

Biochemistry of Wood Components: Biosynthesis and Microbial Degradation of Lignin*¹

Takayoshi HIGUCHI*²
(Received May 31, 2002)

Keywords : biosynthetic pathway of monolignols, *O*-methyltransferase, ferulate 5-hydroxylase, cinnamyl alcohol dehydrogenase, cleavage of side chain and aromatic ring of lignin by lignin peroxidase and laccase

Contents

- I. Introduction
- II. Outline of the present study
- A. Biosynthesis of lignin
 - 1. Biosynthetic pathway of monolignols
 - 2. Dehydrogenative polymerization of monolignols to lignins
- B. Microbial degradation of lignin
 - 1. Side chain cleavage of lignin model compounds
 - 2. Cleavage of aromatic rings of lignin model compounds
 - 3. Cleavage of side chains and aromatic rings of a synthetic lignin (DHP)
- III. Conclusion
- References

I. Introduction

It has been noted that *Sequoia sempervirens* (D. Don) Endl. in California, and some eucalyptus trees in Australia reach heights of 100 meters and the age of 1,000 years. Such magnificent growth of the trees could be ascribed to the presence of lignin which is distributed with hemicelluloses in the spaces of inter-cellulose microfibrils in primary and secondary walls, and in middle lamellae. Lignin acts as a cementing component to connect cells and harden the cell walls of xylem tissues, that helps a smooth transportation of water through vessels and tracheids from roots to upper trunks and branches. Consequently the lignin gives resistance against disease and wood decay by micro-organisms (Fig. 1).

Main chemical components of wood cell walls are cellulose, hemicelluloses and lignin. Cellulose, which accounts for about 50% of chemical components of wood, has been well investigated and widely used as paper and as materials for various cellulosic high polymers.

Lignin, which accounts for 22% in hardwood and 28% of softwood in average, plays an important role to glue cellulose microfibrils in wood cell walls. However, the complexity of its chemical structure has retarded the progress of basic researches on lignin.

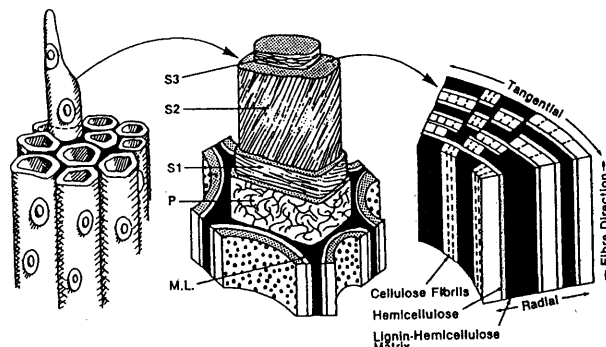


Fig. 1. Molecular architecture of woody tissues and wood cell walls. M.L.: Middle lamella, P: Primary wall, S1, S2, S3: Outer, middle and inner layers of secondary wall. Black parts of middle lamella and cell walls show the presence of lignin. (T.K. Kirk: Marcus Wallenberg Foundation Symposia Proceedings 2, p. 27 (1985)).

Recent studies have indicated that lignins are aromatic polymers of methoxylated phenylpropanoids connected by both ether and carbon-carbon linkages, and classified into three major groups, guaiacyl lignin in conifers, guaiacyl-syringyl lignin in hardwood and guaiacyl-syringyl-*p*-hydroxyphenyl lignin in grasses on their monomeric units (Figs. 2, 3).

The precursors (monolignols) of the respective lignins have been isolated and identified from various woody plants (Fig. 4).

However, the biosynthetic pathway of these monolignols and lignins remained unexplored until 1950s.

II. Outline of the present study

A. Biosynthesis of lignin

1. Biosynthetic pathway of monolignols

On the latter half of 1950 Higuchi and his coworkers started tracer experiments with ¹⁴C-labeled lignin precursors. The radioactive precursors were administered to lignifying plants such as young tree twigs, cultured tissues of conifers and hardwoods, and heading wheat etc. and that the plants were allowed to metabolize for a certain period. The plants were then homogenized in hot ethanol and the cell wall residue was subjected to chemical degradation such as alkaline nitrobenzene oxidation and ethanolysis. Based on the incorporation ratio and dilution values of ¹⁴C-precursors into the lignin

*¹ This review article is the outline of the study "Biochemistry of Wood Components: Biosynthesis and Microbial Degradation of Lignin" for Japan Academy Award granted by the Japan Academy, Tokyo in June 11, 2001.

*² Professor Emeritus of Kyoto University.

