Recent Developments in Lignin Chemistry

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Contents

A. The scope of this article

Work on lignin has now been going on for one-and-a-quarter centuries, and consequently the amount of information available on this natural material is now immense. The powerful new techniques of organic and physical chemistry and biochemistry introduced since the second world war that have brought so much progress in every other field of science have made their mark in lignin research too. For economic and academic reasons that will become apparent below, interest in lignin has been increasing of late; several hundred articles related directly or indirectly to lignin and involving almost every discipline of science appear annually in general or specialized scientific journals throughout the world.

Almost every year, international symposia on wood or lignin chemistry are held in some part of the globe and the transactions recorded in journals or in book form. Recent meetings include those at Atlantic City

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in 1965[1], Grenoble in 1964 [cf. (*10*)], Toronto and London[2] in 1963, Harvard Forest [cf. (*153*)] and Eberswalde [cf. (*106*)] in 1962, Helsinki and Montreal[3] in 1961, Seattle in 1960, and Vienna in 1958 [cf. (*94*)]. In addition, many meetings are also held at a national level, e.g. the annual meetings of the Trade Association of the Pulp and Paper Industries (Tappi) in the USA.

Progress in the lignin field is so rapid and so broad that some of the opinions or data given in older or even relatively recent publications or reviews are now outdated or known to be erroneous. Moreover, for lack of sufficient experimental facts, there is still some controversy about certain aspects of ligninology. It is therefore clear that any short review of lignin chemistry cannot give anything approaching complete coverage or criticism of the subject or convey a complete picture.

Many outstanding reviews (*1–3, 23, 24, 33, 41–46, 80, 91–93, 118, 120, 121, 129*) and books on lignin (*10, 20, 21, 94, 98, 132, 152, 153*) have appeared within the last decade and several other monographs or surveys on the subject are at present in preparation or projected. Because the field has become so expansive, the tendency has arisen for authors to review only their own special aspect of lignin research.

In view of these general developments, the following article is not intended to give an exhaustive treatment of research on lignin, but rather to give a somewhat superficial general picture of the present status of knowledge in this field and to indicate what sort of problems are being worked on at the moment. It would be futile to attempt to give a complete list of references; instead, an endeavor has been made to give the first and some recent leading references to each phase of the subject and to indicate the typical kinds of investigations that are currently being carried out in the principles centres of lignin research. It is hoped that this approach will provide openings for the interested but uninitiated reader to trace his way back into more specific literature on the topic of his concern.

If in this article too the work of the Heidelberg school appears to feature as a main theme, this is merely the result of the author's greater familiarity with these aspects of lignin and is not intended to emphasize the importance of this work or to neglect or deprecate the valuable contributions that have been made from different approaches by other groups, for our current knowledge of lignins has been the outcome of an

[1] The transactions of the Atlantic City "Symposium on Lignin Reactions" will appear in the Advances in Chemistry Series in 1966.

[2] The papers on the "Degradation of Lignin in Geological Environments" appeared in a special issue of Geochimica et Cosmochimica Acta.

[3] The lectures presented at the IUPAC meeting in Montreal were published in the Journal of Pure and Applied Chemistry.

abundance of collateral effort. The chemical mechanistic rather than the biochemical side of the process of lignification has been stressed. Nonetheless, the whole may appear to be more of a review of reviews than a straight review of original papers.

Almost comprehensive and largely unbiased references to original publications and patents in lignin chemistry up until about 1959 can be found in the books by *Brauns* (and wife) (*20, 21*); more recent papers are listed almost in full in the annual reviews of lignin by *Pearl* (*120, 121*).

B. The nature and importance of lignin

The chemistry of lignin is an important aspect of the chemistry of wood; in fact, one might go so far as to say it is the chemistry of wood, for no plant cell or tissue can be described as being woody unless it contains lignin, a lot of lignin. The word "lignin" itself, coined by the French chemist *Anselme Payen* in 1839 to describe this material that makes wood what it is, finds its etymology in the neuter Latin noun "lignum" meaning simply "wood". It is lignin, in close association with cellulose, which confers upon the tissues of plants the properties which we associate essentially with wood: its hardness and rigidity, coupled with elasticity, and even its basic color and appearance. Even more characteristic properties of wood are due to its lignin content. Wood craftsmen from the artisans and boatbuilders of old to the modern furniture designer have known and applied the facts that wood softens on steaming and retains on recooling any new form into which it has been constrained while still warm and pliant. The explanation for this phenomenon is to be found in the thermoplasticity of lignin: lignin is merely softened by the mild heat and can be remolded into a new shape which it maintains on cooling because it resets to its original hardness. Similar plasticity is also obtained with liquid ammonia (*133*).

However, lignin is not only present in materials that are conventionally regarded as wood in the everyday sense of the word; it also occurs in reeds and in the stalks of grasses, grain cereal plants, and ferns. Bamboos, sugar canes, and straws contain large amounts of lignin, although only bamboo is used as a constructional material because of its "woody" properties. Even root crops such as beets, turnips, and radishes, vegetables such as asparagus shoots, or fruit such as pears can have the undesireable properties of being "woody". This is due to the formation of lignified "stone cells". Stone cells also occur in the barks of trees [cf. (*97*)].

