Review

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

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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

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.

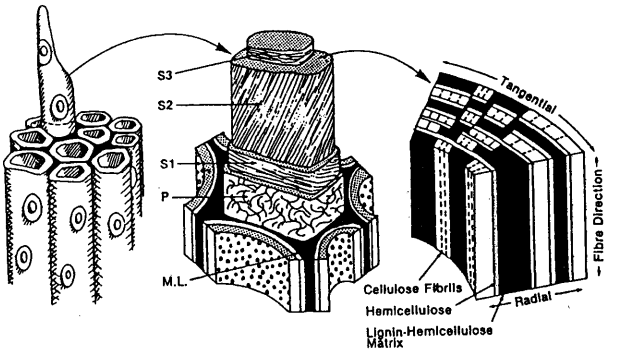

Fig. 1. Molecular architecture of woody tissues and wood cell walls. M.L.: Middle lamella, P: Primary wall, S1, S2, S3: Outer, middle and inner layers of secondary wall. Black parts of middle lamella and cell walls show the presence of lignin. (T.K. Kirk: Marcus Wallenberg Foundation Symposia Proceedings 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 biosynhetic 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 ^{14}C-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 ^{14}C-precursors into the lignin

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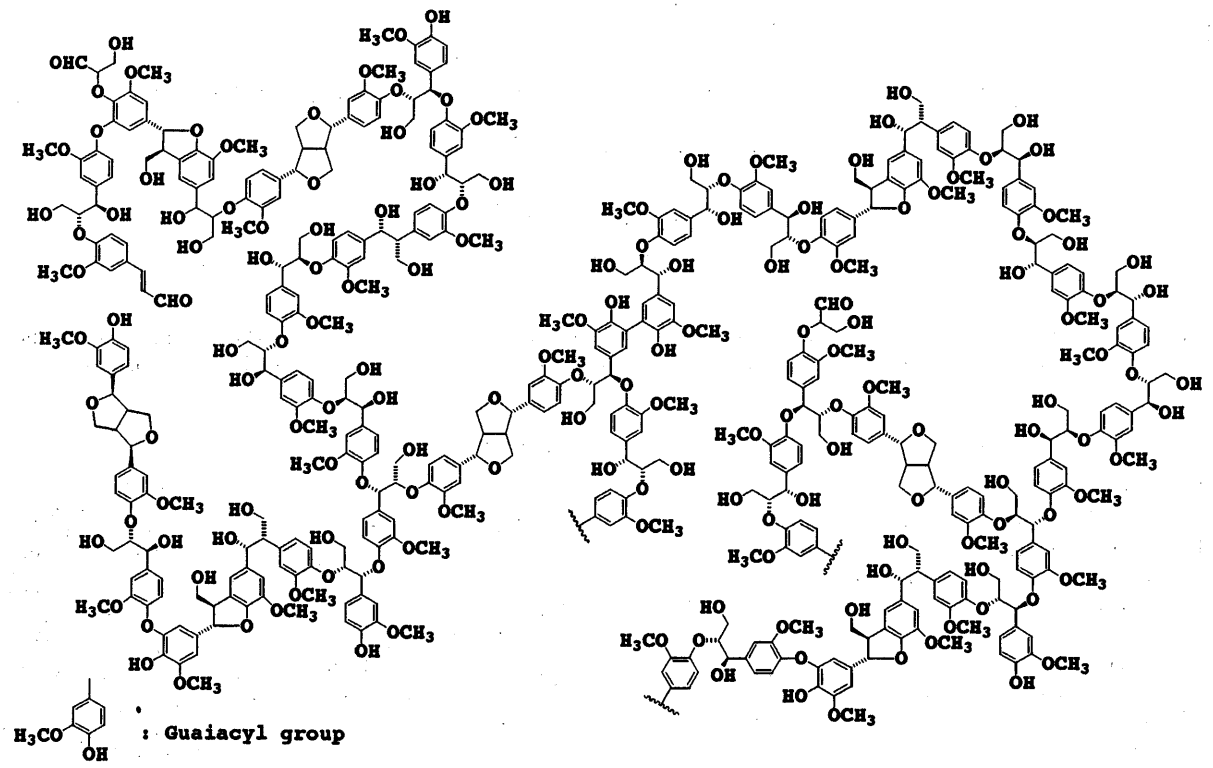

Fig. 2. A structure model of conifer lignin (guaiacyl lignin).

