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Microbiological Degradation of Lignin

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The problems of the microbiological degradation of lignin, closely related to the solution of a number of important theoretical and practical problems, have been continually attracting attention in recent years. The most recent studies in this field are analysed in the present review. Particular attention has been devoted to a critical examination of hypotheses about the possible pathways in the microbiological degradation of lignin.
The present review is the first attempt in the Soviet scientific literature at a survey of data concerning the problem of the microbiological degradation of lignin.
The bibliography includes 151 references.

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1. INTRODUCTION

The microbiological degradation of plant materials, an important component of which is lignin, plays a significant role in the carbon turnover in nature. Studies on the degradation of lignin as a result of the activity of micro-organisms [1-14], which to a large extent differs from the degradation of other plant components, have widely developed in the last 10–15 years. These investigations are closely related to studies in a number of important fields, among which the following should be mentioned: (1) humus formation and the problem of soil fertility (see the relevant reviews [12,15-17]); (2) the formation of peat and coal; (3) the conservation of wood during storage (see the review of Cartwright and Findlay [18]); (4) the possibility of employing wood infected by fungii; (5) the metabolic transformations of plants in man and animals; (6) the use of the fungal mycelia to accumulate protein products; (7) delignification of plant raw materials in order to develop rational methods for the preparation of cellulose; (8) decontamination of natural waters by the removal of lignin-containing waste (see the relevant reviews [19,20]).

There is no need to stress the great theoretical and practical importance of the above studies. Furthermore, recent studies on the microbiological degradation of lignin yielded valuable information about its structure, which makes it possible to treat microbiological activity as one of the promising methods for the investigation of lignin.

Despite the importance of these studies, in the latest monograph on the chemistry of lignin [21] unjustifiably little attention was devoted to problems of its microbiological degradation. Furthermore, there are no reviews in the Soviet chemical literature on this field.

In view of the large amount and great variety of available data, it is impossible to consider here all the above aspects of the microbiological degradation of lignin. Attention will be concentrated below on studies which yielded information about the structure of natural lignin, i.e. lignin isolated from plant tissue.

II. WOOD DECOMPOSING FUNGI

Since the aims of the present review are restricted to the analysis of the results of studies on the microbiological degradation of lignin important for the determination of its structure, among the different biological agents causing decomposition of lignin (wood-decomposing fungi, soil and water micro-organisms, intestinal bacteria) only wood-decomposing fungi will be considered, their action on wood, lignin, and its model compounds having been fairly thoroughly investigated.

Most fungi causing the decay of wood belong to one of the classes of higher fungi (*Basidiomycetes*), but there are also certain wood-decomposing fungi which belong to the class of *Ascomycetes*. Two main types of wood-decomposing fungi, usually referred to as brown rot and white rot, are distinguished. When wood is infected by brown rot, cellulose and other carbohydrate components are preferentially decomposed, while lignin remains relatively unchanged and the decaying wood acquires a brown colour. Infection with white rot results in the decomposition of all the components of wood, and as decay proceeds the residue becomes paler in colour, compared with healthy wood. A list of fungal species belonging to these two groups has been published [1,22] and in addition 126 species of wood-decomposing fungi have been characterised in detail in a review by Nobles [23].

In 1928 Bavendamm [24] observed that white rot fungi cultured on an agar medium containing gallic or tannic acid produce a dark zone around and below the mycelial films, while brown rot fungi do not generate such a zone. This reaction, which has been called the Bavendamm reaction, was subsequently widely used to classify the wood-decomposing fungi. Bavendamm attributed the ability of fungi to degrade lignin to the production of phenol oxidase. This conclusion was confirmed in a study by

Davidson et al.[25], who tested 210 species of wood-decomposing fungi in the Bavendamm reaction and established that 96% of the white rot fungi produced the coloured zones, while 80% of the brown rot fungi did not.

A considerable number of studies[23,26-31] were devoted to the determination of enzymes catalysing the oxidation of the simplest phenolic compounds to quinones, which polymerise to give the coloured complexes in the Bavendamm test. It was noted that the enzymes responsible for this reaction are laccase†[27-29], tyrosinase‡[28,29], and peroxidase§[31], but complete certainty with regard to this problem has not yet been reached.

It is noteworthy that hitherto no systematic studies have been made on the enzymes of wood-decomposing fungi. The available literature data are of a general kind and frequently contradictory. The studies in this field have been examined in detail in monographs by Schubert[7] and Shivrina[32]. It was established that white rot fungi produce two main enzymes: laccase and tyrosinase. Furthermore, Lyr showed that 14 of the 182 species of wood decomposing fungi which have been investigated produce peroxidase[33] and proposed a method for its detection[34]. These enzymes catalyse the abstraction of electrons from phenolic substrates. In the presence of laccase and peroxidase one reducing equivalent is removed from the substrate, while in the presence of tyrosinase two reducing equivalents are eliminated simultaneously.

Since the lignin-decomposing white rot fungi produce in most cases intracellular phenol oxidising enzymes, while the taxonomically related brown rot fungi, which do not produce these enzymes, do not decompose lignin, the relation between the capacity to degrade lignin and the phenol oxidase activity of the wood-decomposing fungi appears to be evident[23,31,35-37]. However, later it was shown that brown rot fungi do produce intracellular phenol oxidising enzymes: tyrosinase and an enzyme of the laccase type[38-43], as well as peroxidase[44]. Furthermore, there is evidence[45-50] that the degradation of lignin is due to enzymes which are not phenol oxidases. For this reason, Kirk and Kelman[51] undertook a study of the ability of 18 different white and brown rot fungi to degrade lignin and produce intracellular phenol oxidase capable of oxidising simple phenolic compounds. Among the fungal species investigated, there were "typical" and "atypical" white and brown rot fungi. The results of the investigation showed that the inability of wood-decomposing fungi to oxidise phenols in an agar medium cannot be used as proof that they cannot utilise lignin.

Thus the role of phenol oxidases in the biodegradation of lignins still remains uncertain. The results of the above studies[31,43,44,51] show that both the lignin decomposing white rot fungi and the brown rot fungi which do not decompose lignin produce phenol oxidising enzymes and that certain brown rot fungi produce more phenol oxidases than white rot fungi. One may therefore conclude that, if the phenol oxidising enzymes from the white rot fungi do not act on lignin in a manner different from that of the phenol oxidising enzymes of the brown rot fungi, the above differences in the ability of both groups of fungi do degrade lignin may depend on the presence of certain other enzymatic systems.

The results of macroscopic studies[52-58] showed that the microbiological degradation of woody tissue begins with the cell lumens in which the fungal hyphae are located and spreads to the region of the middle lamella. In particular, Schmid and Liese[57] established in the course of an electron microscope analysis that woody material is removed essentially layer by layer, which makes the cell walls gradually thinner, while lignin in the middle lamella and in the corners of the cells is apparently relatively resistant to enzymatic attack. On the other hand, Wilcox[58] established that the action of the cellulolytic enzymes of the white rot fungus *Polyporus versicolor* is initially restricted to the surfaces of the cell walls adjoining the lumen, and, as cellulose is removed, gradually spreads to other layers of the cell wall. On the other hand, the cellulolytic enzymes of the brown rot fungi *Poria monticola* and the lignin decomposing enzymes of the white rot fungus can penetrate the cell wall and act within it. The author attributed these differences in the cellulolytic enzymes to their molecular size and chemical properties.

It is noteworthy that, although the principal characteristics of the degradation of wood by various species of brown rot fungi are general, nevertheless the action of each species has its characteristic features due to different enzymatic activities[59]. This is responsible for the differences in the rate of decomposition of wood by various species of brown rot fungi.

III. REACTIONS OF NATURAL AND ISOLATED LIGNINS UNDER THE INFLUENCE OF WOOD DECOMPOSING FUNGI

Studies on the changes in lignin under the influence of wood-decomposing fungi were made either on preparations isolated after incubating various isolated lignins with white rot fungi in synthetic liquid culture media or on preparations extracted by neutral solvents from wood subjected to the action of white rot. In most cases the preparations isolated from liquid culture media and from decayed wood undergo analogous changes in relation to the initial control lignin (cf. Tables 1 and 2) and can therefore be treated together. However, one should bear in mind[13] that (1) the regeneration and purification of the preparations after the incubation of isolated lignins with microorganisms can lead to a series of additional changes and (2) that lignin isolated from decayed wood may differ from the control preparation not only in consequence of the changes due to the action of fungi but also due to the method of extraction, which usually differs from the method used to isolate the control preparation.

A study of the action of various brown rot fungi on sprucewood established that nitrobenzene oxidation of decomposed wood gives a higher yield of vanillin compared with healthy wood[60]. Leopold[61] investigated the products of the nitrobenzene oxidation of wood at different stages with decomposition by brown rot fungi and observed a successive decrease of their yield, although the oxidation products contained the same aldehydes and in virtually the same proportions as the products of the oxidation of healthy wood. The author believes that the decrease of the yield of vanillin is due to the preferential enzymatic attack on the "open" lignin structures, i.e. not condensed in the 5-position of the aromatic ring, which constitute the main source of vanillin in nitrobenzene oxidation. A decrease

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†*p*-Diphenol: oxygenoxidoreductase (*p*-diphenol oxidase), EC 1.10.3.2.

‡*o*-Diphenol: oxygenoxidoreductase (*o*-diphenol oxidase), EC 1.10.3.1.

§ Donor: hydrogen-peroxideoxidoreductase, EC 1.11.1.7.

of the yield of vanillin in the nitrobenzene oxidation of pinewood decomposed by brown rot fungi was also noted by Enkvist et al.[62]

Higuchi et al.[63] studied lignin preparations isolated from beechwood which had been acted upon by various brown and white rot fungi in the course of five months. The authors established that the overall yield of aldehydes in the nitrobenzene oxidation of decayed wood is lower than in the case of healthy wood, and that the yield with respect to lignin is lower for wood subjected to the action of white rot fungi than for wood subjected to the action of brown rot fungi. Moreover, an increase of the molar proportions of syringaldehyde and vanillin in the products of the nitrobenzene oxidation of decayed wood in relation to healthy wood was observed.

The decrease of the yield of vanillin in the nitrobenzene oxidation of the isolated lignins subjected to the action of white rot fungi was also pointed out by Ishikawa et al.[64] They investigated the changes in the native Brauns lignin and milled-wood lignin (MWL), isolated from pinewood and sprucewood, as a result of their incubation with white rot fungi. In the experiments a study was made of both "phenol oxidase-rich" (*Polyporus hirsutus*, *Polyporus versicolor*, and *Poria subacida* J247) and "phenol oxidase-deficient" (*Poria subacida* N199, *Fomes fomentarius*, *Fomes annosus*, and *Trametes pini*) white rot fungi. It was established that the degradation of lignin by the white rot fungi leads to a decrease of the number of methoxy-groups and an increase of the content of the overall and phenolic hydroxy-groups, carbonyl groups, and carboxy-groups (Table 1).

