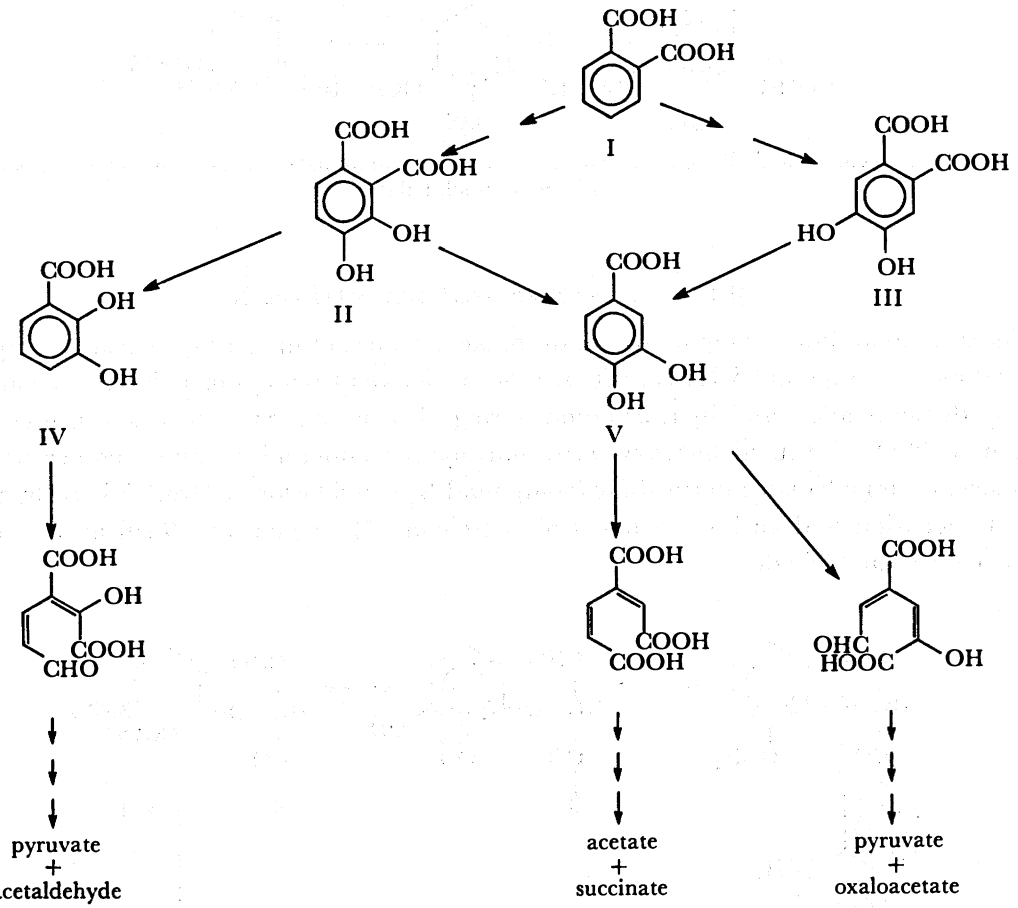


Scheme 2. Examples of products from lignin biodegradation. I, vanillin or veratraldehyde; II, vanillic or veratric acid; III, 4-hydroxy-5-methoxyisophthalic acid or 4,5-dimethoxyisophthalic acid; IV, hemipimic acid; V, a biphenyl dimer; VI, a biphenylether dimer; VII and IX, biphenyl dimer degradation products; VIII and X, biphenylether dimer degradation products.

Hemipimic acid, compound IV

The identification of an *o*-phthalate derivative as a lignin-biodegradation intermediate is interesting because it suggests that there are undescribed minor carbon–carbon bond linkages, i.e. C_β-6-aryl-C-, in the polymers or that some isomerization reactions have occurred during product isolation. The demethylated product of hemipimic acid is 4,5-dihydroxyphthalic acid, long known to be an intermediate in phthalic-acid metabolism by pseudomonads and micrococci (Ribbons & Evans 1960; Ribbons 1970; Ribbons *et al.* 1984). It has not been