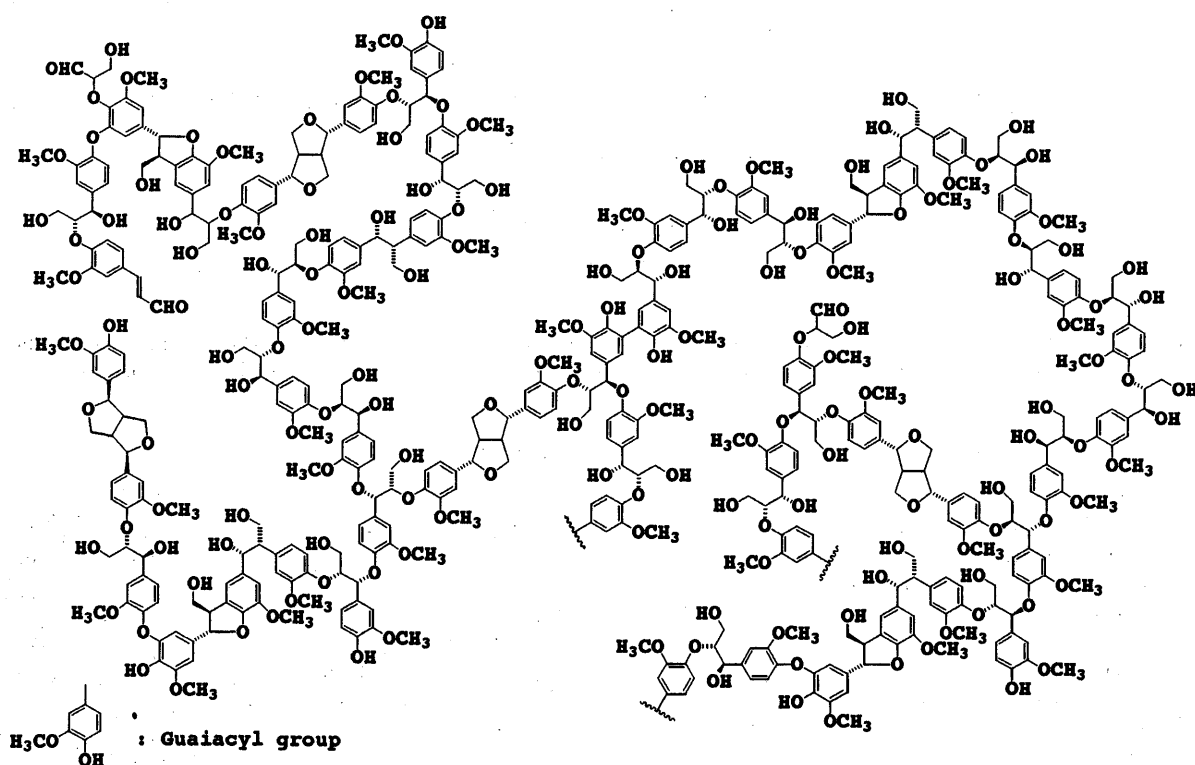


Fig. 2. A structure model of conifer lignin (guaiacyl lignin).

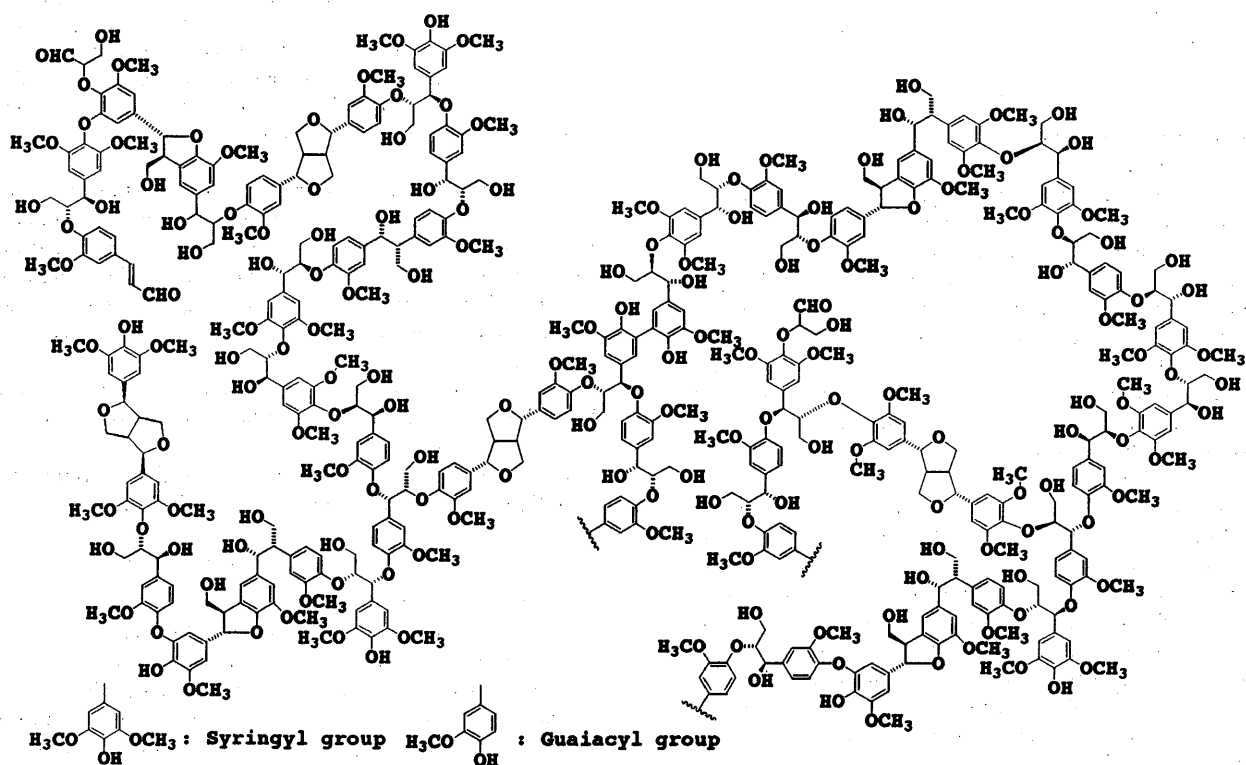


Fig. 3. A structure model of hardwood lignin (guaiacyl-syringyl lignin).

degradation products (vanillin and syringaldehyde by nitrobenzene oxidation, and ethanolysis products) the biosynthetic pathway of monolignols from glucose via shikimic acid was elucidated (Fig. 5).

While, the enzymes involved in monolignol biosynthesis from L-phenylalanine have been characterized by many investigators.

These studies showed that the enzymes involved in

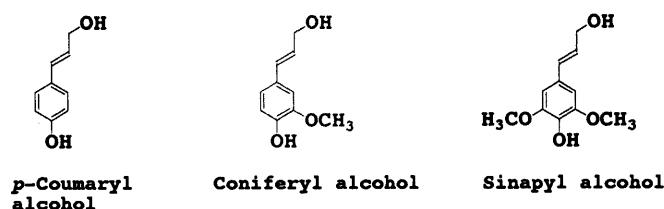


Fig. 4. Chemical structure of monolignols.