Table 1. Results of an analytical study of the initial lignins and lignins acted upon by white rot fungi* (according to Ishikawa et al.[64]).

Lignin	Fungal species	Yield, %	Content, %			Content, moles per lignin unit with mol. wt. of 180			Yield of vanillin, %
			C	H	OCH_3	OH_{phen}	CO	COOH	
Pinewood MWL	control	—	63.70	6.29	15.5	0.24	0.20	0.024	20.0
Ditto	*Fomes fomentarius*	77.0	61.55	5.77	14.1	0.30	0.27	0.106	15.1
"	*Polyporus versicolor*	61.8	61.41	6.11	10.1	0.32	0.23	0.130	11.0
Native pinewood lignin	control	—	63.85	6.22	14.5	0.32	0.24	0.022	22.6
Ditto	*Fomes fomentarius*	73.5	64.00	6.23	12.1	0.40	0.28	0.125	13.6
"	*Polyporus versicolor*	55.2	61.98	6.08	9.6	0.33	0.20	0.150	10.4

*Culture conditions: synthetic liquid medium containing 1% of lignin, 26–29°C, 13 days.

The study of the infrared spectra of lignin subjected to the action of white rot fungi revealed an increase in absorption at about 1670 cm^{-1}. The increase was attributed to the accumulation in the lignin of conjugated carbonyl and (or) conjugated carboxy-groups. Other investigators[65–67] noted that fungal attack enhances the absorption in the range 1710–1735 cm^{-1}. This band was also assigned to conjugated carbonyl and (or) carboxy-groups.

Ishikawa and Oki[68] showed that native spruce lignin acted upon by H_2O_2 and purified peroxidase preparations isolated from horseradish, radish, and lumps of *Collybia velutipes* N8 mycelia undergoes changes analogous to those discussed above, except that the content of methoxy-groups remains constant.

Ishikawa and Oki[69] investigated the changes undergone by ethanol-lignin from Japanese cedar under the influence of a purified enzymatic solution obtained from mycelial clusters or mycelium-free culture filtrates from *Collybia velutipes* N8 and *Polyporus versicolor*. It was established that enzymatic hydrolysis of ethanol-lignin leads to an almost quantitative removal of ethoxy-groups with a simultaneous increase of the number of alcoholic OH groups and a slight increase of the content of phenolic hydroxyls. No changes were observed in the infrared and ultraviolet spectra as a result of the enzyme action. At the same time a study was made of the transformations of a number of aromatic ether compounds under the influence of a purified enzymatic solution (the results are discussed in Section V). The results enabled the authors to conclude that during fungal attack alkyl–alkyl and alkyl–aryl ether linkages are hydrolysed enzymatically in ethanol-lignin.

Table 2. Results of an analytical study of sprucewood MWL and lignin preparations extracted from sprucewood acted upon by *P. subacida* (according to Hata[66]).

Preparation	Yield, % of lignin in decayed wood	Content, %			Content, moles per lignin unit with mol. wt. of 180			
		C	H	OCH_3	OH_{tot}	OH_{phen}	CO	COOH
MWL	—	62.81	5.88	15.24	1.18	0.25	0.14	0.017
DL-I*	1.1	60.44	5.67	12.32	1.42	0.30	0.21	0.053
DL-II**	2.7	58.54	5.23	11.75	1.67	0.40	0.28	0.106
DL-III***	—	55.01	4.98	9.69	—	—	0.30	0.113

*Lignin extracted with an acetone–water mixture from wood acted upon by *P. subacida* for 3 months.
**Lignin extracted with an acetone–water mixture from wood acted upon by *P. subacida* for 6 months.
***Lignin extracted with water from wood acted upon by *P. subacida* for 6 months.

On the basis of ultraviolet and infrared spectroscopic studies native spruce lignin and lignin extracted by acetone from sprucewood powder acted upon by *Poria subacida*, Fukuzumi[70] concluded that there is an appreciable increase in the number of carbonyl groups in lignin subjected to enzymatic action. Hata[66] investigated preparations of lignin extracted from sprucewood acted upon by *Poria subacida* BII. Compared with spruce MWL, the preparations isolated contained fewer methoxy-groups and more phenolic hydroxyls, carbonyl groups, and carboxy-groups (Table 2). In addition, it was established that, as a result of enzyme action, the number of coniferaldehyde and guaiacylcarbinol groups in lignin diminishes. In the nitrobenzene oxidation of degraded lignins a smaller amount of vanillin and a greater amount of vanillic acid (compared with spruce MWL) is produced, and with increase of the duration of enzyme action, this tendency becomes more pronounced (Table 3). However, the overall yield of these products from degraded lignin is lower than from MWL. In hydrolysis by a dioxan–water

mixture (180°C, 50 min and 140°C, 60 min) degraded lignin gives a higher yield of vanillic acid and a lower yield of coniferyl alcohol, coniferaldehyde, and vanillin compared with MWL. Furthermore, a very small amount of ferulic acid, not detected in the products of the hydrolysis of MWL is produced. The study of free radicals in MWL and in degraded lignins showed that their numbers are approximately the same, but the radicals belong to different types.

Konishi and Inoue [71-74] showed that the enzymes produced in culture filtrates by the fungus *Coriolus versicolor* convert MWL isolated from Sugi sapwood into a water-soluble state without a significant change in its molecular weight, but do not break down lignin into low-molecular-weight compounds. Compared with the initial MWL, the water-soluble lignin contains more COOH groups and fewer alcoholic OH groups. The above changes were attributed to enzymatic attack on the side chains of the phenylpropane units of lignin, which evidently proceeds via a radical reaction.

Table 3. Yields of nitrobenzene oxidation products (% of lignin) (according to Hata [66]).

Preparation	Vanillic acid	Vanillin
Sprucewood MWL	2.2	24.1
DL-I*	4.8	14.9
DL-II**	6.7	10.2

*The designations of the preparations are the same as in Table 2.

Steelink [75,76] established that the action of brown or white rot fungi on wood increases by a factor of 2–3 its content of free radicals and that the wood of deciduous trees attacked by fungi has a higher concentration of free radicals than the wood of conifers. These data were confirmed in a study by Kreitsberg et al. [77]

Ferm et al. [78] investigated the transformations of MWL from Liquidambar (sweetgum) under the influence of partly purified preparations of horseradish and peroxidase and laccase from *Polyporus versicolor* and also of an unpurified enzyme from the brown rot fungus *Poria cocos*. Experiments on the enzymatic oxidation of lignin were carried out in a methylcellosolve–water mixture which dissolves MWL and has only an insignificant influence on the stability of the enzyme preparations employed. ESR showed that each enzyme preparation causes the formation of free radicals of the phenoxy-type. The formation of radicals of a similar type was observed previously [79] on incubation of syringyl compounds with phenol oxidising enzymes (see Section V). Furthermore, Ferm et al. [78] reported the preliminary results of a study of the changes in the molecular weight distribution of lignin and lignosulphonate under the influence of phenol oxidising enzymes. It was shown by gel permeation chromatography that phenol oxidising enzymes cause an increase in molecular weight. On this basis, the authors suggested that phenol oxidising enzymes can be involved in later stages of the microbiological degradation process and not in the initial depolymerisation reactions.

Ishihara and Miyazaki [80] investigated the changes in the molecular weight of maple MWL as a result of incubation with laccase B obtained from the filtrate of a *Polyporus versicolor* culture and concluded on the basis of the results that this enzyme can polymerise and depolymerise lignin, the first function being dominant.

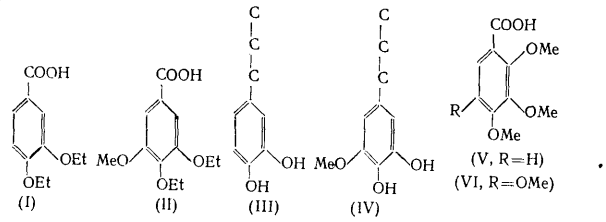
Kirk and Lundquist [67] made a comparative study of MWL preparations isolated from healthy cambium and cambium acted upon by *Polyporus versicolor* (32% weight loss) of sweetgum wood. They established that these preparations do not show distinct differences as regards elemental composition and the content of OCH_3 groups, ultraviolet and infrared spectra, $\Delta\epsilon$ curves, molecular weight distribution curves, and the composition of the ethoxybenzoic acids formed on degradation of lignin by the ethylation–oxidation method [81]. The results showed that the lignin isolated from decayed wood by mechanical milling (27% yield) does not undergo significant changes. This, however, does not allow a conclusion concerning the degree of change of the entire lignin remaining in the decomposed wood. Nevertheless the authors suggested that in the decay of wood by the fungus *Polyporus versicolor* only a limited proportion of the lignin is subjected to enzyme action and is removed before the decay spreads to other parts of the lignin.

Kirk and Lunquist also investigated the substances extracted from healthy and decayed wood with a 2 : 1 benzene–ethanol mixture and then with 96% ethanol (their yields were 3.3 and 3.6% of the wood respectively). The high-molecular (molecular weight >400) fractions (the so called "extractive lignin") obtained as a result of gel filtration on Sephadex G-25 were investigated by ultraviolet and infrared spectroscopy. The experimental results showed that these preparations consist of lignin and revealed differences between the extractive lignin from healthy and decayed wood. An appreciable absorption at 1730 cm^{-1} was noted in the infrared spectrum of lignin extracted from decayed wood. It was established that this band is due to the chemical changes caused by the fungi and not by the presence of non-lignin impurities. The band could not be assigned accurately, although the authors suggest that absorption in this region may be attributed to non-conjugated carbonyl groups and non-conjugated carboxy-groups. It is interesting to note that the changes in lignin leading to the appearance of the 1730 cm^{-1} band in its infrared spectrum are not accompanied by appreciable changes in the ultraviolet spectrum. This is clearly evidence that enzyme action does not result in the formation of appreciable amounts of chromophoric systems whose spectroscopic properties differ greatly from those of the chromophores present initially in the lignin (aryl-conjugated carboxy-groups, aryl-conjugated carbonyl groups, *p*-benzoquinonoid residues, and others).

Interesting results were obtained in recent years in studies by oxidative degradation methods on the lignin remaining after wood has been acted upon by brown rot fungi (the so called enzymatically liberated lignin).

According to the results of a number of investigators [60,62,82-87], the action of brown rot fungi on lignin greatly reduces its content of methoxy-groups. Kirk and Adler [81,88] undertook a study of the structural changes leading to a decrease of the content OCH_3 groups. They investigated the lignin liberated enzymatically from sweetgum wood acted upon by the brown rot fungus *Lenzites trabea*. After preliminary ethylation, the lignin preparations were subjected to oxidative degradation. The resulting ethoxybenzoic acids were identified by gas chromatography and mass spectrometry. The acids (I)

and (II) were detected in the degradation products of the enzymatically liberated lignin, which indicates the presence in the latter of the *o*-diphenol structures (III) and (IV).



