Lignin: Its Constitution and Formation from p-Hydroxycinnamyl Alcohols

Lignin is duplicated by dehydrogenation of these alcohols; intermediates explain formation and structure.

Karl Freudenberg

Lignin (*1–4*) occurs with cellulose and hemicelluloses as an integral component of woody plant tissues. About one-quarter of dry wood consists of lignin, in part deposited in the xylem cell walls, in part located in the intercellular spaces, where it may constitute up to 70 percent of the solids present (*5*). A large proportion of the lignin in woody plants, perhaps even all of it, is in chemical combination with the plant polysaccharides.

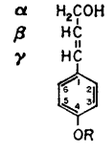
In its role as a filler impregnating the matrix of cellulose fibrils in the cell walls and filling spaces between the elongated wood cells, it is comparable with the cement in reinforced concrete (*6*). It is of undoubted importance for the mechanical properties of wood that the lignin molecules are linked here and there to the polysaccharides by ether bonds (*7–8*). The manner in which lignin originates in wood is compatible with the fact that, first, aggregates of only a few C_6—C_3 units of the lignin (oligolignols) become attached at various points to the polysaccharides and that, later, these attached oligolignols join together as the lignin molecule continues to grow. This concept also explains why lignin is not deposited as separate particles but is spread continuously over wide areas, and why it is at first loosely packed but later stops up the intercellular spaces in a denser form and penetrates the tissue, right down to the surfaces of the fibrils and into the amorphous regions of the cellulose and hemicelluloses. This may explain the intimate relation between lignin and carbohydrates in the tissue. The question of why lignin invariably occurs together with polysaccharides in plants is probably in some way related to the preceding statements, but no completely satisfactory answer is yet available.

Lignin as it occurs unaltered and unmodified in wood I refer to simply as "lignin." This material requires no such special designation as "protolignin" or "native lignin." The best representative of lignin now extractable is the material that is released by mechanical disintegration of the plant tissues—"milled-wood lignin," according to A. Björkman (*9*). Lignin liberated from its carbohydrate associates by mineral acids [even with a cold mixture of dioxan and HCl (*10*)] is of little value for investigations of chemical structure. So-called "soluble lignin" or "native lignin" from wood is, in the case of spruce [*Picea abies (excelsa)*], really less than 0.5 percent of the total lignin content of the wood after removal of the hydroxymatairesinol and other lignans and oligolignols that it contains (*11*); it is also chemically different from milled-wood lignin, which can be extracted in amounts constituting 35 to 40 percent of the total lignin content of spruce (*9, 11*). Chemically, lignin is a copolycondensate of the dehydrogenation products obtained from the alcohols (I)–(III) (*13*).

The molecular weight of carbohydrate-free milled-wood lignin from conifers is a little more than 10,000, but that of undegraded lignin in the plant is probably many times higher. Its "degree of polymerization" exceeds 50 and may be even several hundred. The lignin molecule is highly branched and is thus three-dimensional (*6*); the resulting material is amorphous, crumbly, and lacking solidity.

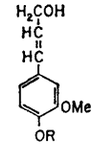
The biogenesis of lignin subdivides into three stages: formation by way of shikimic acid and prephenic acid of C_6—C_3 acids; transformation of these into the alcohols (I)–(III) and their glucosides (IV)–(VI); and dehydrogenation of the alcohols to lignin. Much work has been done on the first two stages, and I cannot review here or even quote more than a fraction of the literature (*12*).

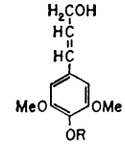
Except for some experiments in which trees were treated with phenylalanine, the following report deals exclusively with the third stage of the biosynthesis of lignin—conversion, by dehydrogenation, of the monomeric alcohols (I)–(III) (*13*) into the polymolecular entity lignin. It is assumed that in plants the alcohols (I)–(III) and the glucosides (IV)–(VI) are almost exclusively the ultimate precursors of lignification.