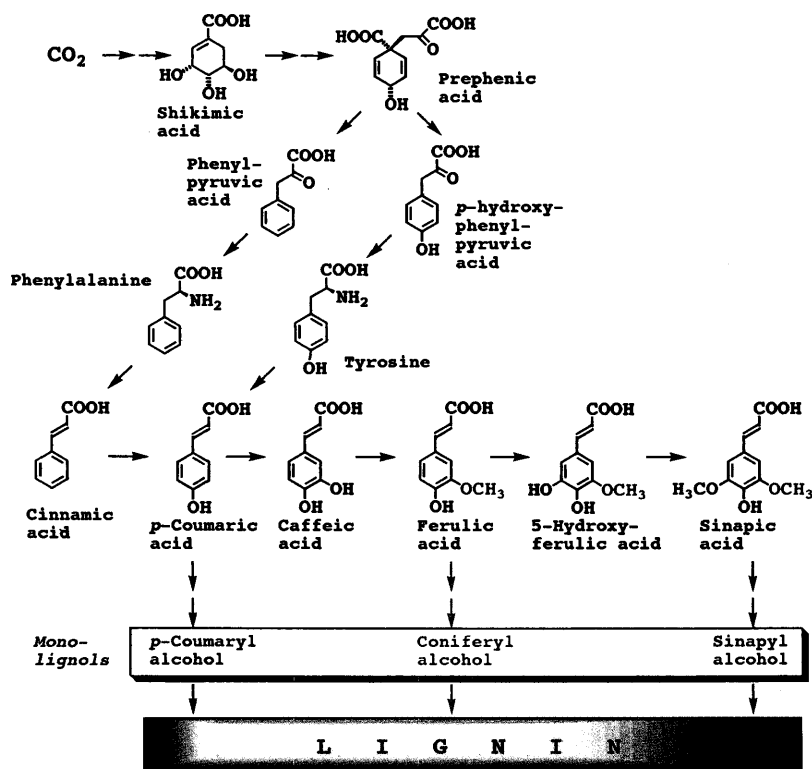


Fig. 5. Pathways for monolignol biosynthesis.

L-phenylalanine biosynthesis from sugars are in common with those in microorganisms and higher plants. While, the enzymes involved in monolignol biosynthesis derived from L-phenylalanine are specifically related to the secondary metabolism in higher plants such as lignin and flavonoid biosynthesis.

In such conditions, Higuchi and his co-workers discovered that the ^{14}C -labeled ferulic acid administered into young conifers was mostly converted to guaiacyl lignin. In contrast, the ^{14}C -labeled ferulic acid administered into hardwoods such as poplar was converted to guaiacyl-syringyl lignin. Then, the studies were focused

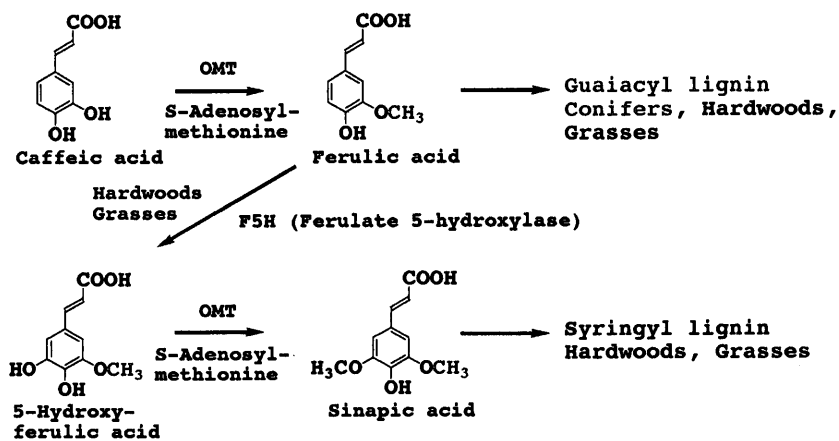


Fig. 6. Biogenesis of guaiacyl and syringyl lignins in plants.

on the elucidation of the biosynthetic differences of guaiacyl- and guaiacyl-syringyl lignins in gymnosperms and angiosperms: The substrate specificities of the respective enzymes involved in monolignol biosynthesis in conifers, hardwoods and grasses have been characterized.

For example, the purified *O*-methyltransferase (OMT) from twigs of young poplar (hardwood) and bamboo shoots (grasses) efficiently catalyzed the methylation of both caffeic and 5-hydroxyferulic acids, but the purified OMT from Japanese black pine seedlings predominantly catalyzed the methylation of caffeic acid leading to the preferential formation of guaiacyl lignin in conifers (Fig. 6).

Fig. 7 shows the relationship between the substrate specificity of enzymes involved in monolignol biosynthesis and the difference and relatedness of biosynthetic pathways of lignins in conifers, hardwoods and grasses.

Higuchi and his co-workers indicated that the following factors are involved in differentiation of guaiacyl, guaiacyl-syringyl and guaiacyl-syringyl-*p*-hydroxyphenyl lignins in conifer, hardwood and grasses.

1. OMT of gymnosperm primarily catalyzes the ferulate formation from caffeate, and sinapate formation from 5-hydroxyferulate is competitively inhibited by caffeate. (mono functional OMT). While angiosperm OMT catalyzes not only the ferulate formation but also sinapate