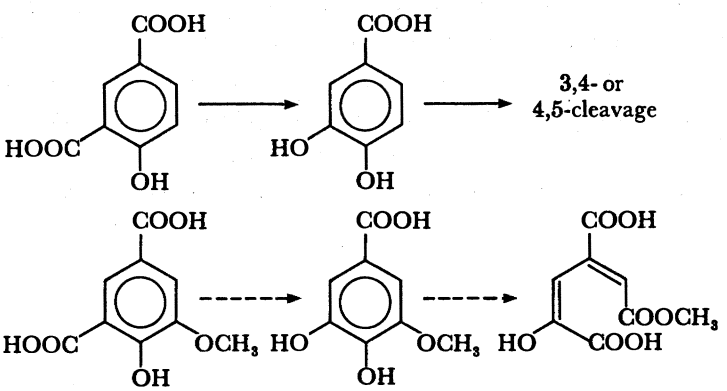
established that white-rot fungi can metabolize 4,5-dihydroxyphthalate; however, phthalic acid is an inhibitor of the ligninolytic activities of *P. chrysosporium* (Fenn & Kirk 1979), and ironically two diesters of this compound rate in the top ten of environmental pollutant chemicals in the U.S.A. Some potential metabolic pathways of *o*-phthalate and 4,5-dihydroxyphthalate are shown in scheme 3, and all of these are known to occur in bacteria (Ribbons *et al.* 1984). They illustrate the diversity of catabolic pathways for simple aromatic monomers.



SCHEME 3. Summary of known pathways for *o*-phthalate and 4,5-dihydroxy-*o*-phthalate by bacteria.

Isophthalic acid analogue, compound III

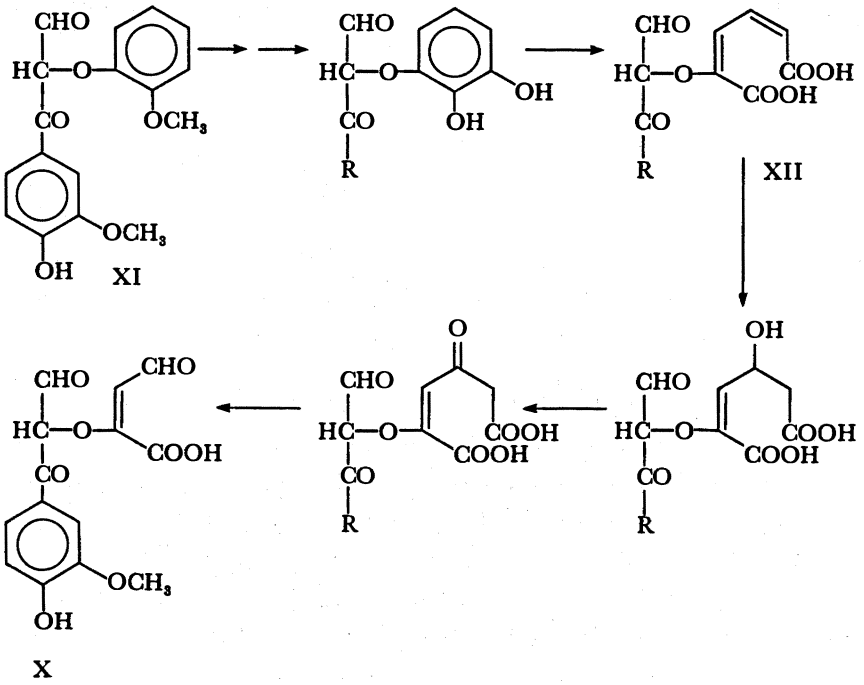
Compound III is easily seen to be derived from a minor structural element of lignin, i.e. the 3,4-dioxygenated nucleus linked in the 5-position to a C_β-carbon of the propanyl side chain of another phenyl-propanoid residue. Information on the metabolism of isophthalic and hydroxyisophthalic acids is limited. None the less, 4-hydroxyisophthalic acid is an intermediate in the biodegradation of 2,4-xylenol (Chapman & Hopper 1968), and its further metabolism to protocatechuate is reasonably well understood (Ribbons *et al.* 1984; scheme 4).



Scheme 4. Potential catabolic route for 4-hydroxy-5-methoxyisophthalate by analogy to the pathway for 4-hydroxy-isophthalate.

β-*O*-4-*Biphenylethers*, *compounds* VIII *and* X

These residual dimer compounds are probable products of the *o*-ring-cleavage of biphenyl-ether dimers. Compound VIII is easily seen to be derived from a dimer similar to compound XI by demethylation and hydroxylation, *o*-ring cleavage to give the *O*-substituted oxalo-crotonate (2-*O*-substituted hydroxy-*cis*,*cis*-muconate) compound X, and lactonization. The oxaloacetic semialdehyde intermediate (compound X) could be formed from XII by hydration, dehydrogenation and carbon–carbon fission (scheme 5) to give the *O*-substituted enol of oxaloacetic semialdehyde.