These structures are formed as a result of the fungal demethylation of the lignin units of the guaiacyl and syringyl type respectively. Furthermore, Kirk and Adler boiled a preparation of the enzymatically liberated lignin with 0.2 M HCl solution in a dioxan–water mixture. This treatment led to the cleavage of the alkyl aryl ether linkages with formation of phenolic OH groups. Subsequent ethylation and oxidative degradation gave higher yields of acids (I) and (II). Thus fungal attack does not lead to the demethylation of units with a free phenolic hydroxyl alone. However, non-phenolic residues are demethylated to a smaller extent than the corresponding phenolic residues. It was also established that both phenolic and non-phenolic structures of the syringyl type are demethylated more vigorously than the corresponding structures of the guaiacyl type. The preferential demethylation of units of the syringyl type can evidently be explained by the fact that they contain two methoxy-groups, so that the demethylating enzymatic system is better able to come into contact with them.

Thus *Lenzites trabea* demethylates both free and etherified units of the guaiacyl and syringyl type in lignin. According to the authors [81,88], the demethylation can occur as a result of the direct oxidation of the methyl group, which is then eliminated as formaldehyde, leaving behind a free phenolic OH group.

Kirk et al. [89] made a comparative study of methoxylated aromatic carboxylic acids formed in the degradation of enzymatically liberated lignin and MWL from sweetgum by the method of Larsson and Miksche [90-92]. The acids (V) and (VI), formed from structures hydroxylated as a result of fungal action, were identified among the degradation products of the enzymatically liberated lignin. Hydroxylation takes place in the *ortho*-position relative to the side chains of the phenylpropane units of lignin. However, neither the mechanism of this process nor the lignin units which undergo hydroxylation (initial guaiacyl and syringyl units or the newly formed *o*-diphenol units) are so far known.

IV. LOW-MOLECULAR-WEIGHT PRODUCTS OF THE MICROBIOLOGICAL DEGRADATION OF LIGNIN

Henderson [93] showed that incubation for six months of sprucewood and birchwood fillings, subjected to preliminary extraction, with the white rot fungi *Polystictus versicolor* and *Trametes pini* led to the liberation of vanillic acid in the first case and a mixture of vanillic and syringic acids in the second. Higuchi et al. [63] reported the presence of coniferaldehyde, vanillin, and syringaldehyde in the alcohol–benzene extract of beechwood powder infected by white rot fungi. Fukuzumi [70] investigated the products of the enzymatic degradation of native sprucewood lignin by the fungus *Poria subacida* using paper chromatography and reported the presence of 4-hydroxy-3-methoxypyruvic acid. Furthermore, β-coniferyl guaiacylglyceryl ether and a compound resembling vanillic acid were detected in the acetone and methylene chloride extracts of sprucewood powder decomposed by the same fungus.

An extensive study on the low-molecular-weight products of the enzymatic degradation of lignin was carried out by Ishikawa et al. [64] They incubated native lignin and MWL preparations isolated from pinewood and sprucewood with various white rot fungi in liquid culture media. The acid fractions of the ether extracts of the lignin degradation products were studied by paper chromatography. The products identified are listed in Table 4.

Table 4. Low-molecular-weight products formed when pinewood and sprucewood lignin is acted upon by white rot fungi* (according to Ishikawa et al. [64]).

	Vanillic acid	*p*-Hydroxybenzoic acid	Ferulic acid	4-Hydroxy-3-methoxyphenylpyruvic acid enolic tautomer)	*p*-Hydroxycinnamic acid	Guaiacylglycerol	Vanillin	Dehydrodivanillin	Coniferaldehyde	*p*-Hydroxycinnamaldehyde	β-Coniferyl guaiacylglyceryl ether
R_f	0.08	0.11–0.12	0.17–0.18	0.18–0.19	0.22–0.24	0.42–0.44	0.45–0.47	0.53–0.55	0.60–0.61	0.66–0.68	0.80–0.82
A** NL	+	+			+		+	+	+	+ (?)	
A** MWL	+	+			+		+	+ (?)	+		
B*** NL	+	+	+	+	+	+	+	+	+	+	+
B*** MWL	+	+	+	+	+	+	+	+	+	+	+
Control****	+	+	+	+	+	+	+	+	+	+	+

*Conditions: culture medium containing 1% of lignin, 27°C, 28 days. Solvent for paper chromatography—butanol saturated with 3% ammonia.

**A—species rich in phenol oxidase (*Polyporus hirsutus*, *Polyporus versicolor*, and *Poria subacida* J247).

***B—species deficient in phenol oxidase (*Poria subacida* N199, *Fomes fomentarius*, *Fomes annosus*, and *Trametes pini*).

****Medium without lignin after degradation by fungi of group A or B.

The results of this investigation showed that ferulic acid, 4-hydroxy-3-methoxyphenylpyruvic acid, guaiacylglycerol, and its coniferyl ether are probably more readily metabolised by phenol oxidase-rich microorganisms (group A), compared with other intermediates. The presence of β-coniferyl guaiacylglyceryl ether in the enzymatic degradation products of lignin and also the formation of coniferaldehyde, α-hydroxypropiovanillone, and vanilloyl methyl ketone in the acidolysis of the phenolic fraction of the lignin degradation products constitutes proof, according to the authors, that lignin contains β-coniferyl guaiacylglyceryl ether linkages.

As noted above, Kirk and Lundquist [67] extracted the cambial components of sweetgum wood, both healthy and acted upon by *Polyporus versicolor*, with a 2:1 benzene–ethanol mixture and then with 96% ethanol. The extracted products were subjected to gel filtration on Sephadex G-25

and the resulting low-molecular-weight (monomeric) fraction was separated by paper chromatography. Vanillin, syringaldehyde, vanillic and syringic acids, coniferylaldehyde and sinapaldehyde were identified in the monomeric fraction of the substances extracted from the decayed wood. Since the same substances were found in approximately the same amounts also in products extracted from the healthy wood, the authors suggest that their formation cannot be attributed to fungal attack. Evidently the low-molecular-weight compounds are formed as a result of hydrolysis during decay and not as a result of the decomposition of the phenylpropane units of lignin.

Kirk and Lundquist attempted to detect in the monomeric fraction of the substances extracted from decayed wood guaiacylglycerol, syringylglycerol, and 4-hydroxy-3-methoxyphenylpyruvic acid, i.e. the compounds which Ishikawa et al.[64] identified among the products of the enzymatic degradation of lignin. These compounds were not detected, which, according to Kirk and Lundquist, requires the amendation of the results of earlier studies. However, since *Polyporus versicolor* is a phenol oxidase-rich species of the white rot fungi, the results of Kirk and Lundquist are not inconsistent with those of Ishikawa et al. (Table 4).

Ishihara and Miyazaki[80] incubated maple MWL with a preparation of laccase B obtained from the filtrate of a *Polyporus versicolor* culture for 4 days at 30°C. 2,6-Dimethoxy-*p*-benzoquinone, the formation of which has been observed previously in the enzymatic degradation of a number of model compounds[94] (see Section V), was identified among the low-molecular-weight degradation products of lignin.

Antropova and coworkers[95] investigated 548 strains of wood decomposing fungi belonging to different taxonomeric groups¶. In these experiments the sulphate lignin from aspenwood served as the only source of carbon. The results of the chromatographic study of the monomeric lignin degradation product revealed some specificity in the action of the fungi investigated. Thus aldehydes and certain acids predominated in the cultures of *Mucor* species, while the main products of the metabolism of fungi of the *Trichoderma* species were ketones and benzoic and vanillic acids. *Aspergillus* species produced coniferaldehyde, *p*-hydroxybenzaldehyde, dehydrodivanillin, ketones, and acids, including certain acids which had not been detected in the presence of *Mucor* and *Trichoderma* species. Alcohols, absent in the presence of fungi of the *Mucor*, *Trichoderma*, and *Aspergillus* species, as well as phenols, certain aldehydes, ketones, and acids were detected in the products of the metabolism of fungi of the *Penicillium* and *Fusarium* species.

The study of the lignin-decomposing properties of micromycetes led to the discovery of two most active strains, *Mucor* sp. 15157 and *Penicillium* sp. 50311, exhibiting laccase and phenolase activities respectively[96].

In the degradation of lignin by *Mucor* sp. 15157 the following products were detected in the culture liquid: *p*-hydroxybenzoic, benzoic, *p*-hydrozycinnamic, vanillic, syringic, sinapic, protocatechuic, homoprotocatechuic, cinnamic, 4-hydroxy-3-methoxyphenylpyruvic, and ferulic acids, vanillin, protocatechuic aldehyde, and a number of unidentified substances. In the determination of the quantitative yield of the main lignin decomposition products *p*-hydroxybenzoic acid was detected in an amount of 0.4% on the 7–12th day of the growth of the fungus, while on the 18th day the yield reached 2.0%. Vannillic acid appeared on the 7th day in an amount of 0.06%, its amount gradually increasing and reaching 0.13% on the 20th day, while on the 23rd day it rose sharply to 3.5% after which it fell and varied in the range 0.06–0.1%. Syringic acid appeared only on the 28th day, in an amount of 0.04–0.1%. The yield of the remaining substances varied in the range 0.04–0.1%.

In the degradation of lignin by *Penicillium* sp. 50311 the following substances were detected in the culture liquid, *p*-hydroxybenzoic, benzoic, vanillic, 4-hydroxy-3-methoxyphenylpyruvic, ferulic acid, sinapaldehyde, vanillyl alcohol, 3-hydroxyphenyl-1-propanol, guaiacyl-3-propanol, guaiacyl-2-propanol, pyrogallol, and phenol. The amount of vanillic acid in the culture liquid gradually increased: its content on the 10th day was 0.09%, reaching a maximum level of 0.89% on the 18th day, after which it gradually fell; the same happened with *p*-hydroxybenzoic acid: its content was 0.06% on the 3rd day, 0.5% on the 30th day, after which it fell. The amount of syringic acid reached a maximum of 3.5% on the 30th day, after which it decreased to 0.06%.

V. MICROBIOLOGICAL DEGRADATION OF PHENOLIC COMPOUNDS STRUCTURALLY RELATED TO LIGNIN. POSSIBLE PATHWAYS IN THE MICROBIOLOGICAL DEGRADATION OF LIGNIN

The studies on the enzymatic transformations of phenolic compounds structurally related to lignin discussed below yielded information, which led to hypotheses concerning the mechanism of the transformations of lignins in the course of fungal attack.

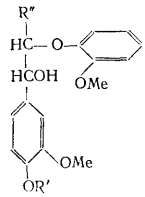
As early as 1956, Minami and Fukuzumi[97] pointed out the reducing function of *Polystictus sanguines*, having detected benzaldehyde in the metabolic products of benzoic acid. Later Fukuzumi et al.[98] reported the reduction of veratric acid to veratraldehyde as a result of incubation with *P. sanguines*.