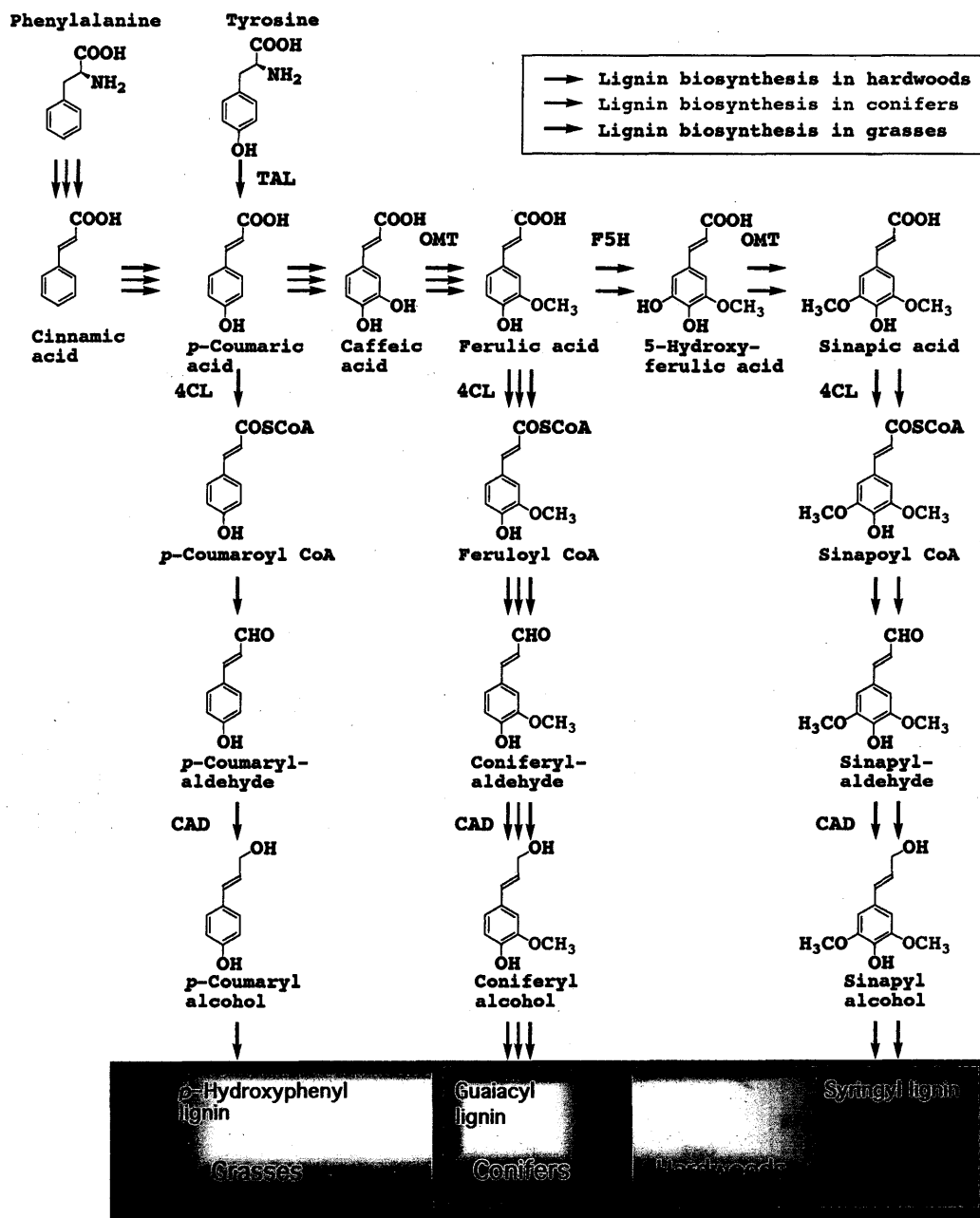


Fig. 7. Biosynthetic pathways for guaiacyl, guaiacyl-syringyl- and guaiacyl-syringyl-*p*-hydroxyphenyl lignins in conifers, hardwoods and grasses. OMT: *O*-methyltransferase, F5H: ferulate 5-hydroxylase, 4CL: 4-coumarate: CoA ligase, CAD: cinnamyl alcohol dehydrogenase.

formation from 5-hydroxyferulate, and ferulate formation is competitively inhibited by 5-hydroxyferulate (difunctional OMT).

2. Ferulate 5-hydroxylase (F5H), a key enzyme in the differentiation of lignin biosynthesis from guaiacyl to syringyl lignins, is distributed only in angiosperms.

3. The synthesis of sinapyl alcohol may occur via successive reactions of 5-hydroxyferulate, 5-hydroxyferuloyl-CoA, 5-hydroxyconiferyl aldehyde, and sinapaldehyde as alternative pathway in some angiosperms.

4. *p*-Hydroxycinnamyl alcohol dehydrogenase (CAD), which mediates the last step of monolignol formation, has different substrate specificity; gymnosperm enzymes primarily catalyze the formation of coniferyl alcohol from coniferyl aldehyde, while angiosperm enzymes catalyze not only the formation of coniferyl alcohol but also the formation of sinapyl alcohol from sinapaldehyde.

It was concluded that the enzymes involved in the synthesis of monolignol intermediates after ferulate are essentially different between gymnosperms and angiosperms: Gymnosperms are controlled to synthesize guaiacyl lignin via mediated reactions by the enzymes which preferentially activate guaiacyl intermediate such as ferulate, feruloyl-CoA and coniferyl aldehyde. While, angiosperms synthesize guaiacyl and syringyl intermediates such as sinapate, sinapoyl-CoA, and sinapaldehyde. Grasses which synthesize guaiacyl-syringyl-*p*-hydroxyphenyl lignin contain enzymes substrate specificities of which are almost similar to those of common angiosperms to catalyze the formation of the both guaiacyl and syringyl intermediates. The formation of *p*-hydroxyphenyl lignin and esterified *p*-coumarate characteristically contained in grass lignin seems to be derived from a high concentration of *p*-coumaric acid directly supplied from L-tyrosine by tyrosine ammonia-lyase.

2. Dehydrogenative polymerization of monolignols to lignins

Higuchi found for the first time that plant peroxidase, which is widely distributed in woody plants, catalyzes dehydrogenative polymerization of coniferyl alcohol to a lignin (DHP). While, Freudenberg and his co-workers demonstrated that coniferyl alcohol was oxidized to its phenoxy radicals by the mediation of horseradish peroxidase. The radicals formed couple to yield quinone methides, which are converted to various dilignols. The dilignols are further dehydrogenated by the enzyme to their radicals, which are finally converted to lignin and lignin-carbohydrate complexes (LCC) via radical couplings.

B. Microbial degradation of lignin

As shown in Figs. 2 and 3 lignins are three dimensional phenylpropanoid polymers linked by several different carbon-to-carbon and ether linkages between monomeric phenylpropane units most of which are not readily hydrolyzed.

While, microbiologists have shown that the white-rot basidiomycetes such as *Coriolus versicolor* and *Phanerochaete chrysosporium* degrade lignin oxydatively by mediation of laccase and lignin peroxidase.

However, the mechanism of lignin biodegradation by the basidiomycetes remained unsolved until the studies carried out by Higuchi and his co-workers, and Kirk and his co-workers, respectively.