Scheme 5. Potential route for the formation of compound X.

Biphenyl structures, compounds VII *and* IX

The metabolism of biphenyl structures by bacteria invariably occurs by dihydroxylation at the 2,3-positions followed by an *m*-ring cleavage. Compounds VII and IX could be expected to arise from (5,5′-dicarboxy-2,2′-dihydroxy-3,3′-dimethoxy) biphenyl, which already possesses the 2,3-oxygenated pattern in both rings. Demethylation and *o*-fission conceivably occur, but reaction paths leading to VII and IX are unclear.

4. Aromatic catabolism by *P. chrysosporium*

There is surprisingly little known about the catabolic activities of white-rot fungi with respect to detailed reaction pathways and the regulation (physiological and genetic) of them, in spite of the extensive interest in, and importance of, this microbial group. The contributions of Buswell *et al.* (1979) show that *P. chrysosporium* can process aromatic monomers derived from lignins, e.g. vanillate, by reaction paths different from those that are used by bacteria (Ribbons & Harrison 1972; Buswell & Ribbons 1986; Crawford & Olsen 1978). Thus vanillate is metabolized (in *P. chrysosporium*) by an oxidative decarboxylation to give methoxyhydroquinone, a reaction previously described for two other white-rot fungi *Lenzites trabea* and *Polyporus dichrous* (Kirk & Lorenz 1973). The decarboxylase activity of the enzyme is reasonably broad as several 4-hydroxybenzoates, including protocatechuate, gallate, 2,4-dihydroxybenzoate, and 2,3,4-trihydroxybenzoate elicited 25% or more of the activity seen with vanillate. The physiological function of this enzyme is not clear because many of the substrates could be merely effectors for O_2 consumptions. The product methoxyhydroquinone could not be metabolized further although it disappeared from fungal cultures. Should demethoxylation of methoxyhydroquinone occur to give hydroxyquinol, then the production of β-ketoadipate is possible by the hydroxyquinol dioxygenase (*o*-) found in extracts derived from *P. chrysosporium* exposed to vanillate (Buswell & Eriksson 1979), and reduction of the maleylacetate formed (Chapman & Ribbons 1976; Buswell & Eriksson 1979). An NAD(P)H–quinone reductase activity may also be relevant in vanillate catabolism. This relates to the potential of methoxyquinone being a substrate for the release of methanol (J. D. Bu'Lock, personal communication 1986; Buswell *et al.* 1979) as discussed in the next section. Hydroxyquinone so released might be the physiological substrate for the quinone reductase to generate the ring-cleavage intermediate hydroxyquinol.

Other ring-cleavage reactions have been described in *P. chrysosporium* but their cell-free activities have been hard to detect other than by sensitive radiochemicals assays (Leathan *et al.* 1983). Ultraviolet spectra of products indicated the formation of muconic acids and over 30 aromatic compounds were degraded by the ligninolytic cultures of *P. chrysosporium*. How these activities are related to the extracellular ligninase activities is not clear (Kirk, Farrell, Palmer *et al.*, this symposium), i.e. do they catalyse *o*-ring-cleavage reactions of catechols?

The metabolism of *trans*-ferulic acid (4-hydroxy-3-methoxycinnamic acid) by *P. chrysosporium* has also been studied (Gupta *et al.* 1981). This is a substrate for the formation of more reduced and methylated products, although small amounts of vanillate and methoxyhydroquinone were also formed. In a phenoloxidase-less mutant, the same products appeared but persisted for longer periods.