Henderson and coworkers[99] investigated the transformations of certain aromatic acids under the influence of *Polystictus versicolor* and showed that these fungi produce an intracellular alcohol dehydrogenase. *Polyporus versicolor* reduced *m*- and *p*-methoxybenzoic, veratric, and β-naphthoic acids to the corresponding aldehydes and alcohols. Only alcohols were detected among the products of the enzymatic transformations of *o*-methoxybenzoic and benzoic acids. The corresponding alcohols were also obtained from *o*- and *p*-hydroxybenzaldehydes. 2,4-Dimethoxybenzoic, phenylacetic, and α-naphthoic acids were not reduced. The rate of reaction varied as a function of the position and nature of the substituent in the aromatic ring. Furthermore, it was shown that 2,4-dimethoxybenzoic, and *o*-, *m*-, and *p*-methoxybenzoic acids were demethylated to a slight extent to the corresponding hydroxy-compounds, while cinnamic, benzoic, and phenylacetic acids were hydroxylated in the *p*-position, the last two acids only to a limited extent. Some degradation of the aromatic structure was observed in the course of the metabolic transformations of cinnamic, β-naphthoic, and *p*- and *o*-hydroxybenzoic acids.

¶ Some of the *Mucor*, *Aspergillus*, and *Penicillium* species comprise soil fungi and are incapable of decomposing wood by themselves.

Shimazono and Nord[100] established that *P. versicolor* under aerobic conditions reduces *p*-methoxybenzoic acid to the corresponding aldehyde and alcohol. Raman and Shanmugasundaram[101] reported that cultures of the fungus *Aspergillus niger* convert in the presence of glucose up to 50% of benzoic acid, 35% of *o*-aminobenzoic acid, and 10% of *p*-aminobenzoic acid into the corresponding aldehydes. *o*- and *p*-Hydroxybenzoic acids were not reduced to the aldehydes. Furthermore, under anaerobic conditions benzaldehyde was reduced to benzyl alcohol, but other aldehydes did not undergo a similar reaction.

Henderson and coworkers[102] established that *Polystictus versicolor* produces an intracellular alcohol oxidase, which can oxidise primary aromatic alcohols (benzyl alcohol and its *p*-methoxy, *m*-methoxy, 3,4-dimethoxy-, 4-hydroxy-3-methoxy, *o*-hydroxy-, and *p*-hydroxy-derivatives, 4-hydroxy-3-methoxycinnamyl alcohol, and β-naphthylmethanol) to the corresponding aldehydes, but does not oxidise secondary alcohols [1-(3,4-dimethoxyphenyl)ethanol, 1-(4-hydroxy-3-methoxyphenyl)ethanol, and 1-(4-methoxyphenyl)ethanol].



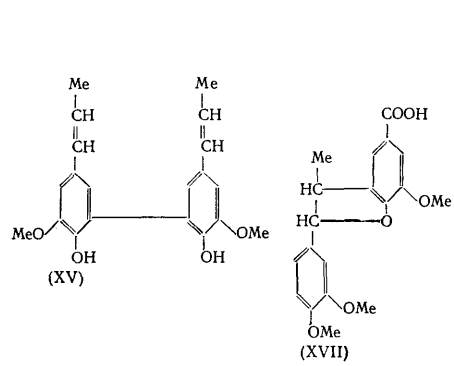
(VII; R′=R″=H)
(VIII; R′=H; R″=CH_2OH)
(IX, R′=CH_3; R″=H)
(X, R′=CH_3; R″=CH_2OH)

In the course of a study of the fungal metabolic transformations of model compounds for lignin, Henderson and coworkers[103] established that the mycelial cells of *P. versicolor* metabolised the β-guaiacyl ether of guaiacylglycol (VII), and β-guaiacyl guaiacylglyceryl ether (VIII) but did not metabolise the β-guaiacyl ether of veratrylglycol (IX) and β-guaiacyl vertrylglyceryl ether (X). The authors noted that the fungus can methylate the phenolic OH groups. Thus the metabolic transformations of compound (VII) yielded veratryl alcohol. Veratraldehyde and veratryl alcohol were also formed as a result of the metabolic transformations of *p*-hydroxybenzoic acid, protocatechuic acid, vanillin, and vanillyl alcohol. None of the compounds (VII)–(X) was oxidised by the intracellular alcohol oxidase of *P. versicolor*.

Ishihara and Miyazaki[104] subjected vanillic acid to the action of the unpurified enzyme from *P. versicolor* and isolated from the reaction mixture a yellow crystalline product melting at 225–226°C. The study of the ultraviolet, infrared, NMR, and mass spectra and analysis of the oxidative degradation products established that this compound is 6-(4′-carboxy-2′-methoxyphenoxy)-2-methoxy-1,4-benzoquinone[105].

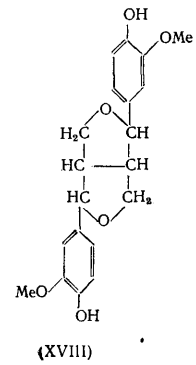
On the basis of the results of the study of the enzymatic degradation of lignin, Fukuzumi[70,106] suggested that lignin decomposes via β-ethers of guaiacylglycerol, β-hydroxyconiferyl alcohol, and 4-hydroxy-3-methoxyphenylpyruvic acid under the action of enzymes from *Poria subaciua*. Fukuzumi suggested that compounds resembling vanillic acid are formed as a result of the cleavage of the side chain of guaiacylpropanones under the influence of the fungal oxidase. He confirmed the presence of homogentisic acid in the culture medium of *P. subacida*.

Ishikawa et al.[107] investigated the transformations of a number of aromatic compounds related to lignin under the influence of *Polyporus versicolor* and *Fomes fomentarius*, which belong to the class of phenol oxidase-rich and phenol oxidase-deficient species of white rot fungi. Vanillyl alcohol (XI), guaiacylmethylmethanol, 4-hydroxy-3-methoxyphenylpyruvic acid (XII), coniferaldehyde (XIII), coniferyl alcohol (XIV), dehydrodi-isoeugenol (XV), guaiacylglycerol (XVI), and its β-guaiacyl ether (VIII) together with certain veratryl and *p*-hydroxyphenyl analogues of these compounds, as well as Erdtman's acid (XVII), pinoresinol (XVIIIa), and symplocosigenol (XVIIIb) were used as substrates.



(XV)

(XVII)



(XVIII)

The studies established that in general guaiacyl compounds were metabolised faster while *p*-hydroxyphenyl compounds were somewhat more resistant to fungal attack under the same conditions. *p*-Hydroxycinnamic, *p*-hydroxyphenylpyruvic, ferulic, and 4-hydroxy-3-methoxyphenylpyruvic acids, coniferyl aldehyde, coniferyl alcohol, isoeugenol, and guaiacylglycerol were particularly rapidly metabolised. After the incubation of these compounds with *P. versicolor* for 4–5 days, only small amounts of the substrate were detected in the filtrates. However, the amount of the metabolic products accumulated in the reaction medium was also comparatively small. It is likewise noteworthy that a proportion of the compounds investigated were converted into polymers. *p*-Hydroxycinnamic, *p*-hydroxyphenylpyruvic, ferulic, and 4-hydroxy-3-methoxyphenylpyruvic acids, Erdtman's acid, coniferaldehyde, coniferyl alcohol, isoeugenol, dehydrodi-isoeugenol, simplocosigenol, and pinoresinol were more readily polymerised by the fungus *P. versicolor* than by *F. fomentarius*. The principal metabolic products formed from the substrates tested are listed in Table 5.

The main products of the enzymatic degradation of the compounds employed are vanillin and vanillic acid, their yield being higher for the degradation monomers and not dimers. Since the authors did not report their control experiments involving the incubation of the medium and substrate alone, this suggested[13] that vanillin and vanillic acids might have been formed as a result of the autoxidation of the specimens or contamination of the substrates.

Both *P. versicolor* and *F. fomentarius* converted β-guaiacyl guaiacylglyceryl ether (VIII) into guaiacylglycerol (VI) and guaiacol. The guaiacylglycerol formed in this way was converted further by both fungi into vanillic acid via 4-hydroxy-3-methoxyphenylpyruvic acid (XII) and vanillin, but the acid (XII) was not detected in the culture medium of *P. versicolor*.

p-Hydroxyphenyl derivatives undergo similar transformations (*p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid are formed from *p*-hydroxycinnamic and *p*-hydroxyphenylpyruvic acids, while *p*-hydroxybenzaldehyde is oxidised to *p*-hydroxybenzoic acid).

It was noted at the same time that guaiacyl compounds are reduced under the influence of enzymes. In particular, in the presence of *F. fomentarius* vanillyl alcohol (XI) was detected in the products of the metabolic transformations of vanillin, vanillic acid, acetovanillone, and isoeugenol. Furthermore, *p*-hydroxybenzoic, *p*-hydroxycinnamic, and ferulic acids as well as *p*-hydroxybenzaldehyde were converted enzymatically into the corresponding aldehyde or alcohol.

Table 5. Products of the enzymatic degradation of a number of phenolic compounds structurally related to lignin (according to Ishikawa et al.[107]).

Substrate	Vanillin (XIX)	Dehydrodivanillin (XX)	Vanillic acid (XXI)	Ferulic acid (XXII)	Coniferaldehyde (XXIII)	4-Hydroxy-3-methoxyphenylpyruvic acid (XII)
Vanillin		+	+			
Vanillyl alcohol	+	+	+			
Acetovanillone	+	+	+			
Guaiacylmethylmethanol	+	+	+			
Ferulic acid	+	+	+			
4-Hydroxy-3-methoxyphenylpyruvic acid	+	+	+			
Erdtman's acid*	+	+	+			
Pinoresinol*	+	+	+			
Symplocosigenol*	+	+	+			
Dehydrodiisoeugenol*	+	+	+			
Coniferaldehyde	+	+	+	+	+	
Coniferyl alcohol	+	+	+	+	+	
Isoeugenol	+	+	+			
Guaiacylglycerol	+		+			+

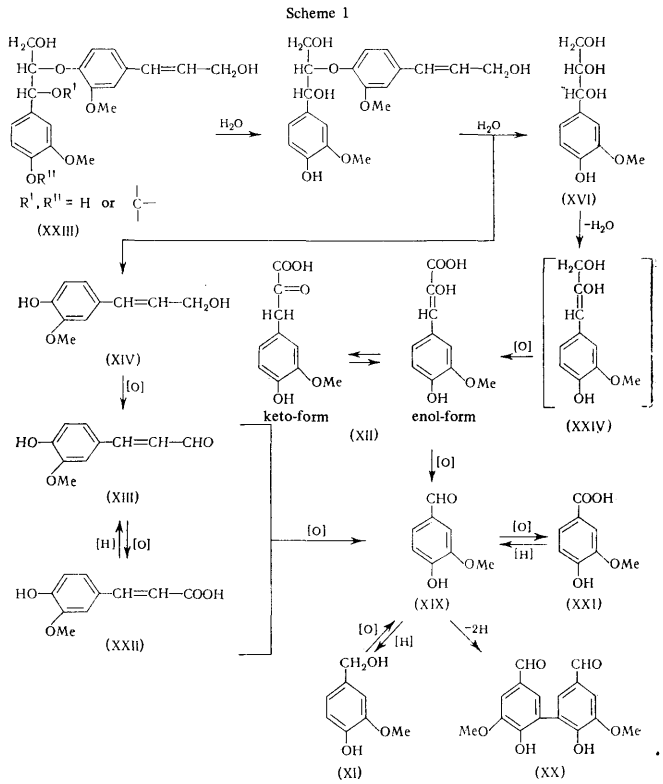
*Compound relatively resistant to fungal attack.