(I)
R = H, *p*-coumaryl alcohol (*13*)

(IV)
R = $C_6H_{11}O_5$ glucocoumaryl alcohol





(II)
R = H, coniferyl alcohol

(III)
R = H, sinapyl alcohol

(V)
R = $C_6H_{11}O_5$ coniferin

(VI)
R = $C_6H_{11}O_5$ syringin

During active vegetative growth in the early summer, the cambium and surrounding tissue in spruce (*Picea abies*) contain a high concentration of coniferin (V) together with small amounts of *p*-glucocoumaryl alcohol (IV) and syringin (VI) (*14*). A little free coni-

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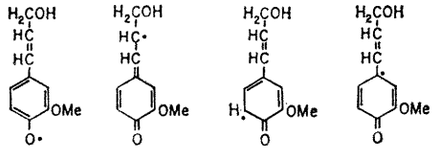
feryl alcohol and coniferaldehyde (XV) and traces of the oligolignols (XVII), (XVIII), (XX), and (XXII) (discussed later) can also be detected (*14*). When fresh young shoots, pared of needles, from the branches of spruce saplings are immersed in a solution of radioactive phenylalanine, they take up the labeled amino acid. After 2 to 3 days cambial sap taken from the branch just above the point of attachment of the shoots is found to contain radioactive coniferin and radioactive *p*-glucocoumaryl alcohol (*15*).

The process occurring in the plant can be explained in the following manner. The three glucosides are formed somewhere between the green shoots and the cambium, or in the cambium. When the glucosides diffuse into xylem cells lying three to four layers under the cambium belt and beyond, they encounter increasing amounts of a β-glucosidase which has been shown to be located in the tissues there (*16*). The glucosidase releases the three alcohols (I)–(III) from their glucosides and makes them available for further processing by phenol dehydrogenases, which are found to be laccase and peroxidase for both spruce and other plants. These enzymes effect removal of the phenolic hydrogen from the alcohols (I)–(III) to form radicals of the type R_a–R_d (VII)–(X) (*17*). These radicals intercombine and undergo other changes which finally lead to lignin.



(VII) R_a (*17*) (VIII) R_b (IX) R_c (X) R_d

Labeled phenylalanine or D-coniferin made by glucosidation of radioactive coniferyl alcohol with D-glucose was incorporated into spruce saplings. After several weeks, radioactive lignin was isolated. Participation of the glucosidase is proved by the fact that, unlike D-coniferin, L-coniferin, prepared from labeled coniferin and L-glucose, is not incorporated into the lignified wood (*18*).

The process of molecular growth is in fact the third and last stage in the complete biological process which may be termed the biogenesis of lignin.

What Is Lignin?

The criterion for designating a substance as lignin should be based on its chemical analysis. Analytical data have been elaborated by many authors during the last few years, especially by E. Adler and J. Marton (*18a*); (see also *39*). The methoxyl content of lignin isolated from different plants can be recalculated on the basis of a C_9 unit, and it is always less than 1.5 OCH_3 per C_9 unit. The value found for a given lignin preparation depends on the ratio of the three *p*-hydroxycinnamyl alcohols from which the lignin originated. Mosses, such as *Sphagnum* and *Polytrichum*, contain lignins with a methoxyl content of 0.25 per C_9 unit. These lignins are derived mainly from *p*-coumaryl alcohol. Spruce lignin is a typical conifer lignin with 0.92 methoxyl per C_9; it originates from coumaryl, coniferyl, and sinapyl alcohols in ratios of about 14:80:6 (*19*). Beechwood (*Fagus sylvatica*) lignin, a typical hardwood lignin, has 1.41 methoxyl per C_9, the alcohol ratios may have been about 5:49:46. Lignin extracted from mistletoe (*Viscum album*) of a pine (*Pinus sylvestris*) is practically identical with conifer lignin; lignin from mistletoe growing on deciduous trees (for example, hawthorn, *Crataegus oxyacantha*) is almost identical with hardwood lignin (*11*). These are examples of roles that lignin plays in taxonomy.