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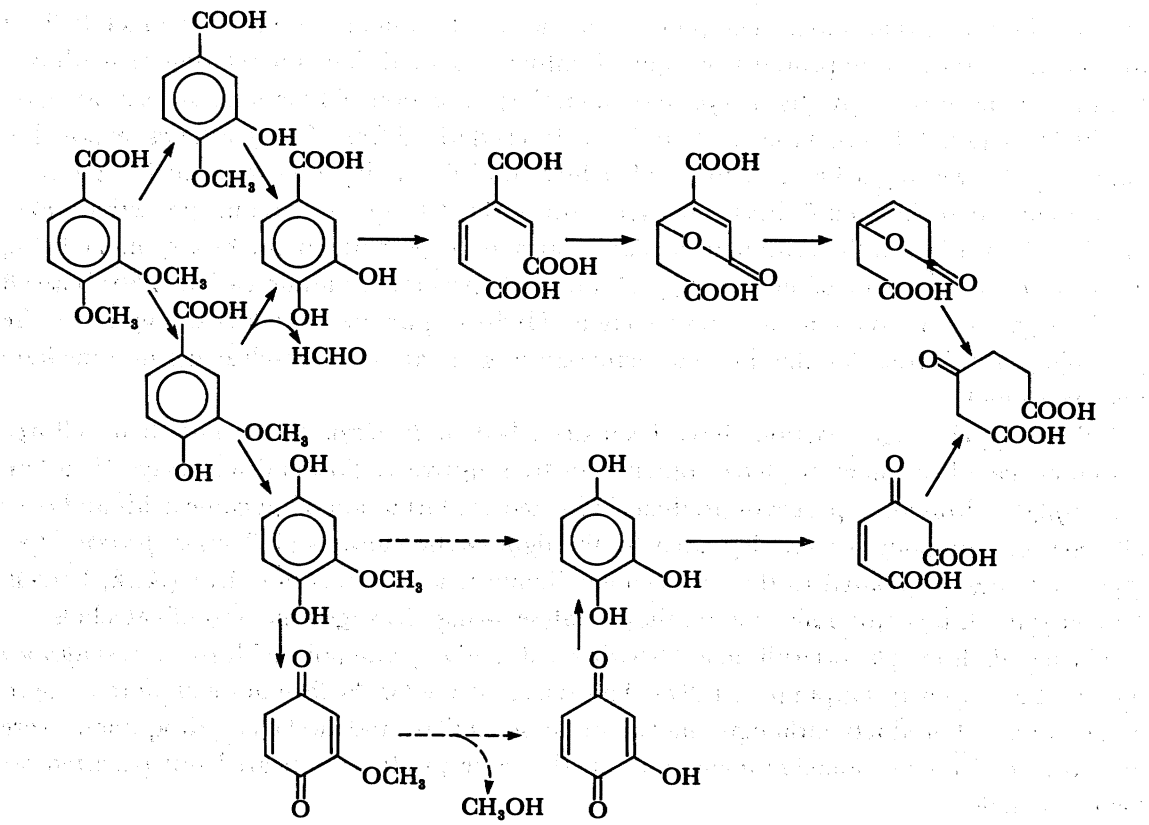
5. Ether-cleavage reactions

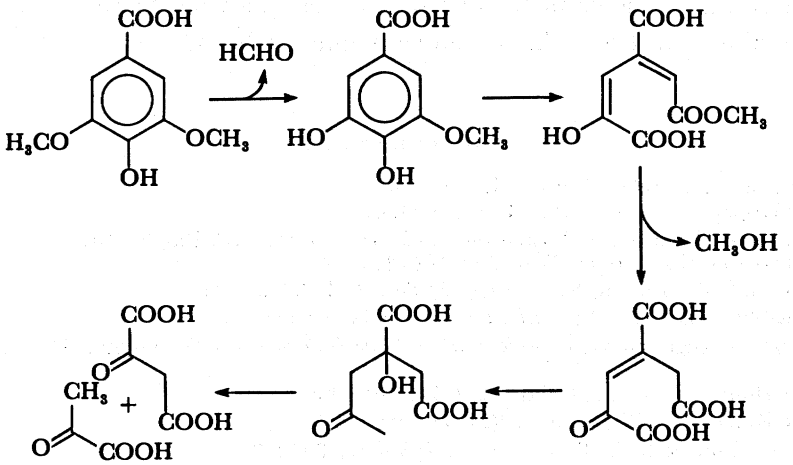
One of the commonest bonds on and between the monomeric units in lignins is the ether bond. This takes many forms, from the methoxyl groups on the phenyl nucleus, to various inter-unit cross linkages that include arylglycerol-β-aryl ethers (48%), benzylaryl ethers, non-cyclic (6–8%) and cyclic phenylcoumarans (9–12%), and diphenyl ethers (3–4%) (Crawford 1981). Many of these ether linkages are broken during the various thermomechanical treatments of lignocellulosics in the pulp, paper and fibre-board industries (Overend & Chornet, this symposium). Enzymic modifications of the ethers in lignins are poorly understood. The brown-rot and white-rot fungi both catalyse extensive demethoxylation of the polymers and one product that is released has been identified as methanol (Eriksson, this symposium).