It was also noted that the action of both fungi leads to partial demethoxylation of a number of methyl derivatives of the *p*-hydroxyphenyl and guaiacyl compounds investigated with subsequent oxidation of the demethylated products. (Subsequent studies[108-111] demonstrated the demethylation of various methoxylated aromatic compounds, occurring as a result of fungal attack.)

The results obtained in studies on the enzymatic degradation of lignin and structurally related phenolic compounds enabled Ishikawa, Schubert, and Nord to suggest that the initial stage in fungal attack leads to the loss of methoxy-groups and the cleavage of certain types of ether bonds as well as the degradation of vanillin forming lignin structures. The low-molecular-weight products formed as a result are degraded further to vanillic acid, which can then be metabolised via the formation of protocatechuic acid.

The β-aryl guaiacylglyceryl ether units (XXIII) of lignin are degraded via guaiacylglycerol (XVI), β-hydroxyconiferyl alcohol (XXIV), and 4-hydroxy-3-methoxyphenylpyruvic acid (XII). The enolic form of this acid, which is most sensitive to the action of oxidases, is converted further into vanillin and vanillic and oxalic acids as a result of the oxidative cleavage of its propane side chain. Coniferyl aldehyde and ferulic acid, which are also formed on decomposition by fungi of the lignins in the wood of conifers, are converted similarly into vanillin and vanillic acid (Scheme 1). However, the hypothesis that compounds

Scheme 1



with the phenylpropane structure are intermediates in the degradation of lignin by white rot fungi and that an appreciable proportion of the polymeric lignin breaks down in the way shown in Scheme 1 is based essentially only on the detection of small amounts of monomeric phenylpropane compounds formed when isolated lignin is incubated with white rot fungi (see Section IV). Nevertheless, small amounts of these products might have been formed from the "side groups" or from secondary structural elements and not from the block of the phenylpropane units of the polymer itself. Furthermore, despite the quantitative importance of the β-aryl glyceryl ether linkages in lignin, the available data show, that their cleavage can liberate only a small amount of monomeric aromatic compounds even when appreciable depolymerisation takes place. (For example acidolysis of lignin, which leads to the quantitative cleavage of β-alkyl aryl ether linkages, the yield of monomeric degradation products is about 11% of the lignin[112].) Thus one may conclude that the cleavage of the β-aryl arylglyceryl linkages by the white rot fungi evidently cannot result in the liberation of appreciable amounts of monomeric products.

Ishikawa et al.[113] also investigated the enzymatic degradation of guaiacylglycerol (XVI), β-hydroxyconiferyl alcohol (XXIV), and 4-hydroxy-3-methoxyphenylpyruvic acid (XII) by purified enzyme preparations obtained from the mycelial clusters and the filtrates of *Fomes fomentarius* and *Collybia velutipes* cultures freed from mycelia. These preparations included mainly tyrosinase, an enzyme of the laccase type, and peroxidase. The authors observed enzymatic transformations of compounds

(XVI), (XXIV), and (XII) to vanillic acid via vanillin. Furthermore, the formation of (XII) from (XVI) and (XXIV) and the formation of compounds of type (XXIV) from (XVI) was demonstrated. The results were regarded by the authors as further evidence indicating that guaiacylglycerol, formed in the enzymatic degradation of the β-aryl guaiacylglyceryl ether linkages of conifer lignin, is converted further into vanillic and oxalic acids via β-hydroxyconiferyl alcohol, 4-hydroxy-3-methoxyphenylpyruvic acid, and vanillin as a result of the oxidative cleavage of the propane side chain.

Ishikawa and Oki [68] investigated the degradation of a number of aromatic compounds by purified preparations of peroxidase obtained from horseradish, radish, and clusters of *Collybia velutipes* N8 mycelia. The enzymatic transformations of the compounds under the influence of peroxidase involved both dehydropolymerisation and oxidative cleavage of the side chains. An example of the first process is provided by the formation of polymers from vanillin, acetovanillone, guaiacylmethylmethanol, eugenol, isoeugenol, ferulic acid, coniferyl aldehyde, guaiacylglycerol, coniferyl alcohol, Erdtman's acid, dehydrodi-isoeugenol, pinoresinol, symplocosigenol, and β-guaiacyl guaiacylglyceryl ether, while the formation of vanillin and dehydrodivanillin from vanillyl alcohol, ethyl vanillyl ether, β-hydroxyconiferyl alcohol, and 4-hydroxy-3-methoxyphenylpyruvic acid is an example of the second process. (It is noteworthy that the methyl derivatives of all these compounds were regenerated quantitatively from the peroxidase solutions after incubation for 2 h.) The authors showed that the degradation is accelerated by the addition of H_2O_2 and an increase of the enzyme concentration, being inhibited by heating (the optimal pH is 5.3 to 5.5).

Ishikawa and Oki [69] subjected a number of aromatic ether compounds (β-guaiacyl guaiacylglyceryl ether, ethyl vanillyl ether, and methyl veratrylmethyl ether) to the action of purified enzyme solutions obtained from mycelial clusters or filtrates of the *Collybia velutipes* N8 and *Polyporus versicolor* cultures freed from mycelia. It was established that the ether linkages in these compounds are hydrolysed with the formation of the corresponding alcohols. In particular, after incubation of compound (VIII) with a 30-day filtrate of the *C. velutipes* culture (25°C, second day), 20–30% of the substrate was converted into guaiacylglycerol. The study of the action of inhibitors and the pH on the enzymatic hydrolysis of ethyl vanillyl ether to vanillyl alcohol led the authors to the conclusion that the enzyme responsible for the hydrolysis of aromatic ether compounds is not of the metal-containing type such as laccases and peroxidases. Fukuzumi and Shibamoto [49] studied the degradation of β-guaiacyl veratrylglyceryl ether by the partly purified enzyme from *Poria subacida*. The reaction was carried out at pH 4.0 in an acetate buffer solution at 30°C in the course of two days. Chromatographic studies of the reaction mixture revealed the presence in the latter of guaiacol and an unidentified compound, which the authors believed to be veratrylglycerol or 3-hydroxy-1-(3,4-dimethoxyphenyl)-2-propanone.

In the course of subsequent experiments Fukuzumi et al. [50] showed that the amount of guaiacol liberated when compound (X) is acted upon by the partly purified enzyme from the mycelium of *F. subacida* increases significantly when the reaction is performed in the presence of the reducing coenzyme NADH (reduced nicotinamide-adenine dinucleotide). The authors also reported the formation of guaiacylglycerol (XVI) on incubation of compound (X) with the enzyme of *P. subacida* in the presence of NADH. The latter finding shows that, apart from the enzymatic cleavage of the β-alkyl aryl ether linkage, *para*-demethylation takes place. However, the identification of compound (XVI) was based exclusively on thin-layer chromatographic data with one solvent system and therefore cannot be regarded as adequate. Fukuzumi et al. [50] reported that the enzyme system employed in these experiments does not attack the β-alkyl aryl ether linkage in compounds containing the α-keto-group, while the β-ether linkage in ethylene glycol and glycerol structures is cleaved. Evidently the presence of the hydroxybenzyl-group in the side chain is a factor which determines the possibility of the cleavage of the β-ether linkage by the enzyme system from *P. subacida*.

The authors suggested that the cleavage of the β-alkyl aryl ether linkage can proceed via two possible mechanisms, one of which invoves the direct hydroxylation of the β-carbon atom in the presence of NADH and oxygen. This reaction should lead to the formation of guaiacol and veratryl- or guaiacyl-dihydroxyacetone (the latter was not identified by the authors, who suggest that it may be reduced to guaiacylglycerol). The second involves a hydrolytic reaction of β-guaiacyl guaiacylglyceryl ether, which is formed via the demethylation of the initial substrate and is more readily hydrolysable than β-guaiacyl veratrylglyceryl ether. The low yield of guaiacol in the enzymatic cleavage of compound (X) in the presence of NADH (at most 30%) is caused, according to Fukuzumi et al., by an enzyme stereospecific in relation to the *erythro*- and *threo*-forms of β-guaiacyl veratrylglyceryl ether.

Thus the results of the above studies indicate the possibility of the existence of an enzyme system which cleaves β-aryl arylglyceryl linkages in lignin. However, the methodological defects of these experiments make it impossible to characterise sufficiently rigorously this enzyme system and the mechanism of its action on lignin.

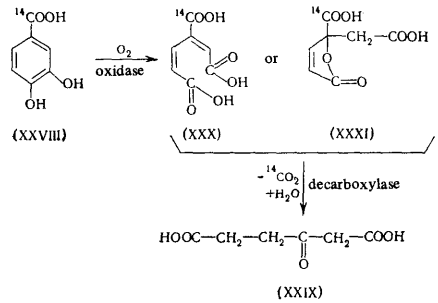
The studies described indicate the ability of the white rot fungi to convert β-aryl guaiacylglyceryl ethers or possibly other structural elements of lignin into monomeric benzenoid compounds of the type of vanillin and vanillic acid. Further transformation of these compounds by the white rot fungi may evidently involve different pathways, an important stage of which is cleavage of the aromatic ring. Data have been published showing that fungal attack involves the cleavage of aromatic rings. Thus Fukuzumi et al. reported that incubation of benzoic acid with the mycelial clusters of *Polystictus sanguineus* results in the formation of acetoacetic acid (XXV) [97] and α-oxogluaric acid [114], which were absent from the control experiment. Subsequently Fukuzumi [115] observed that *Poria subacida* completely metabolised gentisic acid (XXVI) to carbon dioxide. Crowden [116] established that *Polyporos tumulosus* metabolised fully *p*-hydroxyphenylpyruvic, *p*-hydroxyphenylacetic, *p*-hydroxymandelic, *p*-hydroxybenzoic, and protocatechuic acids, and to an extent greater than 50% homogentisic (XXVII), 2,5-dihydroxymandelic, and gentisic (XXVI) acids.