If the analytical values for the elemental composition of lignin from any source are recalculated on the basis of the coumaryl component by replacing the methoxyl content per C_9 by hydrogen, an empirical formula invariably results which contains 1.7 to 2.0 atoms of hydrogen less and 0.4 to 0.9 molecules of water more than the formula of *p*-coumaryl alcohol ($C_9H_{10}O_2$). A synthetic lignin made from *p*-coumaryl alcohol alone has the formula $C_9H_8O_2(H_2O)_{0.4}$. These analytical figures can be taken as limits defining the compositional concept of a lignin.

As mentioned below, drastic conditions must be applied in order to obtain small and simple degradation products from lignin. The simplest degradation products are carboxylic acids, mainly derivatives of methoxylated benzene, biphenyl, and diphenyl ether (*19*). It is clear that any structural formula written for lignin must be able to explain the origin of such degradation products, but lignin chemists are well aware of the fact that knowledge of the structures of lignin degradation products either alone or in combination with analytical data on the number of hydroxyl, carbonyl, and ether linkages in lignin could never suffice for preparing a formula for lignin.

It was only after the discovery of γ-aryl ether linkages in lignin [see for example (XXIII), (XXIV), or units 4 to 3 and 11 to 12 of the scheme (XXXIV)] that a systematic search was started for mild methods for degrading lignin which would yield products that had undergone less drastic chemical changes and would provide better information on the structure of lignin. Mild ether hydrolyses have led to the isolation of mono- and dilignols [(XVII), (XVIII), (XX), (XXIX)–(XXXI)] (*11, 20, 20a*). Despite the small yields obtained, as lignin degradation products, these substances shed some light on the structure of natural lignin. Otherwise, however, the multifarious and variegated forms of linkages that occur between the C_9 units, and the chemical resistance of certain ether bonds, limit the extent to which analytical and degradation procedures can be used to elucidate the structure of lignin.

Lignin Synthesis in vitro and in vivo

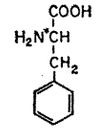
Thus, synthesis of lignin by enzymic dehydrogenation of *p*-hydroxycinnamyl alcohols was used in an endeavor to elucidate its structure.

As early as 1875, F. Tiemann and B. Mendelsohn suspected some relationship between coniferin and lignin. P. Klason (*21*) suggested a connection between lignin and coniferyl alcohol (1897) and coniferaldehyde (1920). In 1923 and 1929 he postulated that lignin is a condensation product of coniferaldehyde formed in the plant from coniferyl alcohol by oxidation. In 1933, H. Erdtman (*22*) suggested that the process leading to lignin synthesis in plants is a dehydrogenation. Enzymic dehydrogenation of coniferyl alcohol was first carried out in 1943 (*23*).

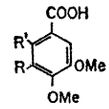
Enzymic dehydrogenation of mixtures of the three *p*-hydroxycinnamyl alcohols in vitro leads to artificial lignins. For duplicating conifer lignin, a mixture of *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (14:80:6 moles) proved to be the most suitable (*19*). Dehydrogenation is carried out

in extremely dilute aqueous solution at *p*H 5.5 and 20°C, either with a laccase purified from juice of the common mushroom *Agaricus campestris* (*24*) or from culture filtrates of the wood mushroom *Polyporus versicolor* (*25*) by aeration or with commercial pure horseradish peroxidase, as 0.1 percent H_2O_2 is added drop by drop (*2*). After loss of about one atom of hydrogen per molecule, the rate of oxidation decreases but continues slowly until about two atoms of hydrogen per unit have been abstracted. The water-insoluble product obtained after extraction of low-molecular-weight constituents has the elemental composition of spruce lignin, as already described.

Comparisons of the artificial (that is, made in vitro) lignin with that of spruce (milled-wood lignin extracted according to Björkman) show that the two materials have very similar properties. For example: (i) When they are similarly treated with hot alkali and then methylated with dimethyl sulfate and oxidized with permanganate, both preparations give more than 20 so-called degradation acids, mostly methoxybenzenecarboxylic acids; veratric acid (XII) is the main product whereas others appear in only minute amounts. The main point is that the amounts and types of acids formed are the same from both sources (*19*). (ii) If [β-^{14}C]-coniferyl alcohol is used to produce the artificial lignin, the isohemipinic acid (XIII) occurring among the degradation acids has half of the specific activity of the starting material, and the corresponding metahemipinic acid (XIV) has one-eighth (*26, 27*). If lignin grown in spruce in the presence of analogously labeled phenylalanine (XI) (*28*) or coniferin (*20, 20a, 27*) is subjected to the oxidative degradation, the isohemipinic and metahemipinic acids isolated bear similar relations (as to specific activity) to the starting lignins as the same acids produced from artificial radioactive lignin.



