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CURRENT PROBLEMS IN RESEARCH

Chemistry of Lignification

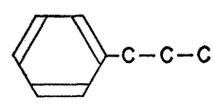
Biochemical research on lignins is yielding clues to the structure and formation of these complex polymers.

Stewart A. Brown

Nearly a century and a quarter has passed since Anselme Payen first used the term *lignin* in reference to the "incrusting material" which he removed from wood by the application of acidic and alkaline reagents. In the intervening years the advances in our knowledge of this complex material have been slow, often uncertain, and attended by considerable frustration. The difficulties involved have paralleled to some extent those encountered in the study of other natural polymers, but because of a unique combination of complicating factors the structure of lignin is even today far from being completely understood.

One such factor is the inhomogeneity of lignin, and it is worth while to point out at the beginning that the name in its singular form is a misnomer. Even if only simple polymers are considered, there are three different lignins, and if one accepts the existence of mixed polymers formed from more than one of the three known monomers, the number of potential lignins can be large indeed. Among other natural polymers it is possible that only proteins exceed lignin in complexity, but in one respect lignins have proved even more intractable than proteins, because no lignin has yet been obtained in crystalline form. The problem of purification has been a major impediment in determining the structure of lignin; different isolation procedures employed with the same starting material have yielded lignin preparations with appreciably different properties, so that lignin chemists have never been sure to what extent they have been working with artifacts. Still another complication has been the multiplicity of products, most of which have successfully resisted purification, that arise from degradative reactions. In spite of the great variety of attacks on the lignin polymer it has never proved possible, even with new techniques, to recover anything approaching quantitative yields of pure reaction products. Such pure products have been monomeric in nature, and the failure to recover oligomeric degradation products has severely handicapped efforts to find clues to the intramolecular linkages present in lignins.

Although there have been some dissenting opinions, there has been increasing acceptance in recent years of a concept of lignin proposed as long ago as 1897 by the Swedish chemist Klason—that of a polymer consisting predominantly, if not entirely, of phenylpropanoid (C_6, C_3) units:



I do not intend in this article to deal with the structural chemistry of lignin except as it relates directly to lignin formation in the plant. Readers interested in the structural chemistry of lignin, or in aspects of lignification other than chemical, are referred to several recent reviews dealing with these subjects (*1*, *2*).

Much speculation occurs in the older literature about the mode of lignin formation, but it has been only during the last decade or so that the availability of new weapons, such as radioactive tracers and refined chromatographic techniques, has made feasible a direct attack on the problem of biosynthesis. It is largely the research carried out during this period that I shall cover here. For a more comprehensive and detailed review of this field the recent treatise by Brauns and Brauns (*2*) is recommended.

Degradative Techniques

In most studies of biosynthesis involving tracers the product in question must be isolated from the organism and purified. Some attempts have been made to interpret the results of carbon-14 analyses on isolated lignins, but in general, because of purification difficulties, workers in this field have had recourse to chemical degradation for the recovery of certain monomeric products, which have known structures and can be purified with confidence. Three types of degradation have been most useful in this respect.

1) *Oxidation.* The use of nitrobenzene as an oxidant leads to the formation of the substituted benzaldehydes (see Fig. 1), vanillin (I), syringaldehyde (II), and *p*-hydroxybenzaldehyde (III). Spruce wood, which has been the most extensively studied, yields vanillin alone, in common with most other conifers (gymnosperms). The wood of deciduous trees (angiosperms) yields in addition syringaldehyde, and monocotyledons such as the grasses yield all three phenolic aldehydes, although much of the *p*-hydroxybenzaldehyde

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appears to originate from oxidation of the amino acid tyrosine if any protein is present (*3*). Another oxidant, permanganate, has been useful in forming benzenedicarboxylic acids which have given some important clues to the intramolecular linkages (*4*).

2) *Ethanolysis.* Prolonged heating of lignified material with ethanol containing hydrochloric acid produces small amounts of compounds, of which IV to VI (R = H or R = CH_3O) shown in Fig. 1 are predominant. These ethanolysis products are phenylpropanoid (C_6, C_3) compounds, and although they are recovered in a lower yield, their structural resemblance to the assumed lignin monomer gives them advantages over the oxidation products, which have lost two side-chain carbon atoms. Gymnosperm lignin yields compounds in which R = H, and from angiosperm lignin are recovered, in addition, compounds in which R = CH_3O (with *two* methoxyl groups).

3) *Hydrogenolysis.* The products VII (R = H or R = CH_3O) in Fig. 1 can be recovered in low yield after hydrogenolysis of lignified material over Raney nickel catalyst. Like the products of ethanolysis, they have the advantage of retaining all the carbon atoms of the phenylpropanoid skeleton.

Conversion of Carbon Dioxide to Lignin

In common with all other organic plant constituents lignin must be derived ultimately from carbon dioxide. In a discussion of its formation the most logical approach would probably be to trace the reactions by which carbon dioxide is converted to the lignin monomers, and finally to discuss the polymerization reactions by which lignin is finally synthesized. Historically, though, the problem has been attacked to some extent from the opposite direction, with the result that much was known about the polymerization before very extensive research had been done on the origin of the monomers. We examine the polymerization studies first, because this approach has the additional advantage of providing an insight into the nature of the lignin structures toward which the earlier metabolic reactions are directed.