1. Side chain cleavage of lignin model compounds

Higuchi and his co-workers synthesized several oligolignols containing major lignin substructures such as β -O-4 linkage. The lignin substructure oligomers were used for elucidation of lignin degradation mechanism by *Phanerochaete chrysosporium* and *Coriolus versicolor*, and their enzymes, lignin peroxidase and laccase: Oligolignols were incubated with ligninolytic cultures of the basidiomycetes, and that the degradation products were isolated successively and identified by NMR and GC-MS to

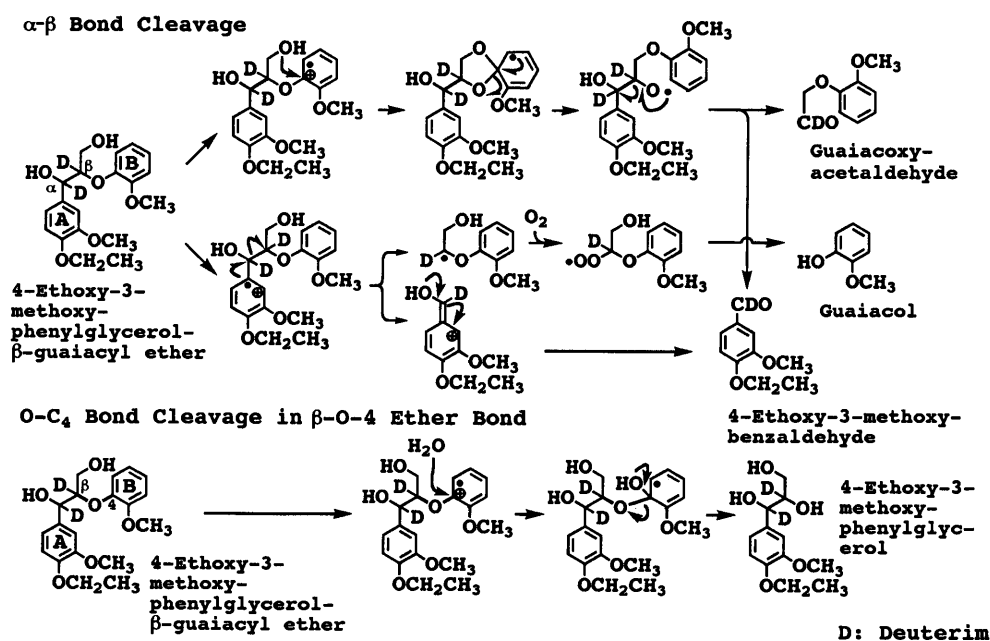


Fig. 8. Mechanism of side chain cleavage of a β -O-4 lignin substructure model compound.

elucidate the degradation mechanism.

The results showed that the mode of side chain cleavage of lignin substructure dimmers mostly agreed with that of lignin side chain: Oxidative cleavage between C α -C β of the propyl side chain, and the oxidative cleavage of β -aryl ether linkages.

Then, α , β -dideuterated 4-ethoxy-3-methoxyphenylglycerol- β -vanillin- γ -benzyl di-ether was synthesized to elucidate the mechanism of C α -C β cleavage and O-C4 cleavage by lignin peroxidase. The results clearly showed that C α -C β cleavage and O-C4 cleavage occurred via the cation radical intermediates by one electron oxidation of the aromatic ring of the substrate by lignin peroxidase (Fig. 8).

2. Cleavage of aromatic rings of lignin model compounds

The mechanism of aromatic ring cleavage of lignin by microorganisms remained unsolved until 1985. Then, Higuchi and his co-workers synthesized 4-ethoxy-3-methoxyphenylglycerol- β -guaiacyl [U-ring ^{13}C , OCD_3] ether, and 4-ethoxy-3-methoxyphenylglycerol- β -syringyl [U-ring ^{13}C , OCD_3] ether as substrate to elucidate the mechanism of aromatic ring cleavage of the model compounds.

The compounds were incubated with ligninolytic culture of *P. chrysosporium* in the presence of H_2^{18}O . As aromatic ring cleavage products β , γ - and α , β -cyclic carbonates of

arylglycerol, formate and oxalate esters of arylglycerol were isolated and identified by GC-MS from the reaction mixtures (Fig. 9).

Muconate ester of arylglycerol was further isolated and identified as an initial ring cleavage product of the dimers by the lignin peroxidase.

After that the cleavage mechanism of the aromatic ring was elucidated by the experiments using ^2H , ^{13}C and ^{18}O labeled dimers with $^{18}\text{O}_2$ and H_2^{18}O (Fig. 10).

The results showed that the mechanism of aromatic ring cleavage of lignin is completely different from the aromatic ring cleavage reaction for catechol derivatives by usual dioxygenases: Lignin peroxidase catalyzes one electron oxidation of the aromatic ring (B) of arylglycerol- β -aryl ether to give the cation radicals which are attacked by H_2O , and that the resulting radicals couple with dioxygen to afford the muconate ester of arylglycerol.

3. Cleavage of side chains and aromatic rings of a synthetic lignin (DHP)

Higuchi and his co-workers elucidated that the most of the initial stage of degradation reaction of β -O-4 lignin substructure model dimers was catalyzed by lignin peroxidase.

Then, a synthetic lignin (DHP: dehydrogenation polymer of coniferyl alcohol with horseradish peroxidase, M.W. >2200) was prepared and subjected to degradation with lignin peroxidase to elucidate the mechanism of lignin

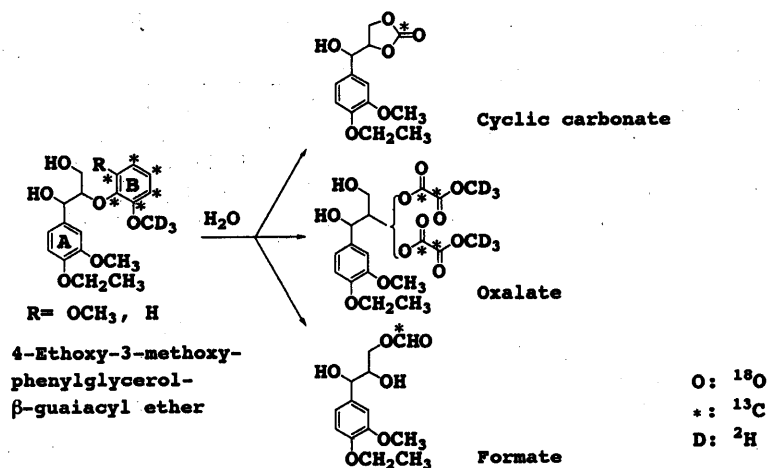


Fig. 9. Aromatic ring cleavage products of a β -O-4 lignin substructure model compound.