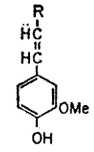
(XI)
[^{14}C] Phenylalanine



(XII)
R, R′ = H, veratric acid

(XIII)
R = COOH, R′ = H, isohemipinic acid

(XIV)
R = H, R′ = COOH, metahemipinic acid



(XV)
R = CHO, coniferaldehyde

(XVI)
R = COOH, ferulic acid

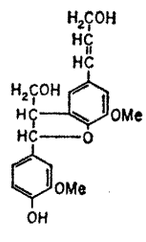
These are only two of many examples that affirm the general coincidence of artificial lignin with milled-wood lignin from spruce; infrared spectra also support these findings (*29*).

Intermediates of Lignin Formation

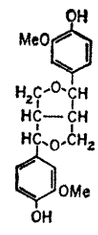
Since the starting materials and the products are the same in both the natural and artificial lignification, the mechanism of synthesis and the nature of the intermediates participating also must be similar. Many of these intermediates can be obtained from the reaction mixture by interrupting the enzymic dehydrogenation. The majority have been isolated, and their structures have been elucidated. These are the structural elements not only of artificial but also of natural lignin and therefore give information on the structure and on the mode of synthesis in each case. These intermediates are designated lignols (*30*). Whereas the constitutions of polysaccharides and proteins are derived from knowledge of the oligomeric fission products formed from them by degradation reactions, mainly hydrolyses, the constitution of lignin is inferred chiefly from the structures of the oligolignols formed during its synthesis.

For simplification, I shall consider only coniferyl alcohol. On dehydrogenation, coniferyl alcohol is converted into the free phenoxyl radical R_a (VII), which is in mesomeric association with the forms R_b (VIII), R_c (IX), and R_d (X) (*17*). This mesomeric radical can undergo hydrogen migration, and to a small extent a second dehydrogenation, to produce coniferaldehyde (XV) and thence ferulic acid (XVI), both of which can form analogous radicals. The half-life of the radicals R_a–R_d at 20°C in a mixture of dioxan and water (1:1 by volume) is about 45 seconds, measured by electron spin resonance (*11, 20a*).

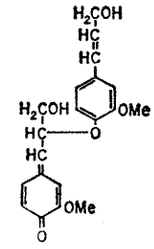
These free radicals combine with each other to form dimers that we call dilignols. These are originally all *p*- or *o*-quinone methides which have a half-life at 20°C in a mixture of dioxan and water (1:1 by volume) of about 1 hour (*7, 31*); most frequently they become stabilized by intramolecular transfer of a hydrogen from a hydroxyl group. So far 11 dilignols have been isolated and identified (*2, 3*), the most important being dehydrodiconiferyl alcohol [$R_b + R_c$] (XVII), pinoresinol (2 × R_b, XVIII), and the quinone methide [$R_a + R_b$] (XIX). The quinone methide (XIX) is unstable and cannot become stabilized by intramolecular rearrangement. Hence it adds the elements of water or other dissociated hydoxyl compounds. Addition of water produces guaiacylglycerol β-coniferyl ether (XX); this, together with the two dilignols (XVII) and (XVIII), makes up the bulk of the low-molecular-weight intermediates. By combination of two R_c radicals, dehydro-bis-coniferyl alcohol (XXI) is formed (*31a*).