Moore and Towers [117,118] investigated the degradation of phenylalanine and tyrosine by the wood decomposing fungi *Schizophyllum commune*, *Sporobolomyces roseus*, and *Ustilago hordei*. Incubation of the mycelial cells of *S. commune* or of washed cell suspensions of *S. roseus* with ring-labelled phenylalanine led to the formation of $^{14}CO_2$. All three organisms converted labelled phenylalanine into cinnamic, benzoic, *p*-hydroxybenzoic, and protocatechuic acids; furthermore, phenyl-lactic and phenylacetic acids were detected in the metabolic products

of *S. commune*. Among the three fungi investigated, only *S. roseus* exhibited tyrosinase activity, converting tyrosine and *p*-coumaric acid into *p*-hydroxybenzoic and protocatachuic acids. On the basis of the results, the authors suggested the following sequence in the enzymatic degradation of phenylalanine and tyrosine: initial deamination to the corresponding acrylic acid derivatives whose side chain is then oxidised with formation of the corresponding C_6-C_1 acids. Further hydroxylation leads to the formation of protocatechuic acid (XXVIII)—the final aromatic compound in the sequence—which then undergoes ring cleavage. The presence of phenyl-lactic and phenylacetic acids in the metabolic products of *S. commune* indicates the existence of yet another pathway, apart from that discussed above, to the degradation of phenylalanine by this organism.

Haider and Flaig [47,119-121] established that, under the influence of *Polystictus versicolor* or the enzyme system isolated from this fungus, protocatechuic acid (XXVIII) is converted into β-oxoadipic acid (XXIX). According to the authors' views, the fungal transformation (XXVIII) → (XXIX) (Scheme 2) proceeds via the same mechanism as the analogous transformation under the influence of bacteria [122-126].

Scheme 2



According to this mechanism, protocatechuic acid oxidase catalyses the initial conversion of protocatechuic acid into *cis*, *cis*-β-carboxymuconic acid (XXX), which can be converted by a number of bacteria under the influence of the specific lactone-forming enzyme into the lactone of β-carboxymuconic acid (XXXI). Both the acid (XXX) and its lactone (XXXI) are then converted into β-oxoadipic acid (XXIX) as a result of the action of two different enzyme systems usually designated as decarboxylase.

Thus the data obtained in the course of the above experiments show that the aromatic ring is cleaved when the compound either contains or can be converted into an *o*- or *p*-dihydroxybenzene derivative. In the microbiological degradation of *o*-dihydroxybenzenes the cleavage can proceed via two different mechanisms. The aromatic ring can be ruptured either between the hydroxy-groups or directly next to a hydroxy-group. *p*-Dihydroxybenzenes undergo ring fission between the carbon atom linked to an OH group and the adjacent carbon atom linked to a substituent [127,128]

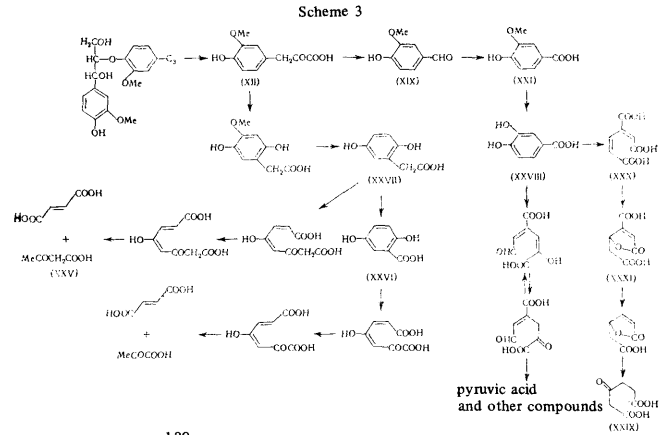
Haider and Grabbe [129] investigated the degradation of labelled biosynthetic [^{14}C] lignin by white rot fungi and concluded that this process probably does not proceed via the initial cleavage of the C–C and C–O bonds in the side chains but involves the fission of the aromatic rings still bound in the polymer. This conclusion did not, however, receive additional experimental confirmation.

Summarising studies on the microbiological degradation of lignin, Haider [48] concluded that the degradation of wood-decomposing fungi involves the following stages without the participation of phenol oxidases: (1) the shortening of the side chain leading to the formation of phenolic aldehydes, which can then be oxidised to the corresponding acids or reduced to the corresponding alcohols; (2) the cleavage of the methyl ether linkages and the elimination of methoxy-groups; (3) the cleavage of aromatic rings.

At the same time phenol oxidases oxidise the lignin decomposition products to coloured polymerisation mixtures. The first stage of this process is dehydrogenation leading to the formation of reactive radicals and quinones.

On the basis of the results of the above investigations, Christman and Oglesby [12] proposed a mechanism for the degradation of the β-aryl guaiacylglyceryl ether linkages in conifer lignin as a result of the action of white rot fungi (Scheme 3).

Scheme 3



Kirk et al. [130] investigated the action of two white rot fungi, *Polyporus versicolor* and *Stereum frustulatum*, on model compounds for lignin: β-guaiacyl guaiacylglycerol ether (VIII) and β-guaiacyl veratrylglyceryl ether (X). Of the two white rot fungi tested, one (*P. versicolor*) is typical and the other (*S. frustulatum*) is atypical. According to the authors [130], these fungi represent extreme varieties of white rot fungi in relation to the formation of phenol oxidising enzymes.

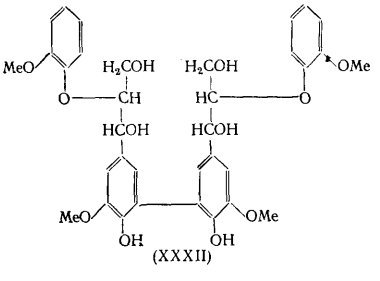
The study established that in the synthetic liquid medium the guaiacyl model (VIII) is converted under the influence of both fungi into a number of products, among which the *oo'*-dihydroxybiphenyl dehydropolymer (XXXII) predominates. This biphenyl derivative was also the main product when compound (VIII) was incubated with laccase. The veratryl model (X) was not changed either by the two fungi tested, in the synthetic aqueous medium, or by purified laccase. However, in a medium containing wood powder compound (X) was oxidised by both fungi to α-guaiacoxy-β-hydroxypropioveratrone (XXXIII). Separate experiments undertaken to elucidate the role of wood powder in this oxidation showed that lignin in wood behaves as a substrate for the formation of radicals when acted upon by the phenol oxidases produced by both fungi.

Thus the fact that β-guaiacyl guaiacylglyceryl ether is susceptible to the action of the white rot fungi tested, while β-guaiacyl veratrylglyceryl ether remains unchanged under the same conditions evidently shows that the phenolic OH group and not the β-ether linkage is involved directly under the above culture conditions. This conclusion is supported also by the finding that β-guaiacyl DL-glyceryl ether is not changed by either of the fungi tested in the synthetic liquid medium. The observed dimerisation of the guaiacyl model to the biphenyl dehydrodimer under the influence of both white rot fungi and the conversion of

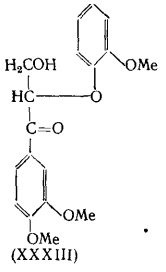
β-guaiacyl veratrylglyceryl ether into the corresponding veratrone in the presence of wood are oxidation reactions and, according to the authors, result from the action of phenol oxidases.

Kirk et al.[94] continued the study of the action of *P. versicolor* and *S. frustulatum* using the β-guaiacyl ether of syringylglycol (XXXIV) as the substrate. The syringyl and not the guaiacyl model was chosen to avoid the condensation reactions catalysed by phenol oxidase, involving the *ortho*-position with respect to the phenolic OH group.

The study established that compound (XXIV) was oxidised by filtrates of the *P. versicolor* culture or by the whole cultures of *S. frustulatum* to α-guaiacoxyacetosyringone (XXXV). Filtrates of the *P. versicolor* culture cleaved the alkyl–phenyl C–C bond in compounds (XXXIV) and (XXXV) with formation of guaiacoxyacetaldehyde (XXXVI) and guaiacoxyacetic acid (XXXVII) respectively.



(XXXII)

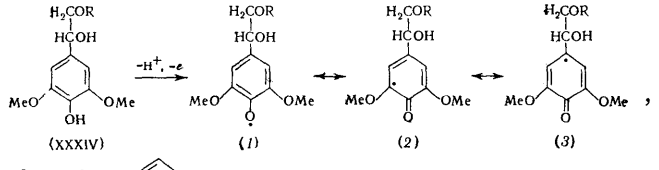


(XXXIII)

The syringyl halve of both initial compounds were converted into 2,6-dimethoxy-*p*-benzoquinone (XXXVIII) by filtrates of *P. versicolor* cultures and whole cultures of *S. frustulatum*. Furthermore it was shown that laccase, isolated from filtrates of *P. versicolor* cultures, catalysed all the above reactions.

At the same time it was established that whole cultures of *P. versicolor* and *S. frustulatum* reduced guaiacoxyacetaldehyde and guaiacoxyacetic acid to 2-guaiacoxyethanol, which accumulated in the reaction medium and was resistant to further changes by any of the above organisms. Since 2-guaiacoxyethanol was formed when whole cultures of both fungi acted on the β-guaiacyl ether of syringylglycol and α-guaiacoxyacetosyringone, the above alkyl–phenyl cleavage can evidently be achieved by whole cultures.

The authors proposed mechanisms for the main reactions occurring in the enzymatic degradation of the model compound (XXXIV). The oxidation of the β-guaiacyl ether of syringyl glycol (XXXIV), catalysed by fungal phenol oxidase, leads to the formation of a free radical, which can exist in at least three main resonance forms. They are analogous to the mesomeric forms R_a, R_c, and R_d of the coniferyl alcohol radical formed, according to Freudenberg's theory[131], during the biosynthesis of lignin.



(XXXIV) (1) (2) (3)

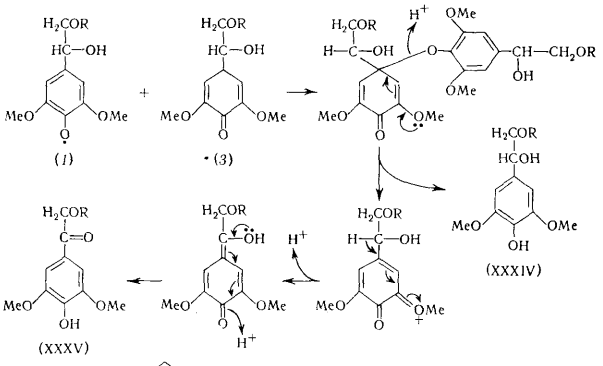
where R =



The mesomeric forms of the radical produced can either undergo disproportionation reactions or can recombine to dimeric products, which can then be altered by stabilisation reactions. The formation of biphenyl derivatives by *ortho–ortho* coupling [including the radical form (2)], observed for guaiacyl compounds[94,132,133], is limited to a large extent by the methoxy-groups in the syringyl compounds, i.e. the fraction of the radicals in form (2) is insignificant in the subsequent reactions[134,135].