Before proceeding with this I should like to mention some early studies in which the over-all transformation of carbon dioxide to lignin was investigated. Carbon dioxide labeled with carbon-14 has been of great value in the study of some aspects of plant metabolism, as witness the well-known work of Calvin's group on the path of carbon in photosynthesis. But its value tends to decrease in the study of plant constituents further removed from carbon dioxide, because in the longer times required for their synthesis, randomization of carbon-14 becomes extensive through the recycling of intermediates, and interpretation of the observed labeling becomes excessively difficult. But much information, especially that concerning time relationships, is obtainable through the use of C^{14}-labeled carbon dioxide.

Stone and his co-workers (*5*) used C^{14}-labeled carbon dioxide to study rates of lignin synthesis and possible breakdown in wheat plants. They exposed the plants to labeled carbon dioxide just before the beginning of rapid lignification, which had been shown to begin about 45 days after seeding, and at varying intervals thereafter determined the carbon-14 present in the phenolic aldehydes formed from lignin by oxidation. The results showed that the formation of lignin was a surprisingly slow process, not becoming rapid until 4 to 6 hours after activation. (The early products of photosynthesis acquire high activity from C^{14}-labeled carbon dioxide within seconds.) Over longer periods, although the total activity in the *tissues* as a whole declined gradually through respiration, the total activity in *lignin*, represented by phenolic aldehydes, remained substantially

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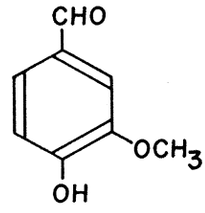
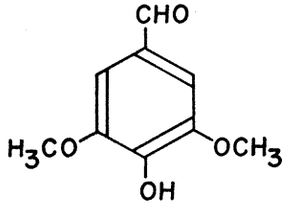
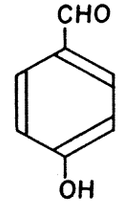


I
VANILLIN

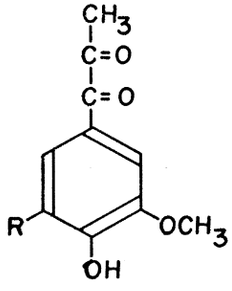


II
SYRINGALDEHYDE

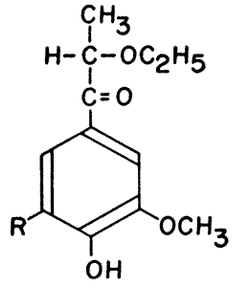


III
p-HYDROXY-BENZALDEHYDE

(2) ETHANOLYSIS PRODUCTS:



IV



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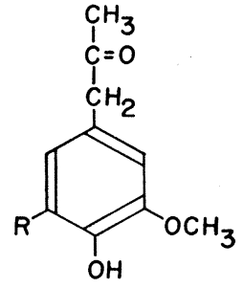
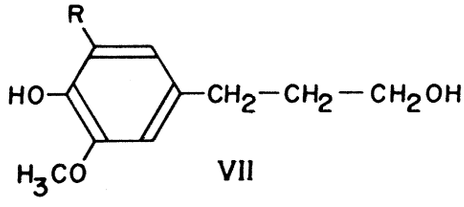


VI

(3) HYDROGENOLYSIS PRODUCTS:



VII

Fig. 1. Degradation products of lignins used in the study of lignification.