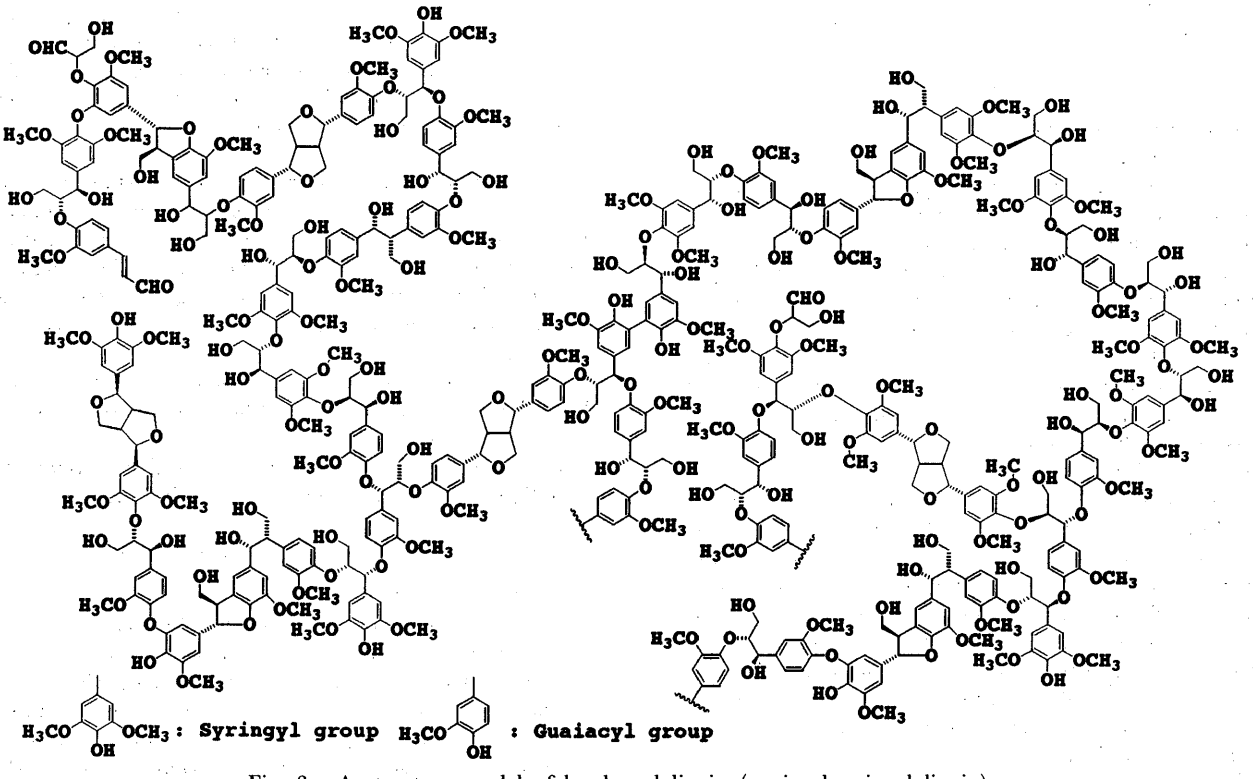

Fig. 3. A structure model of hardwood lignin (guaiacyl-syringyl lignin).

degradation products (vanillin and syringaldehyde by nitrobenezene 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

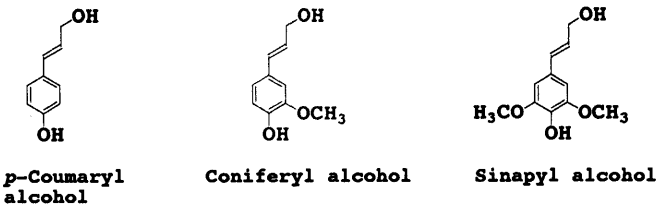

Fig. 4. Chemical structure of monolignols.