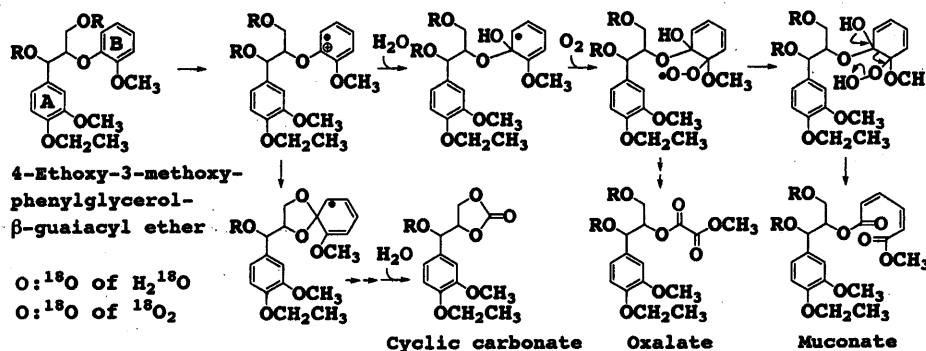


Fig. 10. Mechanism of aromatic ring cleavage of a β -O-4 lignin substructure model compound.

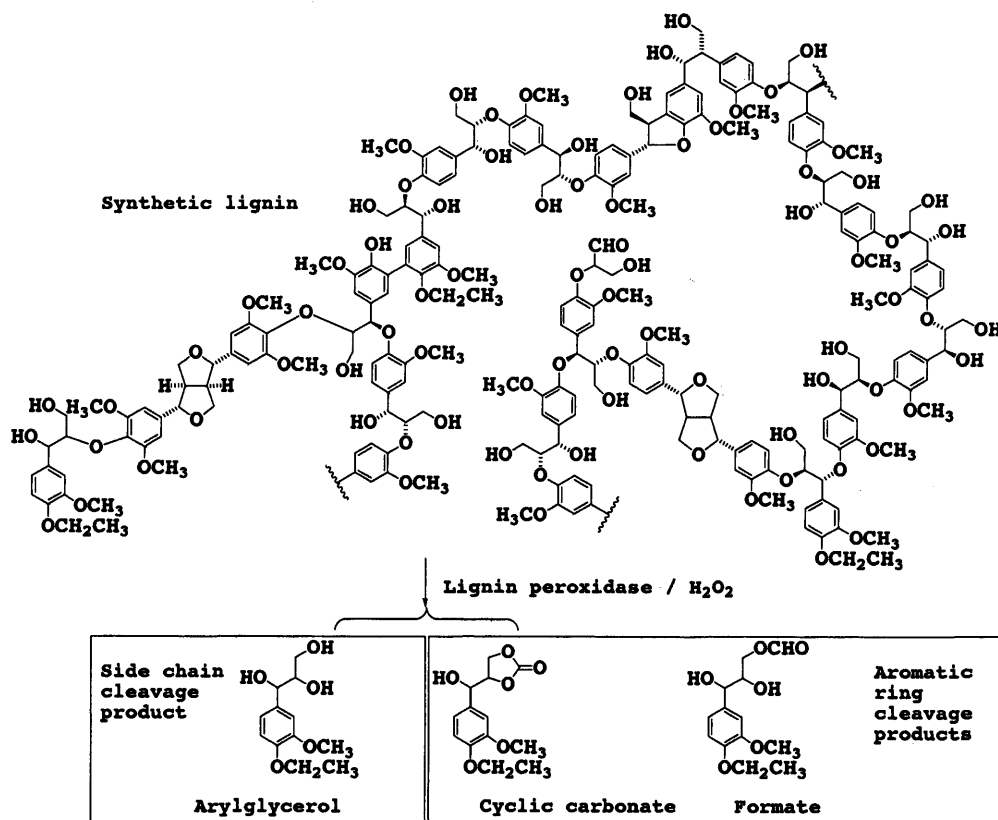


Fig. 11. Degradation of a synthetic lignin (DHP) by lignin peroxidase.

degradation by this enzyme.

As the case of the degradation of β -O-4 lignin substructure model dimers by lignin peroxidase the cyclic carbonates and formate of arylglycerols, and arylglycerol were isolated from degradation products of the DHP with lignin peroxidase and that their chemical structures were identified by GC-MS (Fig. 11).

III. Conclusion

1. Higuchi and his co-workers studied on the biosynthetic pathway of monolignols such as coniferyl-, sinapyl- and *p*-coumaryl alcohols in lignifying plants, and succeeded in the elucidation of the difference and relatedness on the biosynthetic pathways, and the enzymes involved in conifers, hardwoods and grasses.

2. Higuchi and his co-workers prepared major substructure oligolignols and a synthetic lignin (DHP) as substrate for ligninolytic basidiomycetes and lignin peroxidase. Then, they succeeded in the elucidation of lignin degradation mechanism by isolation and identification of the degradation products of these substrates.

The results were concluded that lignin peroxidase catalyzes one electron oxidation to give aryl cation radicals of aromatic rings of lignin, and that the cleavages of the lignin side chains and aromatic rings occur via the aryl cation radicals. The cleavage mechanisms of side chains and aromatic rings of lignin model compounds and the synthetic lignin (DHP) by lignin peroxidase have been established by using ¹⁸O, ²H and ¹³C labeled lignin substructure dimers with ¹⁸O₂ and H₂¹⁸O. The mechanism of aromatic ring cleavage of lignin is

completely different from the aromatic ring cleavage reaction by usual dioxygenases.

Based on these fundamental contributions on lignin biochemistry Higuchi received many awards, especially Japanese Forestry Prize (1959), Japanese Association of Agricultural Science Award (1985), Anselme Payen Award (Cellulose, Paper and Textile Division), American Chemical Society (1987), Purple Ribbon Medal (Japanese Government 1990), and Fujiwara Award (1992).

Dr. Higuchi was elected as President of International Academy of Wood Science (1990–1993) and Foreign Associate of National Academy of Sciences of the United States of America (1991).