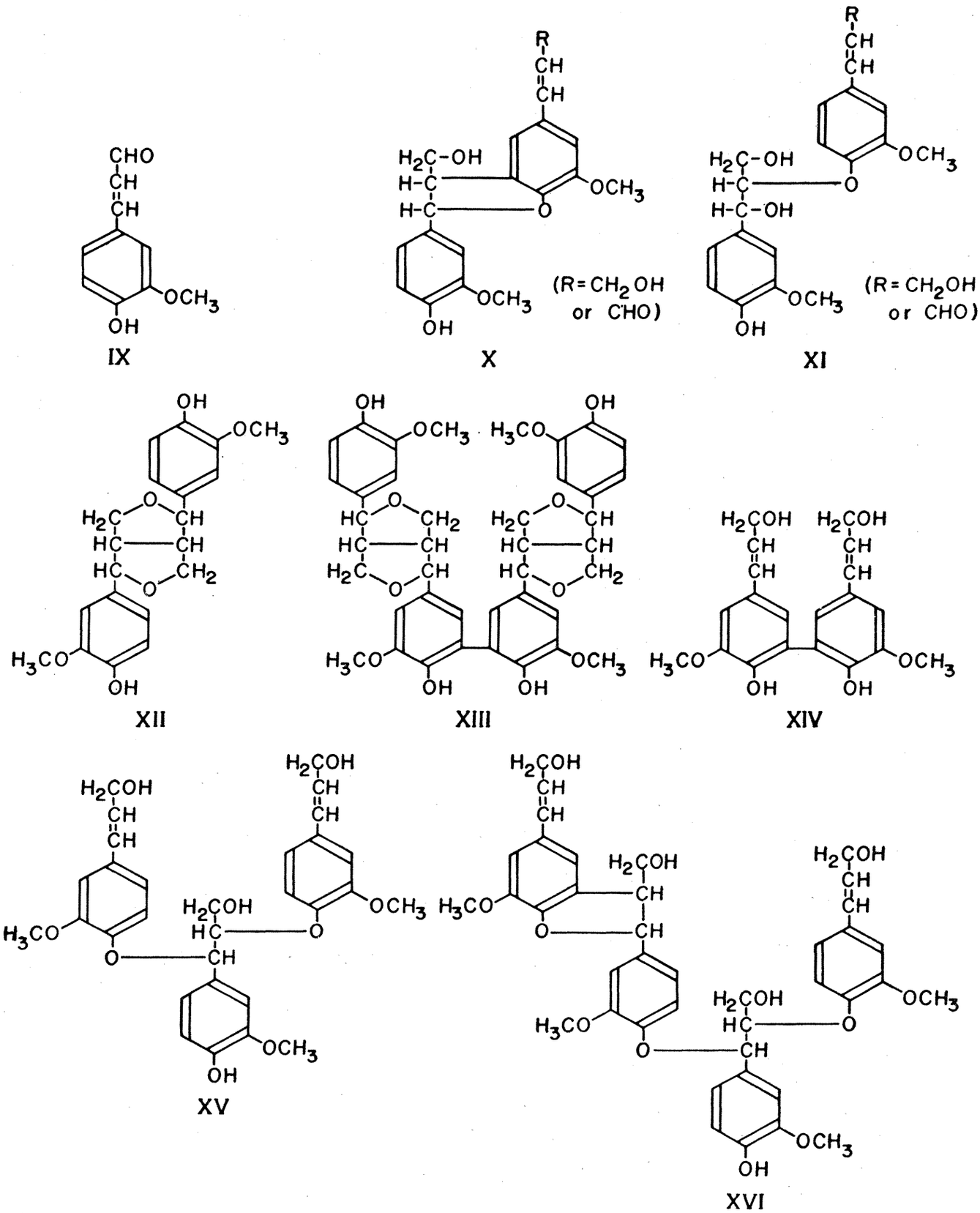


Fig. 2. Products of the action of mushroom laccase on coniferyl alcohol.

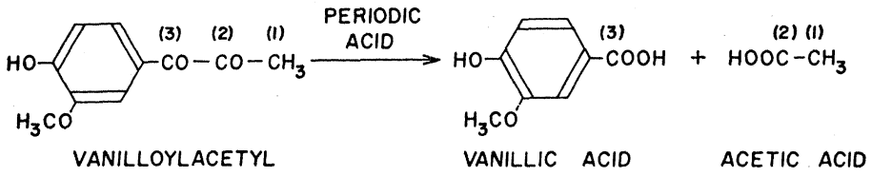
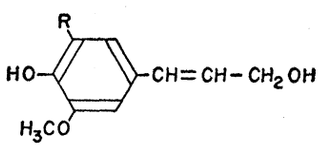


Fig. 3. Chemical degradation of vanilloylacetyl.

constant. Lignin is, then, an end product of the plant's metabolism and cannot be considered to have any metabolic function, once formed. Its functions seem, rather, to be primarily structural and protective.

Polymerization Reactions

The studies begun nearly two decades ago by Freudenberg in Germany have given us a much clearer idea of the nature of the polymerization processes which constitute lignification in the truest sense of the term. Freudenberg and Richtzenhain (*6*) found that press juice from a common mushroom, *Psalliota campestris,* contained an enzyme system which could polymerize added coniferyl alcohol (VIII, R=H)



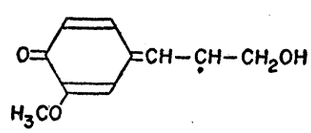
VIII

to an amorphous product bearing a striking resemblance in both chemical and physical properties to the natural lignin of conifers. It has since been established that the enzyme responsible for this polymerization is a phenol oxidase, laccase (*7, 8*), but it is still not completely clear whether the polymerization in higher plants is catalyzed chiefly by laccase or by peroxidase (*8, 9*).

The addition of both coniferyl alcohol and sinapyl alcohol (VIII, R= CH_3O) to the mushroom enzyme system led to the formation of a copolymer resembling the lignin of angiosperms (*10*). In view of the fact that no natural lignin made up exclusively of sinapyl alcohol units is known, it was significant that no lignin-like polymers higher than dimers could be recovered from a reaction mixture to which had been added sinapyl alcohol alone, without coniferyl alcohol.

Freudenberg and his associates found that if they interrupted the reaction at an early stage, a number of products of relatively low molecular weight could be recovered from reaction mixtures in which coniferyl alcohol was the substrate. Some 40 of these products have been distinguished, and with the aid of chromatography on cellulose columns, 11 have been purified (*11*). Structures published for these compounds are shown in Fig. 2. The most important quantitatively are dehydrodiconiferyl alcohol (X, R= CH_2OH), DL-pinoresinol (XII), and guaiacylglycerol-β-coniferyl ether (XI, R=CH_2OH), all dimers.

A free-radical mechanism has been proposed (*12*) for the formation of these condensation products, in which the central intermediate in the formation of coniferyl-type polymers is a quinone methide (XVII)



XVII

and some evidence for the existence of such an intermediate has been presented (*13*). In a similar way the formation of a quinone methide from the dimer XI, R = CH_2OH, could lead to molecules with a higher degree of polymerization, such as compounds XV and XVI. It is of interest, in view of the fact that this theory predicts the formation of an optically inactive lignin, that the intermediates of Fig. 2 are inactive, and no demonstration of optical activity in lignin has yet been made.

As pointed out earlier, attempts to isolate from the chemical and biological degradation of lignin oligomeric products which could provide clues to the linkages between monomers have been consistently unsuccessful. The synthetic approach of Freudenberg's group thus appears to represent a major breakthrough in our understanding of lignin structure. At present this applies only to coniferous lignins, and the structure of lignins which also contain sinapyl alcohol and possibly *p*-hydroxycinnamyl alcohol units is still little understood. Although it is true that the German workers have derived their enzyme system from a plant which does not normally lignify, there seems to be no good reason to believe that the polymerization catalyzed by the mushroom system is essentially different in its mechanism from that in lignifying higher plants.