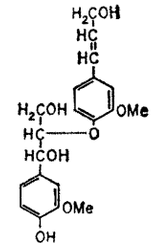
(XVII)
Dehydrodiconiferyl alcohol



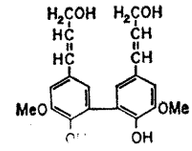
(XVIII)
Pinoresinol



(XIX)
Quinone methide



(XX)
Guaiacylglycerol-β-coniferyl ether



(XXI)
Dehydro-bis-coniferyl alcohol

All dilignols are also phenols and can undergo enzymic dehydrogenation again. If such a free radical formed reacts in its aroxyl form with an R_b radical (VIII), a terminal *p*-quinone methide is formed which adds water. In this way the dilignols (XVII), (XVIII), and (XX) give rise to three trilignols, which have all been encountered among the six trilignols isolated and identified so far; guaiacylglycerol-β-pinoresinol ether (XXII) is a typical example. The alternative mesomeric form of the free radical formed by dehydrogenation of the dilignols is akin to the radical R_c (IX); these too react further immedi-

ately, for example, by intercombination. In this way, for instance, coniferyl alcohol yields dehydro-bis-coniferyl alcohol (XXI), and the dilignol pinoresinol (XVIII) correspondingly yields the tetralignol dehydrodipinoresinol (*32*).

Lignin Growth Mechanisms

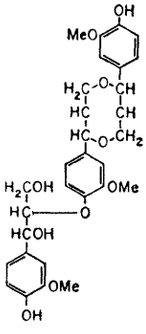
The first mode of molecular growth during lignification is that just described, namely reiterating phenol dehydrogenations. In a second growth mechanism, phenols are added to quinone methides without further loss of hydrogen (*33*). The simplest example is the addition of coniferyl alcohol to the dimeric quinone methide (XIX), resulting in the formation of the trilignol guaiacylglycerol-β,γ-bis-coniferyl ether (XXIII). A tetralignol of this series is guaiacylglycerol-β-coniferyl-γ-dehydrodiconiferyl diether (XXIV). Both of these substances have been isolated and characterized (*33*). The same applies to a pentalignol (*34*) formed by the addition of one of the trilignols to the quinone methide (XIX) and to a hexalignol (*34*) produced by addition of the tetralignol (XXIV) to the quinone methide (XIX). Thus far, more than 20 oligolignols of the coniferyl series have been isolated and elucidated.

The second growth mechanism, that is, the addition of phenols to the quinone methides, occurs in competition with a third mechanism, namely the polymerization of the *p*-quinone methides. This polymerization (*35*) proceeds with extreme ease and without external influences, and results in the formation of chains of benzyl-aryl ethers (XXV). In a fourth growth mechanism, these γ-aryl ethers rearrange (*35, 36*) to form diphenylmethane derivatives. The bond between the γ-carbon of one unit and the phenolic oxygen of the next moves over to effect condensation between the γ-carbon of the first unit and carbon No. 5 in the ring of the second, with regeneration of the phenolic group of the second unit to give structure (XXVI). This condensation can also occur to a lesser extent with carbon No. 6, and to an even lesser extent with carbon No. 2 of the ring. This rearrangement occurs both with polymers such as (XXV) and with simple guaiacylglycerol-γ-aryl ethers such as (XXII), (XXIII), and (XXIV) (*3, 4, 36*).

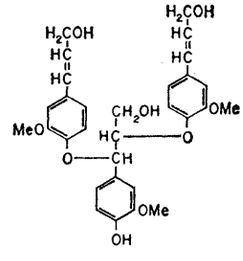
The quinone methides can add not only water and phenols, but also the hydroxyl groups of sugars (*7, 8*), the addition products being easily hydrolyzable *p*-hydroxybenzyl ethers of the sugars. The adducts can be dehydrogenated at the phenolic hydroxyl group and are thus incorporated into growing lignin molecules [for example (XXVII)]. In this way, lignin becomes chemically bonded to the polysaccharides in wood, thus giving rise to a graft polymer.