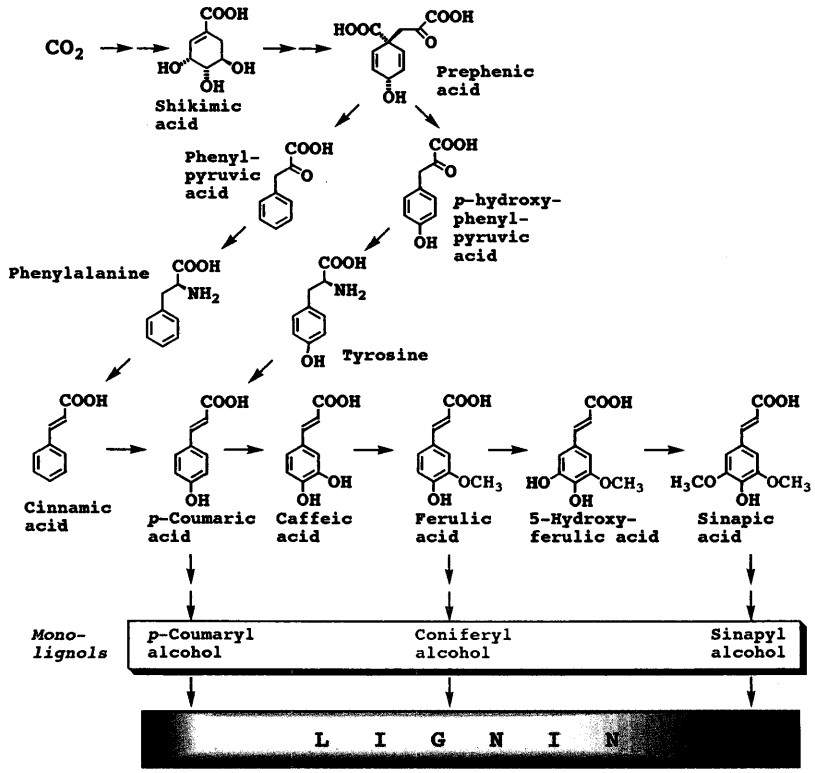

Fig. 5. Pathways for monolignol biosynthesis.

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

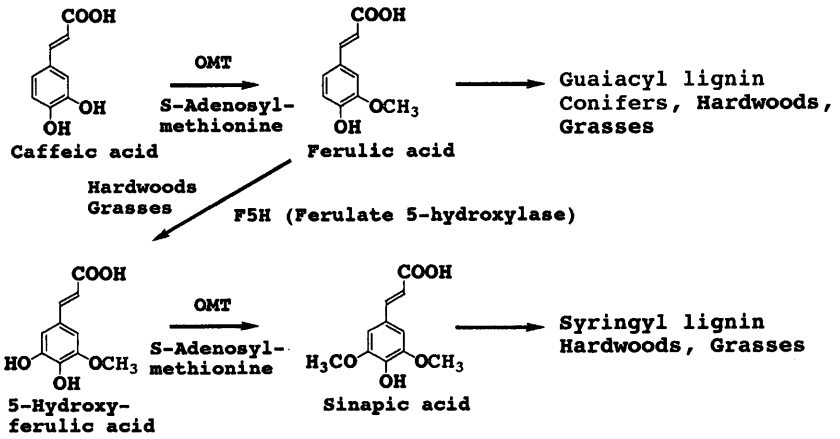

Fig. 6. Biogenesis of guaiacyl and syringyl lignins in plants.

on the elucidation of the biosynhetic 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 gymnopserm 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