Coniferyl alcohol is not known to occur naturally in the free form, but its β-glucoside, coniferin, is found in coniferous species. On the basis of experiments with the conifer *Araucaria,* Freudenberg *et al.* have suggested that the more soluble coniferin is translocated from its site of synthesis to the vicinity of the cambium, where coniferyl alcohol is liberated by the action of a β-glucosidase and then undergoes polymerization (*14*). Higuchi and his co-workers (*15*) were able to show a correlation between the rate of lignification and the concentration of β-glucosidase in different zones of the bamboo shoot. In general, though, the role of coniferin, at least in species other than conifers, cannot be considered to be fully established. Even in conifers its primary function may be that of a storage product.

More extensive studies on the utilization of coniferin in the intact plant have been carried out by Kratzl, Billek, and their associates. They used ethanolysis to degrade the lignin from spruce wood into which coniferin-3-C^{14} had been implanted (*16*). One of the ethanolysis products, vanilloylacetyl, was further degraded chemically, as shown in Fig. 3. The vanillic acid from this oxidation accounted for all the carbon-14 in the vanilloylacetyl, as would have been expected if coniferyl alcohol from the coniferin had been incorporated into lignin without rearrangement of its side chain. When coniferin-2-C^{14} was fed (*17*), the 2-carbon fragment arising from C-1 and C-2 of the side chain contained all the radioactivity. These findings, together with similar results of Brown and Neish (*18*) in studies in which cinnamic acid-3-C^{14} was fed to wheat, make it clear that no significant reconstruction of the side chain occurs during lignification.

Tissue-culture techniques, which have proved so useful in many other biochemical applications, have been used by several investigators (*19, 20*) to obtain additional evidence that coniferin is a lignin precursor, not only in conifers but in several other species not known normally to contain this glucoside. Peroxide was shown to enhance the polymerization, and substances

known to be peroxidase inhibitors were shown to inhibit it, providing further evidence that the polymerization is, at least in some tissues, mediated by peroxidase.

Pathway from Carbohydrates to Lignin Monomers

An equally important aspect of lignification concerns the biosynthetic route leading from carbon dioxide to the lignin monomer or monomers. It has long been assumed that lignin is synthesized, in common with other organic plant constituents, from carbohydrates formed in photosynthesis. But it has been only within the past decade that the pathways by which aromatic rings are formed have begun to be revealed in detail. The first clues to the reactions involved came not from higher plants but from the now well-known studies of Davis, Sprinson, and their associates on mutants of the bacterium *Escherichia coli* (*21*). These radiation-induced mutants, which lacked enzymes necessary for aromatic ring formation, accumulated in the growth-medium compounds which have proved to be obligatory intermediates in the conversion of sugars to benzenoid compounds. The pathway, as understood at present, is shown in Fig. 4. One of the cyclic pre-aromatic compounds is shikimic acid (XXIV), which is now known to accumulate in many plants. By condensation of shikimic acid phosphate (XXV) with phosphoenolpyruvic acid (XIX) there is formed prephenic acid (XXVII), which in turn reacts to form phenylpyruvic acid (XXVIII), the first fully aromatic compound synthesized. In an alternative pathway the hydroxyl group of prephenic acid is retained, and the product is *p*-hydroxyphenylpyruvic acid (XXIX) (*22*).

The fact that the first aromatic compounds resulting from the shikimic acid pathway were phenylpropanoid in nature, with a carbon skeleton resembling that of the cinnamyl alcohols, led Brown and Neish to suspect that the shikimic acid pathway might participate in lignification. It was demonstrated (*23*) that C^{14}-labeled shikimic acid and L-phenylalanine were incorporated into the lignin of wheat and maple with a quite low dilution of the carbon-14, whereas the dilution of carbon-14 from a C_6,C_1 compound, 3,4-dihydroxybenzoic acid, whose ring substitution resembled that of coniferyl alcohol, was much greater, indicating much less efficient utilization. Eberhardt and Schubert (*24*) complemented this finding by showing that no rearrangement of carbon atoms took place during the conversion of radioactive shikimic acid to lignin. More recently, active ethanolysis products have been recovered from pine tissue cultures after administration of labeled shikimic acid (*25*), and two groups of workers have obtained further supporting evidence for the participation of the shikimic acid pathway in lignin biosynthesis, by feeding to plants glucose specifically labeled with carbon-14 (*26, 27*). From the lignin formed by spruce from this glucose they recovered vanillin labeled as would have been predicted if lignification had proceeded by way of shikimic acid. Russian workers have found that labeled pentoses, too, can act as lignin precursors (*28*).

On the basis of the work reviewed above and elsewhere (*29*) on the biosynthesis of lignin and other plant constituents, it seems clear that the shikimic acid pathway is involved in aromatic ring formation in higher plants, and that it is at least closely similar to that established in *Escherichia coli*. It is less certain that this is the only way aromatic lignin precursors are formed from carbohydrates. A route to certain aromatic compounds involving the condensation of three molecules of acetic acid is known to exist in plants (*29*), but there is evidence that its significance in lignification is slight (*25, 27, 30*). Other