Recently H. Nimz (*20, 20a*) applied mild hydrolysis to beech lignin. In addition to coniferyl alcohol, coniferaldehyde, sinapyl alcohol, and sinapaldehyde, he obtained 4-hydroxy-3,5-dimethoxyphenylglycerol (XXIX), syringaresinol (dimethoxypinoresinol) (XXX), and 1,2-bis-(4-hydroxy-3,5-dimethoxyphenyl)-propan-1,3-diol (XXXI), all in crystalline form.

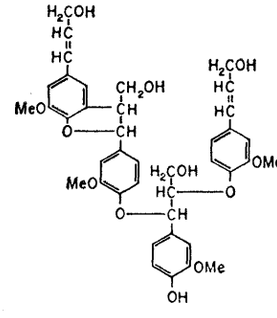
The formation of (XXXI) can only be explained by combination of the radicals (XXXII) and (XXXIII), with simultaneous elimination of the side chain of (XXXIII). This and recent findings (*20, 20a, 27*) with radioactive coniferyl alcohol are indications of the occurrence of a *p*-quinonoid mesomeric form of the radical such as R_a (X) or (XXXIII).



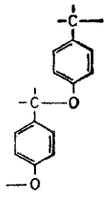
(XXII)
Guaiacylglycerol-β-pinoresinol ether



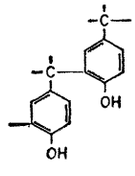
(XXIII)
Guaiacylglycerol-β, γ-bis-coniferyl ether



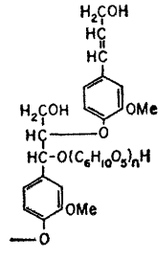
(XXIV)
Guaiacylglycerol-β-coniferyl-γ-dehydrodiconiferyl diether



(XXV)
Polymer from quinone methide

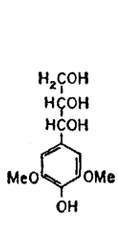


(XXVI)
Rearrangement product of (XXV) (C-γ to C-5 condensation)

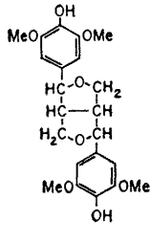


(XXVII)
Ether of a polysaccharide

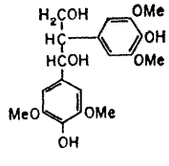
(XXVIII)
Thioglycolic acid



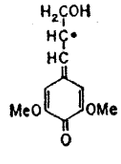
(XXIX)
4-Hydroxy-3,5-dimethoxyphenylglycerol



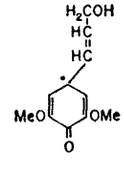
(XXX)
Syringaresinol



(XXXI)
1,2-bis-(4-Hydroxy-3,5-dimethoxyphenyl)-propan-1,3-diol



(XXXII)
Radical corresponding to R_b



(XXXIII)
Radical corresponding to R_a

Constitutional Model for Spruce Lignin

Attempts have been made (*3, 4, 37*) to utilize the intermediates and the knowledge derived from them, together with analytical data from lignin research, to design a structural formula for lignin such as that shown here (XXXIV), which is made up of 18 (or 25) units interlinked in a fashion corre-

Table 1. Comparison of reactions of lignin with predictions by the model presented.

	Finding/ estimate	Model prediction
Units able to undergo thioglycolic acid reaction		
γ-Ether, γ-carbinol, phenylethylene, carbonyl (twice)	0.8	1.0
Lignosulfonic acid formation		
Group X } A (*40*)	.15	0.18
Group Z }	.15	.18
Group B	.30	.24
Reaction with methanol (0.5 percent HCl, 20°C)	.62	.78
Similar reaction with methanol after treatment with $NaBH_4$	.42	.29

sponding to the biochemical growth of the naturally occurring lignin molecule. Such an attempt automatically leads to a step-by-step construction, for example, of a hexalignol (units 1–6) linked to a trilignol (units 7–9), to which a single unit (unit 10) is attached. In this way a decalignol (units 1–10) is formed, which is dehydrogenated at its terminal phenolic group to give an aroxyl radical. The aroxyl radical reacts with an R_h radical [(VIII), unit 11] to give a terminal quinone methide grouping, which in turn adds another preformed polylignol such as the heptalignol (units 12–18). This illustrates how a single unit in its R_h form (unit 11) is capable of welding a preformed decalignol and a heptalignol together to give an octadecalignol. Such a process doubtlessly also occurrs in nature: small and medium-sized aggregates are "glued" together by R_h radicals to form larger entities. The sequence of the individual units in lignin is fortuitous, for they are not molded like proteins on a matrix. The activity of the enzymes is restricted to dehydrogenation of the phenolic groups and is therefore sterically nonspecific. All the lignols, the artificial and natural lignins, and the lignin degradation products are optically inactive.