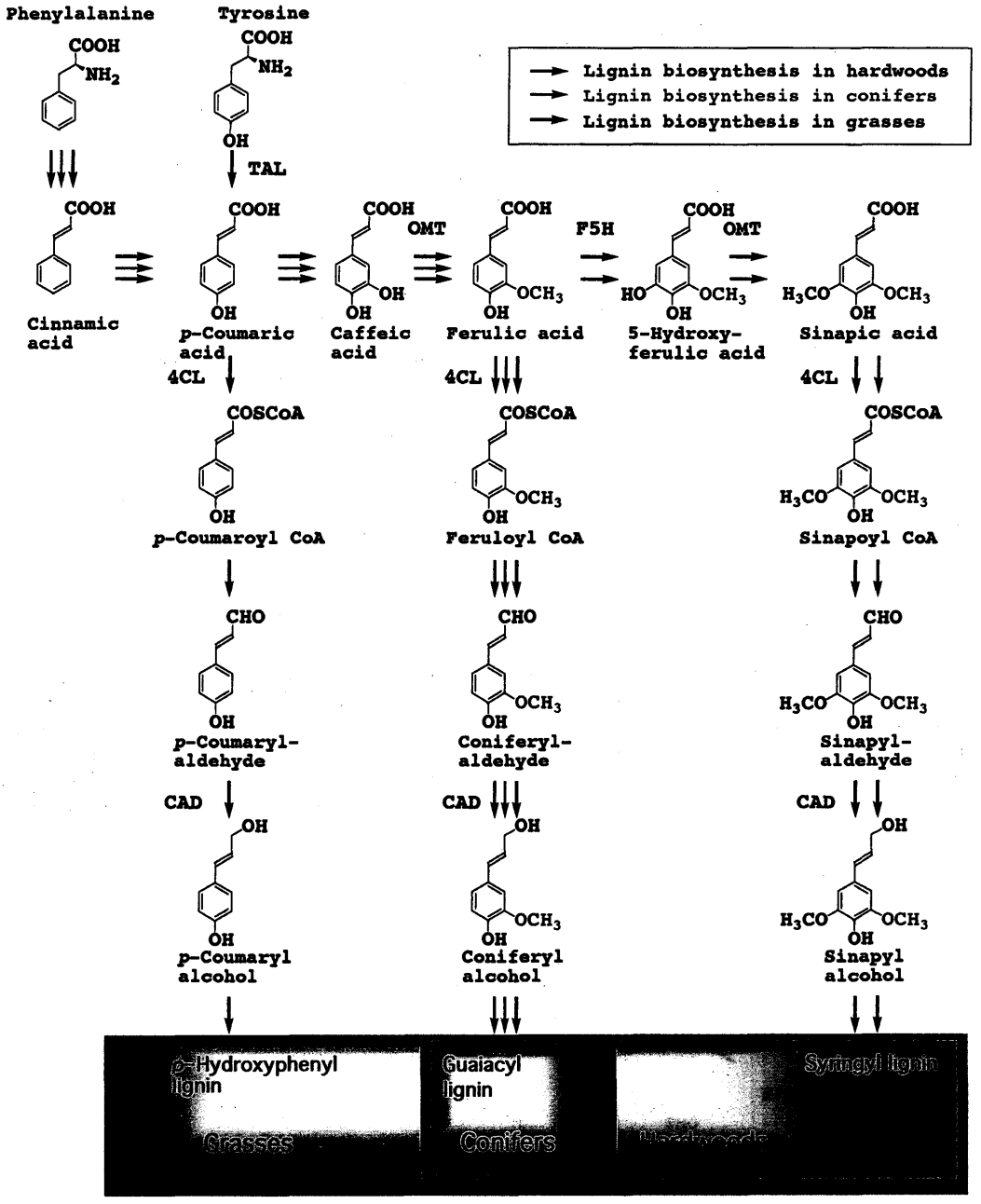

Fig. 7. Biosynthetic pathways for guaiacyl, guaiacyl-syringyl- and guaiacyl-syringyl-*p*-hydroxyphenyl lignins in conifers, hardwoods and grasses. OMT: *O*-methyltransferase, F5H: ferulate 5-hydroxylase, 4CL: 4-coumarate: CoA ligase, CAD: cinnamyl alcohol dehydrogenase.