References

- A. Biosynthesis of lignin
 1. Biosynthetic pathway of monolignols
 - 1) T. HIGUCHI: Studies of lignin biosynthesis using isotopic carbon X. Formation of lignin from phenylpropanoids in tissue culture of white pine. *Can. J. Biochem. Physiol.*, **40**, 31–34 (1962).
 - 2) T. HIGUCHI and S.A. BROWN: Studies of lignin biosynthesis using isotopic carbon XII. The biosynthesis and metabolism of sinapic acid. *Can. J. Biochem. Physiol.*, **41**, 613–620 (1963).
 - 3) T. HIGUCHI and S.A. BROWN: Studies of lignin biosynthesis using isotopic carbon XIII The phenylpropanoid system in lignification. *Can. J. Biochem. Physiol.*, **41**, 621–628 (1963).
 - 4) Y. NAKAMURA, H. FUSHIKI and T. HIGUCHI: Metabolic differences between gymnosperms and angiosperms in the formation of syringyl lignin. *Phytochem.*, **13**, 1777–1784

- (1974).
- 5) T. HIGUCHI, M. SHIMADA, F. NAKATSUBO and M. TANAHASHI: Differences in biosynthesis of guaiacyl and syringyl lignins in woods. *Wood Sci. Technol.*, **11**, 153–167 (1977).
 2. Enzymes involved in monolignol biosynthesis
 - 1) T. HIGUCHI: Role of phenylalanine deaminase and tyrase in the lignification of bamboo. *Agric. Biol. Chem.*, **30**, 667–673 (1966).
 - 2) T. HIGUCHI, Y. ITO and I. KAWAMURA: *p*-Hydroxyphenylpropane component of grass lignin and role of tyrosine ammonia-lyase in its formation. *Phytochem.*, **6**, 875–881 (1967).
 - 3) T. HIGUCHI, M. SHIMADA and H. OHASHI: Role of *O*-methyltransferase in the lignification of bamboo. *Agric. Biol. Chem.*, **31**, 1459–1465 (1967).
 - 4) M. SHIMADA, H. OHASHI and T. HIGUCHI: *O*-methyltransferases involved in the biosynthesis of lignins. *Phytochem.*, **9**, 2463–2470 (1970).
 - 5) M. SHIMADA, H. KURODA and T. HIGUCHI: Evidence for the formation of methoxyl groups of ferulic and sinapic acids in Bambusa by the same *O*-methyltransferase. *Phytochem.*, **12**, 2873–2875 (1973).
 - 6) H. KURODA, M. SHIMADA and T. HIGUCHI: Purification and properties of *O*-methyltransferase involved in the biosynthesis of gymnosperm lignin. *Phytochem.*, **14**, 1759–1763 (1975).
 - 7) H. KUTSUKI, M. SHIMADA and T. HIGUCHI: Distribution and role of *p*-hydroxycinnamate: CoA ligase in lignin biosynthesis. *Phytochem.*, **21**, 267–271 (1982).
 - 8) H. KUTSUKI, M. SHIMADA and T. HIGUCHI: Regulatory role of cinnamyl alcohol dehydrogenase in the formation of guaiacyl and syringyl lignins. *Phytochem.*, **21**, 19–23 (1982).
 - 9) T. HIBINO, D. SHIBATA, T. UMEZAWA and T. HIGUCHI: Purification and partial sequences of *Aralia cordata* cinnamyl alcohol dehydrogenase. *Phytochem.*, **32**, 565–567 (1993).
 - 10) T. HIBINO, J.-Q. CHEN, D. SHIBATA and T. HIGUCHI: Nucleotide sequence of a *Eucalyptus botryoides* gene encoding cinnamyl alcohol dehydrogenase. *Plant Physiol.*, **104**, 305–306 (1994).
 3. Dehydrogenative Polymerization of monolignols to lignins
 - 1) T. HIGUCHI: Further studies on phenol oxidase related to the lignin biosynthesis. *J. Biochemistry (Japan)*, **45**, 515–528 (1958).
 - 2) T. HIGUCHI and Y. ITO: Dehydrogenation products of coniferyl alcohol formed by the action of mushroom phenol oxidase, rhus-laccase and radish peroxidase. *J. Biochemistry (Japan)*, **45**, 575–579 (1958).
 - 3) T. HIGUCHI: Studies on the biosynthesis of lignin. In: *Biochemistry of Wood*. K. Kratzl and G. Billek (eds.). Pergamon Press, 161–188 (1959).
 - 4) Y. NAKAMURA and T. HIGUCHI: Ester linkage of *p*-coumaric acid in bamboo lignin. *Holzforschung*, **30**, 187–191 (1976).
 - 5) Y. NAKAMURA and T. HIGUCHI: Ester linkage of *p*-coumaric acid in bamboo lignin III. Dehydrogenative polymerization of coniferyl *p*-hydroxybenzoate and coniferyl *p*-coumarate. *Cellulose Chem. Technol.*, **12**, 209–221 (1978).
 - B. Microbial degradation of lignin
 1. Syntheses of oligolignols
 - 1) T. HIGUCHI and F. NAKATSUBO: Synthesis and biodegradation of oligolignols. *Kemia-Kemi.*, **9**, 481–488 (1980).
 2. Degradation mechanism of lignin model compounds by *Phanerochaete chrysosporium* and *Coriolus versicolor*
 - 1) T. UMEZAWA, F. NAKATSUBO and T. HIGUCHI: Degradation pathway of arylglycerol- β -aryl ether by *Phanerochaete chrysosporium*. *Agric. Biol. Chem.*, **47**, 2677–2681 (1983).
 - 2) T. HIGUCHI: Degradative pathways of lignin model compounds. In: *Biosynthesis and Biodegradation of Wood Components*. T. Higuchi (ed.) Academic Press, 557–578 (1985).
 - 3) T. UMEZAWA and T. HIGUCHI: Role of guaiacol in the degradation of arylglycerol- β -guaiacyl ether by *Phanerochaete chrysosporium*. *FEMS Lett.*, **26**, 123–126 (1985).
 - 4) T. HIGUCHI: Catabolic pathways and role of ligninases for the degradation of lignin substructure models by white-rot fungi. *Wood Research (Kyoto)*, **73**, 58–81 (1986).
 3. Elucidation of degradation mechanisms of ^2H , ^{13}C , ^{18}O -labeled lignin model compounds by lignin degrading basidiomycetes and their lignin peroxidase and laccase
 - 1) T. HIGUCHI: Mechanism of lignin degradation by lignin peroxidase and laccase of white-rot fungi. *Biogenesis and Biodegradation of Plant Cell wall Polymers*. G. Lewis and M.G. Paice (eds) American Chemical Society, 482–505 (1989).
 4. Elucidation of degradation mechanisms of lignin substructure model compounds by lignin degrading basidiomycetes and their lignin peroxidase and laccase by using $^{18}\text{O}_2$ and H_2^{18}O
 - 1) S. KAWAI, T. UMEZAWA and T. HIGUCHI: Arylglycerol- γ -formyl ester as an aromatic ring cleavage product of nonphenolic β -*O*-4 lignin substructure model compounds degraded by *Coriolus versicolor*. *Appl. Environ. Microbiol.*, **50**, 1505–1508 (1985).
 - 2) T. UMEZAWA and T. HIGUCHI: A novel C α -C β cleavage of a β -*O*-4 lignin model dimer with rearrangement of the β -aryl group by *Phanerochaete chrysosporium*. *FEBS Lett.*, **192**, 147–150 (1985).
 - 3) T. UMEZAWA and T. HIGUCHI: Aromatic ring cleavage in degradation of β -*O*-4 lignin substructure by *Phanerochaete chrysosporium*. *FEBS Lett.*, **182**, 257–259 (1985).
 - 4) T. UMEZAWA, M. SHIMADA, T. HIGUCHI and K. KUSAI: Aromatic ring cleavage of β -*O*-4 lignin substructure model dimers by lignin peroxidase of *Phanerochaete chrysosporium*. *FEBS Lett.*, **205**, 287–292 (1986).
 - 5) T. UMEZAWA and T. HIGUCHI: Aromatic ring cleavage of β -*O*-4 lignin model dimers without prior demeth(ox)ylation by lignin peroxidase. *FEBS Lett.*, **205**, 293–298 (1986).
 - 6) T. UMEZAWA and T. HIGUCHI: Formation of a muconate in aromatic ring cleavage of a β -*O*-4 lignin substructure model by lignin peroxidase. *Agric. Biol. Chem.*, **51**, 2281–2284 (1987).
 - 7) T. UMEZAWA and T. HIGUCHI: Mechanism of aromatic ring cleavage of β -*O*-4 lignin substructure models by lignin peroxidase. *FEBS Lett.*, **218**, 255–260 (1987).
 - 8) S. KAWAI, T. UMEZAWA and T. HIGUCHI: Degradation mechanism of phenolic β -1 lignin substructure model compounds by laccase of *Coriolus versicolor*. *Arch. Biochem. Biophys.*, **262**, 99–110 (1988).
 - 9) S. KAWAI, T. UMEZAWA, M. SHIMADA and T. HIGUCHI: Aromatic ring cleavage of 4,6-di (*tert*-butyl) guaiacol, a phenolic lignin model compound, by laccase of *Coriolus versicolor*. *FEBS Lett.*, **236**, 309–311 (1988).
 - 10) T. UMEZAWA and T. HIGUCHI: Cleavages of aromatic ring and β -*O*-4 bond of synthetic lignin (DHP) by lignin peroxidase. *FEBS Lett.*, **242**, 325–329 (1989).
 - 11) T. HIGUCHI: Mechanism of lignin degradation by lignin peroxidase and laccase of white-rot fungi. In: *Biogenesis and Biodegradation of Plant Cell Wall Polymer*, G. Lewis and M.G. Paice (eds.) American Chemical Society, p. 482–505 (1989).