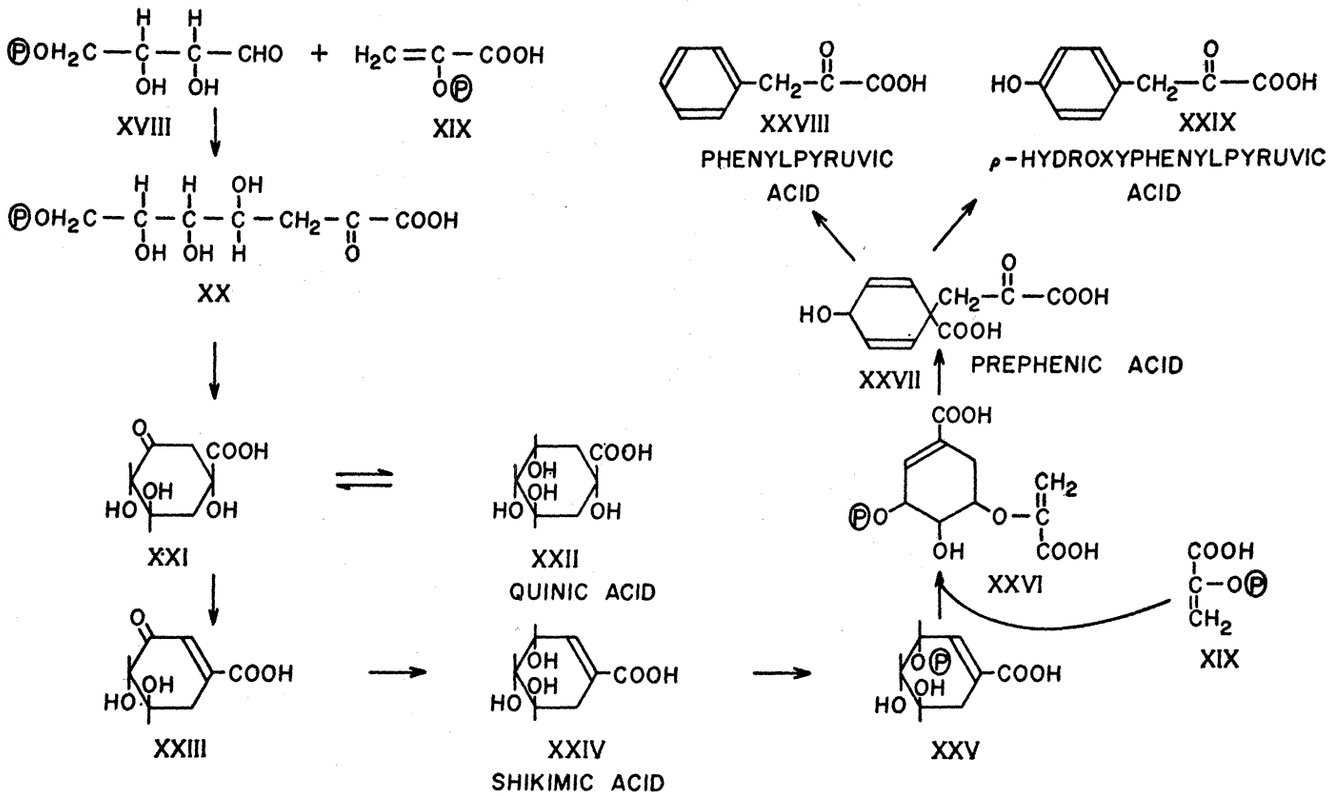


Fig. 4. The shikimic acid pathway of aromatization operating in *Escherichia coli* (P=PO_3H_2).

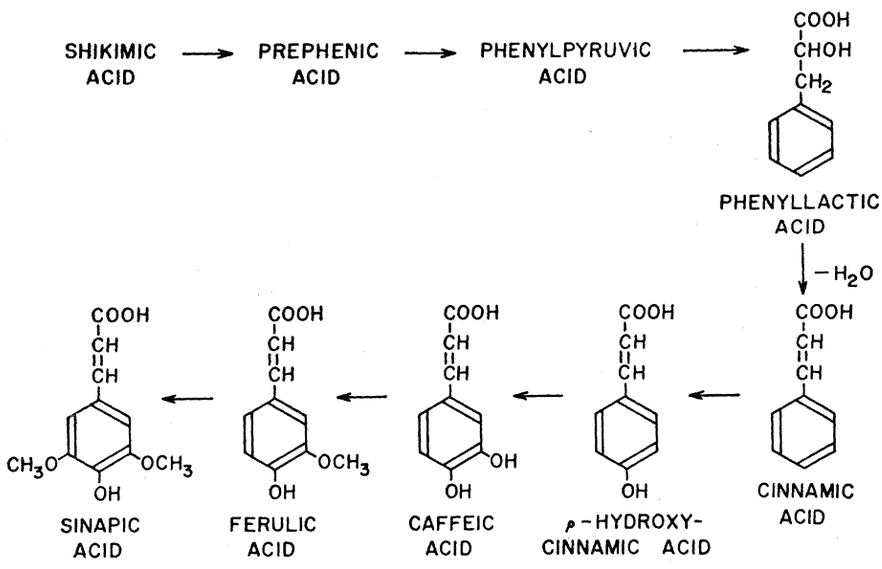


Fig. 5. A possible route for the formation of phenolic cinnamic acids in the plant.

routes to phenylpropanoid compounds have not been ruled out. Recently Weinstein, Porter, and Laurencot (*31*) showed that when quinic acid (Fig. 3, XXII) was fed to roses, the carbon-14 in phenylalanine reached a peak before that of shikimic acid. This may indicate that shikimic acid here is not on the direct route between quinic acid and phenylalanine, which can be formed from phenylpyruvic acid by transamination, or possibly that an alternative pathway is operating independently of the shikimic acid route.

The origin of the methyl carbons of lignin has been shown by Byerrum and his co-workers (*32*) to be the methyl group of methionine, and ultimately the 1-carbon metabolic pool. Thus, we can account with reasonable certainty for the origin of all the carbon atoms in the lignin monomers, and we can now examine the question of how the first phenylpropanoid compounds are converted to lignin monomers.