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 dimentional 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

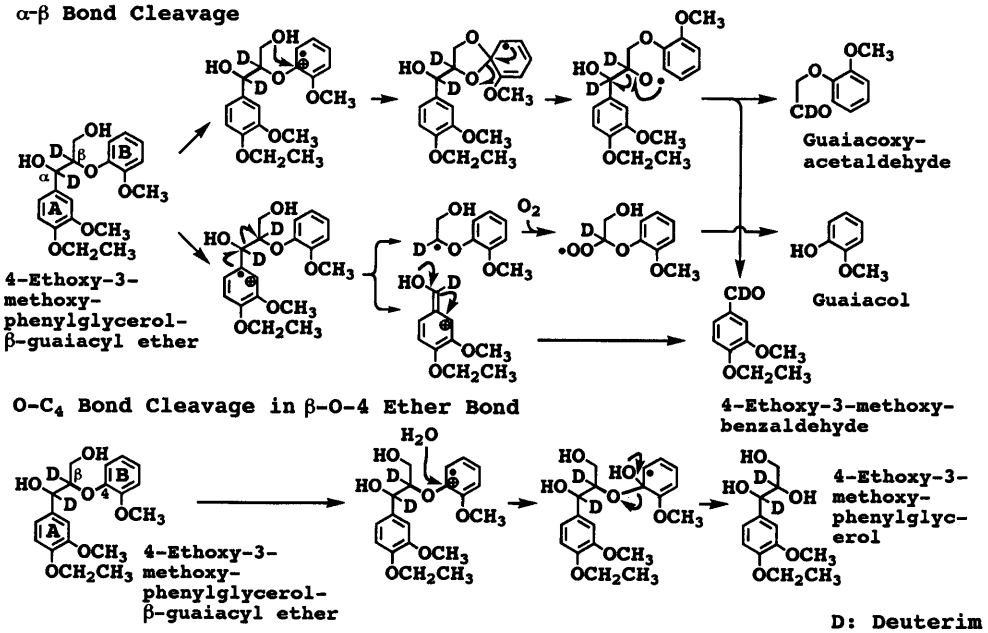

Fig. 8. Mechanism of side chain cleavage of a *β-O*-4 lignin substructure model compound.

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 Cα-Cβ 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 Cα-Cβ cleavage and *O*-C4 cleavage by lignin peroxidase. The results clearly showed that Cα-Cβ 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 $^{18}O_2$ 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

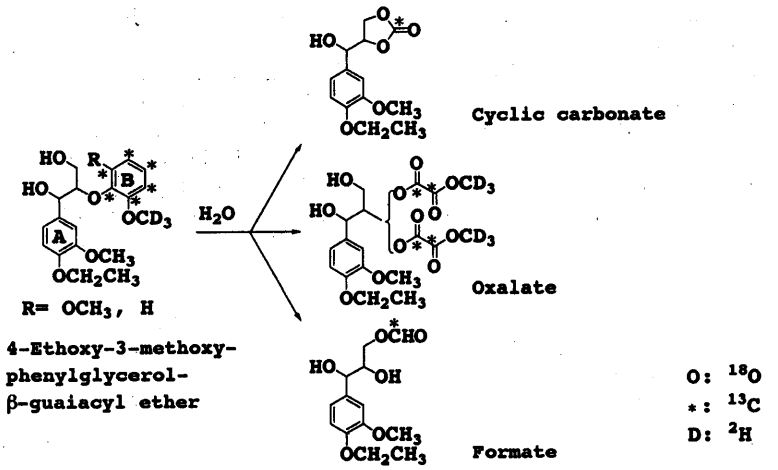

Fig. 9. Aromatic ring cleavage products of a β-*O*-4 lignin substructure model compound.