- 12) T. HIGUCHI: Biodegradation of lignin and its potential applications. In: Bioprocess Engineering, T.K. Ghose, (ed.) Ellis Horwood Ltd, p. 39–58 (1989).
 - 13) S. KAWAI, S. SHOJI, K. NABETA, H. OKUYAMA and T. HIGUCHI: Degradation of nonphenolic β -O-4 lignin substructure model compounds by lignin peroxidase of *Coriolus versicolor*. *Mokuzai Gakkaishi*, **36**, 126–132 (1990).
- C. Review article
- 1) T. HIGUCHI: Biosynthesis of lignin. In: Biosynthesis and Biodegradation of wood Components, T. Higuchi (ed.) Orlando, Academic Press, 141–160 (1985).
 - 2) T. HIGUCHI: Phenylpropane Derivatives. In: Biochemistry and Molecular Biology of Wood, T. Higuchi, Heidelberg, Springer, 131–180 (1997).
 - 3) T. HIGUCHI: The Discovery of lignin. In: Discoveries in Plant Biology, S.-D. Kung and S.-Fa Yang (eds.), Singapore, World Scientific II, 233–269 (1998).
 - 4) T. HIGUCHI: Recent progress and problems in lignin biosynthesis. Proc. Intern. Symp. Environm. Friendly and Emerging Technologies for a Sustainable Pulp and Paper Industry, Taipei, 315–324 (2000).
 - 5) T. HIGUCHI: Degradative pathways of lignin model compounds. In: T. Higuchi (ed.) Biosynthesis and Biodegradation of Wood Components. Orlando, Academic Press, 557–578 (1985).
- D. Book
- 1) T. HIGUCHI: Lignin biochemistry: Biosynthesis and biodegradation. *Wood Sci. Technol.*, **24**, 23–63 (1990).
 - 2) T. UMEZAWA and T. HIGUCHI: Degradation of synthetic lignin (DHP) by the lignin peroxidase of *Phanerochaete chrysosporium*. In: Biotechnology in Pulp and Paper Manufacture, T. Kent Kirk and Hou-min Chang (eds.) Boston, Butterworth-Heinemann, 351–358 (1990).
 - 3) T. HIGUCHI: Biosynthesis and microbial degradation of lignin (in Japanese). *Gakujyutsu Geppou*, **44**, 21–26 (1991).
- 1) Tree Biochemistry (in Japanese), pp.190, Kyoritsu Shuppan, Tokyo (1969).
 - 2) Biosynthesis and Biodegradation of Wood Components, T. Higuchi (ed.), pp. 679, Academic Press, Orlando (1985).
 - 3) Wood Biochemistry (in Japanese), pp. 246, Buneido Shuppan, Tokyo (1992).
 - 4) Wood Molecular biology (in Japanese), T. Higuchi (ed.), pp. 271, Buneido Shuppan, Tokyo (1994).
 - 5) Biochemistry and Molecular Biology of Wood, pp. 362, Springer, Berlin, Heidelberg (1997).