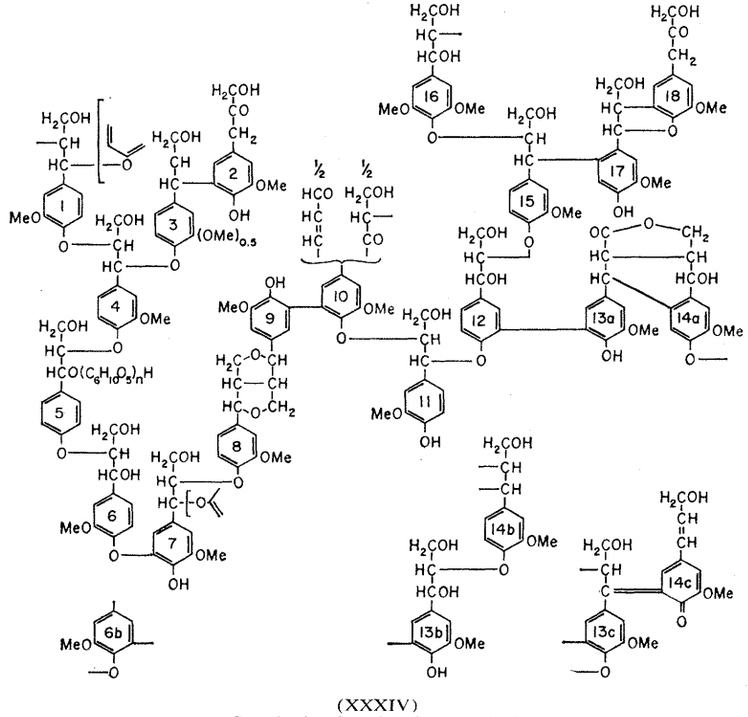
Apart from the biogenetic aspects, which formed the major criterion in constructing the model, numerous other data from lignin chemistry are incorporated into the draft of the lignin formula shown. These data include the elemental composition of spruce lignin, the number and type of hydroxyl groups, the mutual ratios of the three basic alcohols (I)–(III), the carbonyl-group content, the amounts of the three most important dilignols (XVII), (XVIII), and (XX), the condensations due to rearrangements of guaiacylglycerol γ-aryl ethers (XXVI), and some structural elements inferred from the degradation acids.

While the model was being evolved, it was recognized that it is possible to express all the data cited in a model of reasonable dimensions and that the degree of randomness is by no means as large as expected, once a start has been made from one end of the molecule.

Appraisal of the Schematic Formula

In addition to finding that all the data mentioned could be expressed in the structural model, it was found that the scheme contained a quantity of further information about lignin that had not been included a priori during construction of the formula. In other words, if this additional information had not already been available, the model would have predicted it. The first main point to notice is that, of the 18 (or 25) units, only two (units 1 and 4) have the same structures. Ether linkages (noncyclic, except methoxyl) are present in 11.5 units (0.64 per unit) and predominate over cyclic ether linkages (0.17 per unit). The 0.64 value had not been incorporated intentionally; it resulted automatically. The scheme also shows that only a very small fraction of the oligolignols linked at the periphery as γ-aryl ethers can be freed by mild degradation reactions, for the major proportion is condensed by C—C bonds or strong ether bonds. By this method so far small amounts of vanillin, coniferyl alcohol (II), coniferaldehyde (XV), dehydrodiconiferyl alcohol (XVII) (*11*), pinoresinol (XVIII) (*11*), and a derivative of guaiacylglycerol-β-coniferyl ether (XX) (*20*) have been isolated, by hydrolysis, in crystalline form from extracted spruce wood. In order to understand this from a constitutional model, the latter would have to be enlarged to several hundred units. Acids, on the other hand, cause preferential rearrangement of these ethers to yield the structure in which the γ-carbon of the side chain is condensed with carbon No. 5 of the ring (for example, units 2 and 3). The number of units that can react with thioglycolic acid (XXVIII) (*38*) is correctly represented in the scheme; the same applies to reaction with sulfurous acid (Table 1).