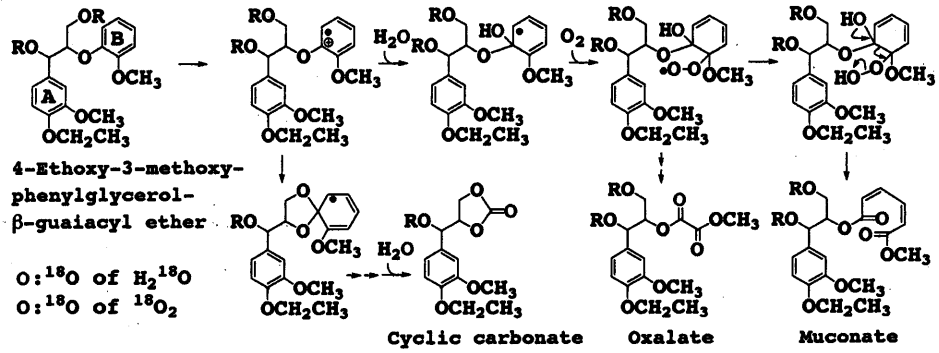

Fig. 10. Mechanism of aromatic ring cleavage of a β-*O*-4 lignin substructure model compound.

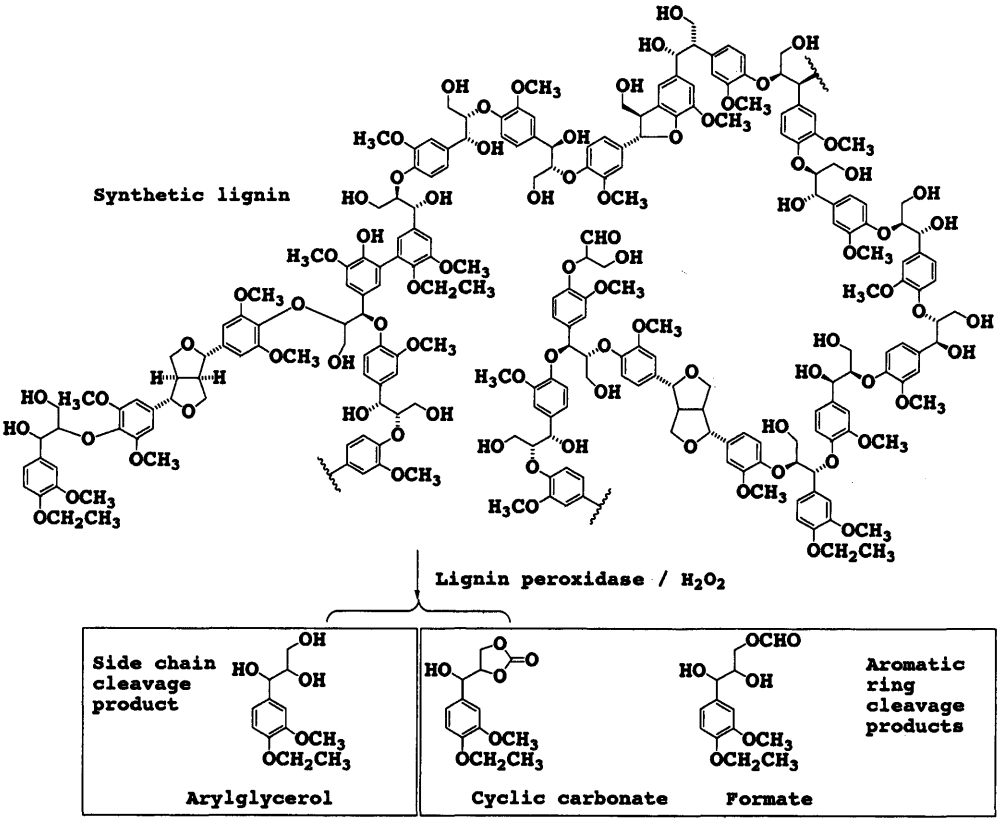

Fig. 11. Degradation of a synthetic lignin (DHP) by lignin peroxidase.

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 ^{18}O, ^{2}H and ^{13}C labeled lignin substructure dimers with $^{18}O_2$ and $H_2{}^{18}O$. 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).

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