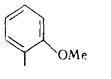
The mechanism of the oxidation of the β-guaiacyl ether of syringylglycol, which constitutes essentially a disproportionation reaction, is presented in Scheme 4.

Scheme 4



(1) (3) (XXXIV) (XXXV)

where R =



The initial stage of the above process involves the coupling of the phenoxyl (1) and the methylenequinonoid (3) radical forms. The distribution of the electron pair of the methoxyl oxygen atom over the cyclohexadienone ring leads to the elimination of a molecule of the β-guaiacyl ether of syringylglycol (XXXIV). Subsequent stabilisation of the molecule formed is achieved by the loss of a proton from the benzyl carbon atom. Rearomatisation leads to the formation of α-guaiacoxyacetosyringone (XXXV).

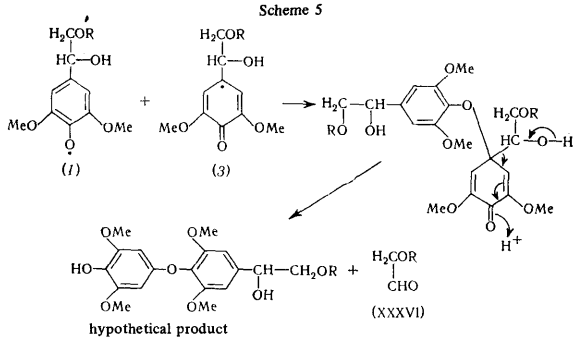
Scheme 5 presents the mechanism for the formation of guaiacoxyacetaldehyde (XXXVI) from the β-guaiacyl ether of syringylglycol.

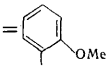
The initial coupling of the radicals in the mesomeric forms (1) and (3) is followed by the transfer of a proton from the benzyl OH group to the *p*-methylenequinonoid half. The subsequent cleavage of the benzyl–ring C–C bond liberates the side chain in the form of the aldehyde and leads to the rearomatisation of the initial molecule.

There is also a possibility of the coupling of two radicals in the mesomeric form (3). The dehydrodimer formed yields 3,3′,5,5′-tetramethoxy-*p*-biphenyl, as a result of the elimination of both side chains, which is converted on further oxidation into cerulignone. The latter was detected in very small amounts by Kirk et al.[94] in the products of the oxidation of model compound (XXXIV) by laccase from *P. versicolor*. However, this reaction is apparently limited by steric hindrance.

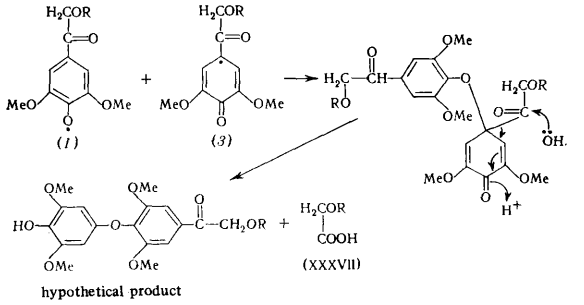
A similar mechanism (see Scheme 6) was proposed to account for the formation of guaiacoxyacetic acid (XXXVII) from α-guaiacoxyacetosyringone (XXXV).

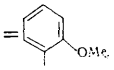
Scheme 5



where R = 

Scheme 6

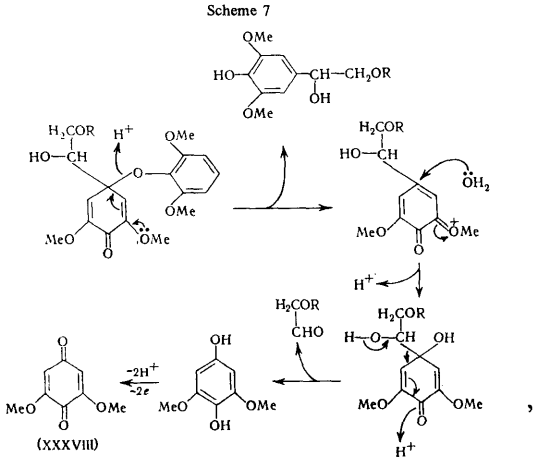


where R = 

After the coupling of the phenoxyl (1) and *p*-methylenequinonoid (3) forms of the radical, produced as a result of the oxidation of the phenol, nucleophilic attack by a water molecule on the carbonyl substituent in the cyclohexadienone ring leads to its migration, yielding guaiacoxyacetic acid (XXXVII) and diphenyl ether. There is a possibility of the recombination of two *p*-methylenequinonoid radicals in form (3) with subsequent elimination of both side chains; this leads to the formation of 3,3′,5,5′-tetramethoxy-*p*-biphenyl, which is then oxidised to cerulignone. The later was obtained in a very small yield in the oxidation of compound (XXXV) by laccase from *P. versicolor*.

The formation of 2,6-dimethoxy-*p*-benzoquinone (XXXVIII) by the oxidative cleavage of the β-guaiacyl ether of syringylglycol is illustrated in Scheme 7.

Scheme 7



where R = 

The initial stage in this process is also the coupling of the phenoxyl and *p*-methylenequinonoid forms of the radical, leading to an unstable cyclohexadienone derivative (this stage is not included in Scheme 7). The distribution of the electron pair of one of the methoxyl oxygen atoms over the cyclohexadienone ring leads to the elimination of a molecule of the β-guaiacyl ether of syringylglycol. Next, there is nucleophilic attack by water on the 4-position of the cyclohexadienone ring with subsequent loss of the proton. The elimination of guaiacoxyacetaldehyde with rearomatisation of the dienone half yields 2,6-dimethoxyhydroquinone, which is oxidised enzymatically to the corresponding *p*-quinone. A similar mechanism can account for the formation of a quinone from two molecules of α-guaiacoxyacetosyringone.

On the basis of the results of these investigations, Kirk, Harkin, and Cowling put forward the so called "phenol oxidase" hypothesis to account for the mechanism of the depolymerisation of lignin by white rot fungi. According to this hypothesis, the phenol oxidising enzymes present in the hyphae of the white rot fungi, and probably liberated from them when the latter penetrate the woody tissue, catalyse the oxidation of the phenolic OH groups in the lignin macromolecule. The resulting unstable free radicals are then stabilised by reactions which condense certain lignin structures but at the same time lead to the cleavage of the carbon–carbon bonds between the side chains and the rings of other oxidised phenylpropanoid units. The subsequent elimination of the terminal aliphatic component exposes additional phenolic OH groups to continuing degradation by enzymatic oxidation. The overall effect of these processes should theoretically lead to fragments of certain components of the lignin macromolecule and to the condensation of the residue.

It is interesting to note that the alkyl–phenyl cleavage reactions on which the proposed hypothesis is based, were first observed in the course of detailed studies on the biosynthesis of lignin. In particular, Freudenberg et al.[136] showed that in the enzymatic dehydogenation of sinapyl alcohol its condensation stops at the syringaresinol dimer and that the continuing enzyme action led to the breakdown of the dimer. The detection among the degradation products of 2,6-dimethoxy-*p*-benzoquinone is evidence that the oxidative dissociation of the alkyl–aryl carbon–carbon bonds took place. Furthermore, the authors reported that, when the oxidation of lignin by purified laccase is continued, 2-methoxy-*p*-benzoquinone is formed.

Structural elements of a new important type—the 1,2-diarylpropane-1,3-diol structures—were detected in lignin comparatively recently[137–139]. The mechanism of their formation in the enzymatic dehydrogenation of *p*-hydroxycinnamyl alcohols, proposed by Lundquist and Miksche[137], involves the alkyl–phenyl cleavage. Subsequently additional experimental confirmation was obtained for this mechanism[139,140]. Finally, in the course of a study of the products of the enzymatic dehydrogenation of propioguaiacone, Pew and Connors[141] identified a triphenyl derivative, the formation of which involves in all probability the cleavage of the alkyl–phenyl bond.

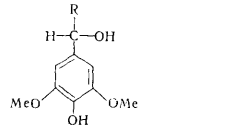
On the basis of the schematic formula of the fragment of the lignin molecule proposed by Freudenberg[131,142], Kirk et al.[94] calculated that 41% of the phenylpropane units of lignin are sensitive to the types of cleavage described above.

In order to elucidate the role of peroxidase and laccase in the degradation of lignin, Gierer and Opara [143] investigated the action of these enzymes on monomeric (guaiacol, 2,6-dimethoxyphenol, and aryl glyceryl ethers) and dimeric (1-guaiacyl-2-syringylethanol, pinoresinol, and dihydrodehydrodi-isoeugenol) phenolic compounds structurally related to lignin. The enzyme action led to the formation of products of a higher molecular weight, as a result of carbon–carbon and carbon–oxygen coupling. Non-phenolic model compounds were not involved by either enzyme system. This is evidence that their catalytic effect is oxidative and not hydrolytic.

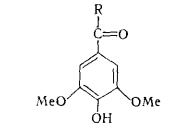
The results of the above study showed that both enzyme systems function mainly if not exclusively by catalysing oxidative coupling with formation of aryl–aryl or aryl–O–aryl bonds. No evidence was obtained for the cleavage of the ether linkages—the coupling products contained all the alkyl–aryl bonds present initially, including the aryl methyl and β-alkyl aryl ether linkages. Thus the degrading effect of the enzymes must be related to the oxidative coupling reactions which they catalyse. Such dehydrogenation reactions, which model compounds of p-hydroxy-α-carbonyl [94,144] and p-hydroxy-α-carbonyl [94,145] types are caused to undergo by laccase [94] or peroxidase [144,145], were examined above.

The identical compositions of the coupling products obtained with peroxidase–H_2O_2 and with laccase and the lack of substrate specificity in both enzymes are evidence that only the first stage of these reactions—the abstraction of hydrogen from the phenolic hydroxyl—are catalysed by the enzymes. The subsequent couplings evidently take place spontaneously. The authors point out that in the course of the above investigation no experimental evidence was obtained for the direct involvement of either enzyme in the microbiological degradation of lignin. However, this does not rule out the possibility that the oxidative coupling reaction can constitute the first step in the reaction sequence where peroxidase and laccase are involved in the microbiological cleavage of lignin in combination with other still unknown enzymes.