In working out biosynthetic pathways a fruitful approach can be a comparison of the efficiencies with which a large number of compounds are converted to the component in question. This approach is most useful when radioactive compounds are available. From a consideration of the most efficiently utilized compounds (there is often a difference of one or more orders of magnitude) it is often possible to arrive at a working hypothesis about the biosynthetic route, which can be tested by the feeding of additional compounds. It is necessary also, when using tracers, to carry out degradations to locate the position of the tracer in the molecule and eliminate the possibility of breakdown and resynthesis. The two approaches are both essential for the most rigorous demonstration of a precursor. The tracer technique cannot by itself establish a compound as an obligatory intermediate in a pathway, but the participation of naturally occurring compounds can often be demonstrated. When several such intermediates have been identified, studies on the plant enzyme systems can be undertaken to confirm the individual steps in a proposed pathway.

Various potential lignification intermediates have been compared by Brown and Neish (*3, 18, 23, 30, 33, 34*), who used tracer techniques, and by Stafford (*35*), who used unlabeled compounds. The former workers were able to eliminate several compounds as contributing significantly to lignification; these included acetate, C_6C_1 compounds in general, and C_6C_3 compounds bearing hydroxyl groups on the 3- and 2,3-carbons of the side chain, or in the *meta*-position only of the ring. On the other hand, a number of other C_6C_3 compounds were much more efficiently utilized: phenylalanine, phenylpyruvic acid, phenyllactic acid, cinnamic acid, and four hydroxylated cinnamic acids—*p*-hydroxycinnamic, caffeic, ferulic, and sinapic. On the basis of these findings it was proposed (*3*) that the formation of these compounds proceeded according to the sequence shown in Fig. 5. Support for the second part of this sequence was obtained by McCalla and Neish (*36*), who found that labeled cinnamic acids fed to *Salvia* underwent successive hydroxylations and methylations to form the later members of the sequence. Thus far it has not been possible to demonstrate these reactions in cell-free systems. But Koukol and Conn (*37*) have very recently found in legumes an enzyme which removes from phenylalanine the elements of ammonia to produce cinnamic acid directly. This enzyme, originally called phenylalanase, has now been named phenylalanine deaminase. Thus, it is no longer necessary to postulate the participation of phenyllactic acid in lignification, although its involvement still remains possible. This compound can be converted by way of phenylpyruvic acid to phenylalanine in the plant (*29, 38*); results of the tracer studies can be explained in this way.

In the course of tracer investigations on various grasses another pathway to lignin synthesis was discovered (*18, 33*), in which tyrosine can serve as a lignification intermediate. This reaction may proceed through *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids (see Fig. 5), both of which are readily convertible to lignin in wheat. But an enzyme analogous to phenylalanine deaminase, named tyrase has been reported recently by Neish (*39*). This enzyme, found in significant amounts thus far only in grasses, reaminates tyrosine to *p*-hydroxycinnamic acid. Examination of over 20 plant species has shown that tyrosine is utilized as efficiently as phenylalanine only in grasses, although several other species can use tyrosine to a considerably lesser extent (*40*). Similarly, *p*-hydroxyphenylpyruvic acid, which is a lignin precursor in wheat and sugar cane, is much less efficiently utilized by spruce, buckwheat and *Salvia* (*3, 41*). It seems highly probable that the ability of grasses to lignify by the tyrosine route depends on their tyrase content. Grasses also contain phenylalanine deaminase (*39*) and can synthesize lignin by way of phenylalanine. The route to lignin through phenylalanine would appear to be of earlier evolutionary origin, as it is found in the gymnosperms as well as in the higher angiosperms. Phenylalanine-C^{14} fed to spruce yields both lignin degradation products and coniferin which are radioactive (*42*). Apparently only in grasses, representing a very high stage of development, has the alternative tyrosine pathway been fully evolved. Interestingly, it is among the Compositae, which occupy an analogous place in development among dicotyledons, that some utilization of tyrosine as a lignin precursor is also detectable (*40*).