Some enzymes responsible for the demethoxylation of lignin-degradation products and related compounds are known in bacteria. Other reaction sequences may be tentatively formulated for the generation of methanol by *P. chrysosporium* on the basis of experimental observations. Thus veratric acid derived from lignin or veratryl alcohol is metabolized to a mixture of vanillate and isovanillate before protocatechuate formation by *Nocardia* sp. (Crawford *et al.* 1973). In pseudomonads, vanillate *O*-demethylases yield protocatechuate and formaldehyde with the consumption of equimolar quantities of oxygen and NADH (Ribbons 1970; Ribbons & Harrison 1972; Buswell & Ribbons 1987). A different pathway for vanillate degradation occurs in *P. chrysosporium* whereby the carboxyl group is replaced by hydroxyl to give methoxyquinol (Buswell *et al.* 1979; scheme 6). The nature of the demethoxylation reaction



Scheme 6. Demethoxylation of vanillate by pseudomonads and potential pathway for *P. chrysosporium*. Broken arrows indicate postulated reactions.

has not been defined, but methanol is known to be a product (Eriksson, this symposium). In scheme 6, a tentative scheme for demethylation is given for the *P. chrysosporium* pathway. Methoxyquinol is oxidized to methoxyquinone, which serves as a substrate for demethylation giving methanol and hydroxyquinone (Bu'Lock, personal communication 1986). The latter could be reduced to hydroxyquinol by the quinone reductase (Buswell & Eriksson 1979). Ring cleavage of hydroxyquinol to maleylacetate and its reduction to β-ketoadipate have also been observed (Buswell & Eriksson 1979; scheme 6). Other routes of demethylation yielding methanol have been demonstrated. For a variety of 3,4,5-trimethoxylated acids, two adjacent methoxyls are removed presumably by reactions analogous to vanillate *O*-demethylase to give 3,4-dihydroxy-5-methoxy acids, e.g. 3-*O*-methylgallic acid, scheme 7. 4,5-Dioxygenative cleavage of this methoxylated protocatechuate gives a methyl ester, which is hydrolysed by an esterase to give methanol (Donnelly & Dagley, 1981; scheme 7). Release of methanol from methoxylated benzenoid compounds has also been demonstrated with the peroxidative ligninases (Kirk, Palmer *et al.* this symposium).



SCHEME 7. Demethoxylation of syringic acid by hydrolysis of ring-cleavage product esters.

6. CONCLUSIONS

Most other speakers at this symposium emphasized the chemical and physical complexities of the three main structural polymers of lignocellulose, their intimate interrelations, and the multiplicities of microbes and enzymes for lignocellulose degradation. For lignin to serve as a source of feed-stock aromatic chemicals from lignocellulose wastes there seems to be little prospect of success in the near future other than exceptions mentioned, e.g. vanillin. On the one hand the types of monomeric units released for processing are very limited, i.e. 3,4-di- and 3,4,5-tri-substituted phenylpropane, phenylethane, phenylmethane carbon skeletons; on the other, the lignin polymers are very diverse randomly formed molecules, so that the putative depolymerizing ligninases acting also in random fashion generate a vast array of depolymerization products. These accumulate transiently in small quantities presumably because they are metabolized further by the mixed populations of fungi and bacteria responsible for their formation. The costs of developing and maintaining laboratory-designed populations of microbes for the large-scale production of chemicals are probably far too great for any process

to be viable for the utilization of lignocellulosic wastes such as straw or pulp effluents (P. J. Senior, unpublished results). These conclusions should not depress research activities of lignocellulose biodegradation. There is much to be learned from the quantitatively most-important degradation process of the carbon cycle in Nature. There are many questions that can be asked, and to be answered, that may well reveal avenues for exploitation, lateral from those discussed in this paper.

If the peroxidative ligninase(s) catalyse C–C and C–O bond cleavages by cationic radical formation in fully *O*-alkylated benzenoid model compounds, and the formation of C–C bonds to give biphenyl dimers (and oligmers) from the analogous phenolic (4-hydroxy) (Kirk, this symposium), as actually occur in lignins, how is it that depolymerizations are achieved with these enzymes? Is there a rapid sequestration of lignin monomers formed by cellular uptake and catabolism? Is such a metabolic sink a function of the white-rot fungi alone? How do other organisms (in particular bacteria) contribute and interact with white and brown rots for depolymerization reactions and the catabolism of their products? What contribution do anaerobes make to the processing of lignin depolymerization products? How much does veratryl alcohol provide veratrate and vanillate intermediates as opposed to lignin? How are compounds derived from lignins identified as higher added value products?

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