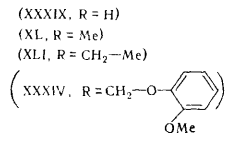
As mentioned above, Kirk and coworkers, who investigated the enzymatic degradation of a number of lignin model compounds, suggested the existence of free-radical intermediate compounds, which might arise as a result of the loss of one electron from the corresponding phenols. Furthermore, it has been noted that decayed wood is characterised, according to ESR data, by a higher free radical concentration [75–77]. In order to establish which lignin structures form the most stable types of free radicals in enzymatic degradation, Caldwell and Steelink [79] oxidised a number of syringyl derivatives, including the benzyl alcohols (XXXIX)–(XLI) and (XXXIV), the α-carbonyl compounds (XLII)–(XLIV) and (XXXV), syringylpropane (XLV), and syringaresinol (XLVI) with H_2O_2 in the presence of horseradish peroxidase and established that these compounds give rise to stable intermediate phenoxy-radicals.



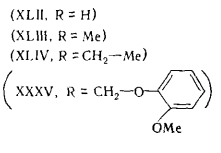
(XXXIX, R = H)
(XL, R = Me)
(XLI, R = CH_2—Me)



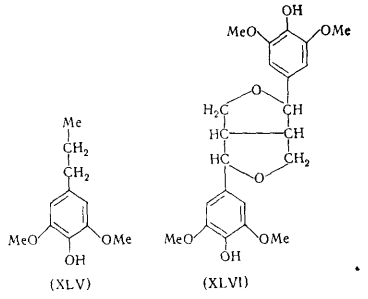
(XLII, R = H)
(XLIII, R = Me)
(XLIV, R = CH_2—Me)



(XXXIV, R = CH_2—O—)



(XXXV, R = CH_2—O—)

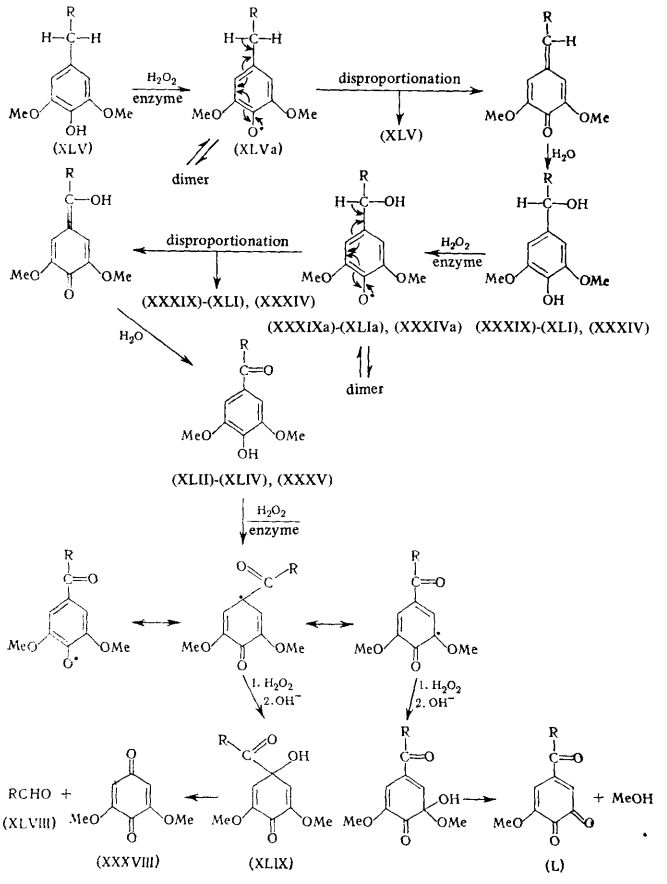


(XLV) (XLVI)

The results of these investigations showed that the phenoxy-radicals are formed most readily from α-carbonyl syringyl derivatives, less readily from α-carbinol, and α-alkyl ether derivatives, and slowly from alkyl derivatives.

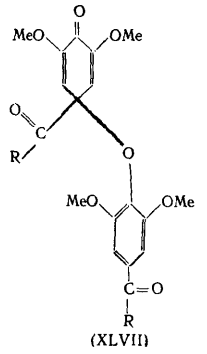
In order to establish the sequence of the reactions occurring in the oxidation of the compounds tested, the reaction mixture was examined simultaneously by ESR and optical spectroscopy. In addition, aliquots of the reaction mixture were periodically subjected to thin layer chromatographic separation in order to identify the reaction products. The mechanism proposed for the enzymatic oxidation of the compounds investigated on the basis of the results is presented in Scheme 8.

Scheme 8



The peroxidase–H_2O_2 system probably abstracts the phenolic hydrogen atom from the substrate. The resulting phenoxy-radical, which is in equilibrium with the hydroquinone-ether dimer (XLVII), then disproportionates, yielding a phenol with a higher oxidation potential. This

second phenol does not undergo further oxidation until the entire initial substrate is used up. This process is repeated until the side chain is split off.



(XLVII)

Thus enzymatic oxidation proceeds via a sequence of separate stages. The abstraction of the hydrogen atom probably determines the rate of reaction in each change of the oxidation state of the substrate. The disproportionation of two radical forms (or a dimeric form) is rapid.

It is noteworthy that Pew[132] reported earlier that H_2O_2 and peroxidase are capable of hydroxylating the α-position of syringylpropane. Caldwell and Steelink showed that this reaction is also a one-electron transfer process. The reaction of peroxidase and H_2O_2 with compound (XXXIV), which the authors investigated, is of special interest. The oxidation of this compound by laccase was studied in detail by Kirk et al.[130], who showed that the main product is compound (XXXV) with additional formation of a certain amount of (XXXVI), (XXXVIII), and of unidentified products. The high yield of compound (XXXV) is probably due to its insolubility in water, which prevented its further oxidation. Since Caldwell and Steelink oxidised compound (XXXIV) in a water–methanol medium, in which compound (XXXV) dissolved, they obtained a high yield of the phenoxy-radical from (XXXV) and of compound (XXXVIII).

According to Scheme 8, the quinone (XXXVIII) and the aldehyde (XLVIII) are formed in the last oxidation stage. Caldwell and Steelink suggested two possible ways for the decomposition of the radicals formed from compounds (XLII)–(XLIV) and (XXXV)—via the intermediate (XLIX) or its hyperoxide precursor, which can yield directly compounds (XXXVIII) and (XLVIII), and also via the *o*-quinone (L), which can be a source of a small amount of an unidentified red substance.

Later Young and Steelink[146] established that, when horseradish peroxidase and H_2O_2 are allowed to act on 4-substituted 2,6-dimethoxyphenols and also MWL and alkaline lignins from the wood of deciduous trees, phenoxy-radicals are formed, the lignin substrates being degraded by the same oxidation mechanism as the simple phenolic substrates.

The results of the above studies suggest that the α-carbonyl groups in units with a free phenolic hydroxyl are most sensitive to the fungal attack on the lignin macromolecule. If the decomposition of all the lignin units with a free phenolic hydroxyl proceeds via the free-radical mechanism proposed by Caldwell and Steelink, then this can explain the appreciable depolymerisation of lignin on the basis of a series of one-electron hydrogen abstraction steps. On the other hand, one must emphasise[147] that the main dehydrogenation stage is regulated by the structure of the phenolic substrate and also by the oxidation potential of the enzyme or the chemical oxidant. In biological systems the possibility of such oxidation depends largely on factors such as the presence of the appropriate enzymes, the pH of the medium, and the presence of oxidants, for example O_2 or H_2O_2. The combination of these factors determines the preferential occurrence and extent of the polymerisation or degradation reactions under the influence of the same enzyme–oxidant system.

Sergeeva and coworkers[148] investigated the action of the brown rot fungus *Fomitopsis pinicola* I on coniferin, glucovanillin, vanillin, syringaldehyde, and coniferyl alcohol. Syringaldehyde and vanillin did not undergo any changes as a result of the enzyme action, while coniferyl alcohol was oxidised to vanillin. The action of the fungus *F. pinicola* I on coniferin and glucovanillin led to the dissociation of the β-phenylglucosidic linkage with formation of coniferyl alcohol and vanillin respectively. These primary decomposition products underwent further oxidation. Thus vanillic acid and a further two unidentified acids were detected together with vanillin in the products of the decomposition of glucovanillin after exposure to the action of the fungus for 10 days. Coniferyl alcohol and coniferaldehyde were detected in the coniferin decomposition products after 10 days, and after more prolonged enzyme action (45 days) vanillin was found as well.

The authors suggest that coniferyl alcohol is formed from coniferin and vanillin is formed from glucovanillin under the influence of the fungus *F. pinicola* I via an intermediate stage involving the formation of unstable phenoxy-radicals. The hypothesis has been put forward, that when *F. pinicola* I acts on wood, the dissociation of β-phenylglucosidic lignocarbohydrate linkages can lead to the formation of stable phenoxy-radicals. The subsequent oxidation of coniferyl alcohol to coniferaldehyde and of coniferaldehyde to vanillin also evidently proceeds via free-radical intermediates. The authors believe that the abrupt increase in the concentration of paramagnetic centres observed on extensive enzymatic decomposition of wood[77] may be a consequence of the oxidation of the lignin structures and the formation of stable phenoxy-radicals. On the basis of the content of functional groups and the infrared spectroscopic data for sprucewood and birchwood obtained in the course of successive stages of their degradation by *F. pinicola* I, Kreitsberg and coworkers[149,150] concluded that the number of quinhydrone structures in the lignin macromolecule increases, the biolignin isolated from degraded sprucewood containing more of these structures than the analogous preparation from birchwood.

In conclusion of the discussion of the possible pathways in the microbiological degradations of lignin, certain general comments have to be made. As already mentioned, there is evidence for the presence in the wood decomposing fungi of an enzyme system capable of cleaving β-aryl arylglyceryl ether linkages in lignin. However, the dissociation of these bonds does not evidently lead to the liberation of appreciable amounts of monomeric products, although it can cause the depolymerisation of lignin.

For this reason the so called "phenol oxidase hypothesis", put forward by Kirk, according to which the phenol oxidising enzymes of the wood decomposing fungi catalyse the oxidation of the phenolic OH group of the lignin macromolecule, appears to have more justification. As a result, unstable free radicals are formed and are then stabilised in the course of reactions leading to the condensation of certain lignin structures and the simultaneous cleavage of carbon–carbon bonds between the side chains and rings of other oxidised phenylpropane units. According to Caldwell and Steelink, the degradation of lignin takes

place as a result of a series of one-electron transfer processes leading ultimately to the removal of the side chains.

One should bear in mind that, in consequence of the wide variety of linkages between the monomeric units in lignin [151], its complete decomposition may not involve a direct attack on each linkage with the resulting liberation of low-molecular-weight compounds. Direct attack on the general structural elements of the polymer, such as aromatic rings, is more likely and the dissociation of the linkages between the phenylpropane units may take place indirectly. However, the available data are inadequate to reach definite conclusions.

Thus the studies examined in the present review do not unfortunately permit an unambiguous conclusion either about the enzyme systems present in wood-decomposing fungi or about the mechanism of the microbiological degradation of lignin, although the solution of these problems is evidently necessary for successful research in the fields of study discussed in the introduction.

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