The pathways leading from carbo-

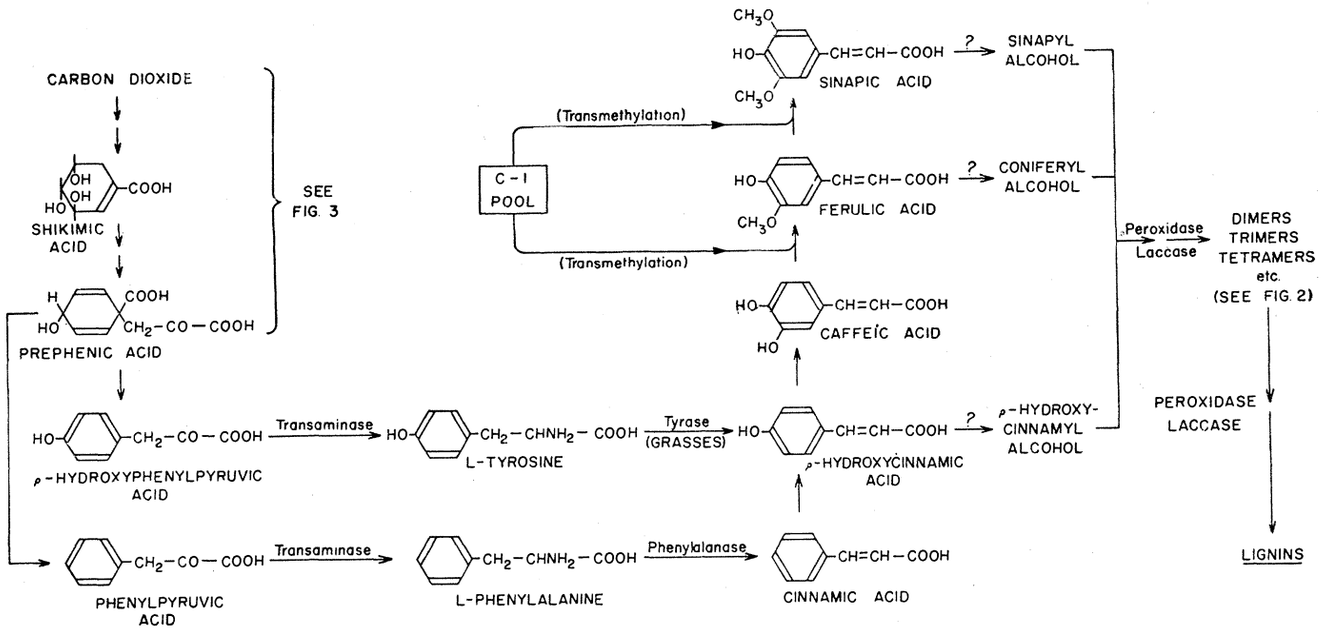


Fig. 6. Probable lignification pathways. Double arrows signify that more than one reaction is known to be involved. Not all these reactions occur in all species.

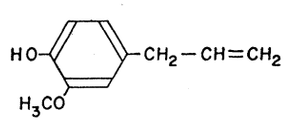
hydrates to lignin synthesis which are best supported by present evidence are shown in Fig. 6. This representation is still incomplete in several respects. Other routes to lignin remain possible, and this question is discussed in the next section. There is also no reference to the possible role of glucosides. The phenolic cinnamic acids are quite insoluble at the acid *p*H of typical plant saps, and in fact they must be administered to plants as salts. It would not be unreasonable to expect glucoside formation as an aid to translocation, as has been suggested already for coniferin, but reports that ferulic acid glucoside is a poor lignin precursor in spruce (*43, 44*) do not support this idea. Recently Higuchi and Brown (*45*) have found evidence that labeled ferulic acid fed to wheat can be chemically combined with glucose. The nature of possible "activated" forms of the intermediates has been largely a matter of conjecture. McCalla and Neish (*36*) found evidence for esters of substituted cinnamic acids in *Salvia,* and Levy and Zucker (*46*) showed that cinnamic acid in the form of a depside with quinic acid (Fig. 3, XXII) undergoes successive hydroxylation to chlorogenic acid, the corresponding depside of caffeic acid. Whether this process has any significance in lignification is not known. One serious gap in the reaction sequence of Fig. 6 is between the substituted cinnamic acids and the corresponding alcohols, which undergo the actual polymerization. In fact there is still uncertainty whether sinapic acid, as such, is an intermediate (*30*), and at least some of the extra hydroxylation found in the sinapyl-type lignin may be introduced after polymerization.

It should be pointed out that most of the steps shown in Fig. 6 are by no means peculiar to lignin biosynthesis. Flavonoid substances and coumarins, for example, are considered to be synthesized by the same route up to at least the cinnamic acid stage (*29, 47*). Clearly, too, the stages prior to phenylalanine and tyrosine must be viewed in the light of the relation of these amino acids to protein synthesis. Thus, these early compounds, such as *p*-hydroxyphenylpyruvic acid, can hardly be considered central intermediates in lignification. *p*-Hydroxycinnamic acid is a more likely candidate for that role (*3, 39*).

Other Possible Lignification Pathways

Although C_6,C_1 compounds in general are not efficient lignin precursors, vanillin, which occurs in some plants, is utilized well enough to suggest that it may participate in a minor lignification pathway (*18, 30*). We might infer a condensation with some 2-carbon metabolite, but the nature of this hypothetical compound is not known. It has not been possible to confirm preliminary findings indicating that glycine (*48*) or acetaldehyde (*25*) is the 2-carbon compound in question.

Siegel, in a series of papers several years ago (*20, 49*), studied the polymerization of eugenol (XXX)



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by several plant tissues in the presence of hydrogen peroxide. The amorphous product had solubility properties and gave color reactions characteristic of lignins. A number of considerations, such as the action of heat and enzyme inhibitors, the enhancing effect of hydrogen peroxide (up to 100-fold), and histochemical studies showing a correspondence between the sites of greatest polymerization and the most intense peroxidase activity, led Siegel to conclude that the polymerization is mediated by peroxidase. This agrees with the concept that peroxidase is one of the enzymes involved in natural lignification.

Although this is a very interesting model system, it is very doubtful that eugenol itself polymerizes. Eugenol undergoes ready spontaneous oxidation to coniferaldehyde (Fig. 2, IX) (*50*), and polymerization of this or a derivative probably leads to structures very similar to those reported by Freudenberg *et al.* Higuchi and Kawamura (*50*) found that freshly distilled eugenol was not converted to lignin-like polymers by peroxidases of bamboo shoot and horse radish, whereas the distillation residue

did undergo such polymerization. Stafford (*51*) has found that freshly distilled eugenol gives only small amounts of a polymer in the presence of timothy grass leaf sections but that ferulic acid yields large quantities of polymer similar to the natural lignin of that species. There seems no reason to believe that eugenol is a natural lignification intermediate; its formation in plants such as cloves doubtless represents a side reaction from the main pathway.

Factors Involved in the Control of Lignification

An intriguing aspect of lignification is that of the factors which exert the controlling influence on this process. Very young plants often appear to contain little lignin, and in wheat, for instance, lignification does not proceed at an appreciable rate until the plants are about ready to head out. What factors intervene to prevent or induce lignin synthesis?