According to Erdtman, Lindgren, and Mikawa (*39*) the reaction of lignin with sulfurous acid proceeds in different stages called *Z*, *A*, and *B*. The structural background of these stages is fairly well known. The difference is due to open or etherified phenylhydroxyl of benzylhydroxyl. These stages



(XXXIV)
Constitutional model of spruce lignin

Table 2. Comparison of known characteristics of lignin with predictions by the model presented.

Characteristic	Finding/ estimate	Model prediction
Condensations at C-5	0.45	0.43
Condensations at C-6	.08	.06
Condensations at C-2	.04	
Units participating in biphenyl bonds	.25	.25
Units able to form Hibbert ketones	.1	.3
Units able to form vanillin	.25 to .28	.30

can be discerned approximately from the scheme (*40*). The same applies to the uptake of methanol at 20°C with 0.5 percent HCl before and after treatment with sodium borohydride. The number of biphenylyl linkages, such as that in dehydro-bis-coniferyl alcohol (XXI), agrees in quantity with Pew's estimate from optical measurements (*41*). The number of bonds in which the carbon No. 5 participates turns out to be 0.43 per unit in the model (unit 17 excluded) (Table 2).

Experiments with 5-deuteroconiferyl alcohol indicated 0.45 substitutions per unit at carbon No. 5 (*42*). The units in the model that are capable of yielding so-called Hibbert's ketones on ethanolysis are more than sufficient to cover the yields actually obtained from lignin. The same applies to the units capable of yielding vanillin on oxidation of lignin with nitrobenzene and alkali at elevated temperatures. The number of aromatic and similar protons detected by nuclear magnetic resonance spectroscopy (*43*) (2.5 per unit) turns out to be 2.7 in the model. The number of aliphatic protons in the side chain [about four for lignin (*43*)] turns out to be 3.8 in the scheme.

Groupings such as that in the combination between units 2 and 3 are readily dehydrogenated to give colored quinonoid products; these could explain part of the carbonyl content of lignin (unit 14*c*). Thus, there appears to be no certain known fact regarding lignin that cannot be explained by reference to the scheme.

A formula containing only 18 or 25 units and representing a fraction of a lignin molecule can be regarded only as an approximation, no matter how good a one. That is why some alternative structures have been included beside the main chain; these alternative configurations have been taken into account in making the aforesaid calculations of the figures given above. Any scheme is subject to improvements and amendments, but, by and large, that proposed appears to approach reality.

Summary

The conversion of *p*-hydroxycinnamyl alcohols and their glucosides into lignin is illustrated in vitro and in vivo for spruce.

Dehydrogenation of the free phenolic groups of the alcohols leads to free radicals, which are detectable by electron paramagnetic spin resonance. The radicals combine to form quinone methides that become stabilized by intramolecular prototropy or condensation with water, other phenols, or carbohydrates. The stabilized products are again phenols which in turn undergo dehydrogenation to form radicals and quinone methides. Over 20 intermediates of such reactions (di- to hexalignols from coniferyl alcohol) have been isolated and characterized. The structures of these oligolignols indicate the various mechanisms by which the lignin molecule grows. Knowledge of these aspects of spruce lignin has been applied to construct a structural formula for a fragment of a lignin molecule made up of 18 (with variants, 25) C_9 units.

Numerous data on lignin can be built into such a schematic formula without conflict with the rules pertaining to the biogenesis of lignin. Moreover, numerous details of lignin chemistry that were not composed into the scheme are satisfactorily reflected by it. By and large, the scheme comes close to reality.

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