The question whether photosynthesis is always necessary for lignification has not been fully answered. Kratzl (*52*), on the basis of three criteria—methoxyl content, solubility properties in acid, and oxidative degradation products—concluded that "a substance which doubtless can be classified as lignin" was formed in etiolated potato sprouts. This was in contrast to the results of early work by Klason, and also to the subsequent findings of Phillips (*53*), who showed that when ash shoots were shaded for periods of several weeks, the wood formed during this time gave only weak color reactions for lignin. The possibility that species differences may play a part here has not been excluded. The effect of light may be due to its influence on the supply of lignin precursors, such as intermediates of the photosynthetic cycle; an effect on other possible controlling factors is an equally plausible interpretation.

In any case, the availability of intermediates in the photosynthetic cycle cannot be the only factor controlling lignification, since much actively photosynthesizing tissue contains virtually no lignin. But it is quite possible that blocks at a later stage of the sequence imposed by the relative lack of a necessary enzyme or activator, or the presence of an inhibitor, can prevent lignification during certain periods of growth. Little is yet known about such possible control mechanisms. Kratzl (*54*) has reported the interesting observation that when syringin (glucoside of sinapyl alcohol) is fed to spruce, which normally does not make sinapyl-type lignin, the ethanolysis products of the lignin contain radioactive syringoylacetyl (Fig. 1, V; R=CH_3O), a sinapyl-type lignin degradation product. Perhaps the reason spruce cannot normally synthesize sinapyl-type lignin is that it is unable to convert ferulic acid to a sinapyl-type monomer, since ferulic acid is readily utilized by spruce for lignification (*43*).

Auxins appear to be involved in the control of lignification. Siegel (*49*) showed that the polymerization resulting from the action of *Elodea densa* tissues on eugenol was inhibited by 3-indoleacetic acid, which inhibited competitively oxidations catalyzed by peroxidase. He suggested that the high levels of this auxin in rapidly elongating organs would suppress peroxidase activity, and hence lignin deposition, whereas with the decline of auxin concentration that accompanies maturity, lignification would increase. Siegel and his associates (*55*) have concluded that 3-indoleacetic acid acts as an antioxidant to constrain peroxidase from a premature attack on metabolites essential to growth. Such a delaying action would prolong favorable conditions for growth, and when this is virtually complete, removal of the influence of the auxin could permit the onset of lignification in a given zone or throughout the plant. Other less-detailed reports (*56*) have suggested that in some tissues 3-indoleacetic acid can exert the opposite effect and stimulate lignification.

On the basis of measurements of glutathione and ascorbic acid in lignifying and nonlignifying parts of the bamboo shoot, Higuchi (*57*) believes that an oxidation-reduction system involving these compounds may be involved in controlling the amount of coniferyl alcohol available for the action of peroxidase in this species.

We could summarize by saying that the control of lignification is accomplished by the action of several factors, which may include the availability of intermediates from the photosynthetic cycle, the presence or absence of key enzymes or other factors involved in the formation of aromatic intermediates, and the presence or absence of oxidation inhibitors acting on the final polymerization steps. The relative importance of these factors undoubtedly varies from one species to another.

Cell-Wall Carbohydrates and Their Relation to Lignin

It is becoming generally accepted that lignin is chemically linked to polysaccharides in the cell wall (*2, 58*). Thus far little is known about the biochemical aspects of this linkage, but one or two suggestive findings have been published. Siegel (*59*) has shown that when cellulose, in the form of filter paper, is added to a system of eugenol, peroxidase, and hydrogen peroxide, there is deposited on the paper a lignin-like polymer similar to that observed in the presence of tissue sections. In the absence of the cellulose no such material is formed. This suggests that lignification in natural tissues may also be intimately associated with the presence of preformed cell-wall carbohydrates. Freudenberg's group more recently have used other model systems containing mushroom laccase or manganese dioxide (*60*) to demonstrate that ethers can be formed between guaiacylglycerol coniferyl ether (Fig. 2, XI; R=CH_2OH) and sorbitol or sucrose. Siegel's findings are consistent with the possibility that ether formation may even be essential for lignification in the true sense. If so, it would be meaningful to conceive of lignification only in the broadest sense as those reactions leading to the formation of the lignin-carbohydrate entity which constitutes the cell wall.

Present Status, Future Prospects

Looking back over the work of the past decade, one can appreciate that very considerable strides have been made in our understanding of the biochemical pathways leading to lignin synthesis. As has been the case with other natural products, this has also furnished the structural organic chemist with important clues which would not have been available otherwise. The broad outlines of the lignification picture have been largely sketched in, but much detail remains to be added. Further developments can be expected in the isolation of more complex intermediates from the polymerization reactions and in the elucidation of linkages to polysaccharides of the cell wall. Additional tracer studies may lead to a bridging of the remaining gaps in our theories of the reactions by which lignin monomers are formed from carbohydrates. In this phase of lignification there is much scope for work in cell-

free systems, in which one can hope to demonstrate enzymes that would confirm more of the postulated reaction steps, or possibly bring to light still unsuspected reactions. A better understanding of the control mechanisms might open to us the possibility of regulating the formation of lignin, whose presence in many plants often leads at present to undesirable consequences. Another decade of research on lignification, if as successful as the last, may well provide us with a quite clear conception of this important process and provide extra dividends in the form of significant practical applications.

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A study of the list of periodicals abstracted by *Chemical Abstracts* shows that the 1926 edition reviewed a total of 1246 periodical titles. The American Chemical Society reports that over 9000 periodical titles were reviewed in 1960. This is an increase of more than 7700 titles in 34 years.

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