However, it is not only considerations such as these that have aroused the interest of scientists in lignin, but much more practical matters. The vast amount of lignin that is produced annually in the pulp and paper industry of the world in the form of lignosulfonates makes it a material of astounding potential economic importance. Currently almost 75 million tons of wood pulp are produced annually in the world, hence about 40–50 million tons of "lignin" are simultaneously produced as a by-product. This is mainly in the form of sulfonated derivatives polluted with carbohydrate residues. This is more of a bane than a boon to industry, for the lignosulfonates discharged from pulping plants still represent a waste product that presents immense disposal problems.

Because lignin cannot be used, it must be disposed of as safely and economically as possible. It is now no longer permissibly to merely "throw it away" by releasing the effluent from pulping mills into the local waterways. Lignin contains much carbon and hydrogen, and has thus a high oxygen requirement for biodegradation. Even if lignosulfonate solutions are adjusted to a physiological pH before dumping into rivers, the biological oxidation of the lignin proceeds extremely slowly, especially when it is chemically altered by pulping processes. It therefore acts as a sort of cumulative poison, and repeated additions of even relatively small amounts exhaust the oxygen content in rivers and streams and hence asphyxiate not only all fish but also all water insect and aerobic microbial life. The legislature in most industrialized – and hence densely populated – countries prohibits water pollution of this kind, hence the pulp manufacturer has to resort to other methods to dispose of his unwanted lignosulfonates. Nowadays, for example, the bulk of the lignosulfonates is precipitated from the liquors discharged from the pulping digesters by addition of lime to pH 10.5–12.2 and filtered off and converted into neutral sodium or magnesium lignosulfonate by treatment with sulfuric acid plus sodium or magnesium sulfate – provided some market is available for the product. Some of the few uses to which lignin can be put will be mentioned later. Normally, however, the concentrated liquor is merely evaporated to dryness and burnt, e.g. in a Tomlinson furnace, the heat produced being used to evaporate further batches of liquor and to make electricity and steam for the energy requirements of the pulping plant. The chemicals required for the pulping cook are largely recovered in the combustion residues. Both of these approaches are far from ideal, for in both, apart from the technical difficulties involved, residual solutions are obtained that are too dilute to warrant economical processing. Such dilute residues must still be disposed of and this means extra costs and inconveniences for pulping firms.

Lignin thus represents one of the greatest enigmas of applied chemical research: despite over a century of study, it is still impossible to apply more than a trifling fraction of the world output of lignosulfonates for useful purposes. The man of perspicacity who discovers a useful application for lignin has the prospect of agglomerating more money from it than Andrew Carnegie did from steel or Alfred Nobel from dynamite.

The main reason that a useful outlet for lignin could not be found was that far too little was known about the chemical structure and properties of the unmodified, natural material. The ordinary methods used for isolating and identifying natural products all failed when applied to lignin, but it is only looking back in retrospect from the present-day knowledge of lignin that the reasons for this state of affairs can be recognized. Lignin is a high polymer of a most unusual type. The native molecule is apparently completely amorphous and is highly branched. In addition, it is a graft polymer in intimate physical admixture and chemical combination with the cellulose and hemicelluloses in plants. In this respect the function of lignin in the plant cell can be compared with that of the polyester resins used in making boats, car bodies, or gliders from fiberglass. The network of cellulose fibres in the plant cell wall is comparable with the glass-fibre textile which is modelled into the desired shape (matrix) before being impregnated with the polyester in order to solidify it. Similarly lignin stops up the spaces between the cellulose fibrils and solidifies the structure of the plant cell. Moreover, in the plant cell the lignin molecule probably intertwines among the cellulose fibrils like a climbing plant in a trelliswork. Just as many such climbing plants attach themselves to the trellis frame with tendrils, so lignin adheres to the cellulose framework with its chemical bonds. Some of the lignin molecules may later become stretched out under slight tension between the cellulose fibrils owing to these bonds when the cell stretches. In other words, the lignin molecule may perhaps become slightly orientated in the plant. The lignin would then no longer merely stop up the spaces between the cellulose fibres like the polyester resin in fiberglass but would play a structural role more similar to that of honeycomb paper reinforcement between laminated board. The intrinsic dichroism of lignin in cell walls (*63*), although thought to be due merely to form anisotropy of the lignin, might perhaps be interpreted as being caused in part by orientation of this kind.

No matter what its state is in the cell wall *in vivo*, when the lignin molecule is released from its attachment to the cellulose matrix, either by breaking its tendril-like bonds (e.g. by pulping) or destruction of the cellulose matrix (e.g. by hydrolysis with acids or enzymes), it rolls up to a globular entangled mass something like tumbleweeds.

This situation makes lignin extremely difficult to isolate from plants for purposes of chemical investigation. The normal physicochemical methods used for separating other natural polymers from the mixtures in which they occur fail when applied to lignin, owing to its intimate admixture and chemical combination with the plant polysaccharides. If chemical methods are applied to split the bonds between the carbohydrates and the lignin, the latter undergoes drastic changes which alter its structure and properties, e.g. its solubility is greatly reduced and its thermoplasticity disappears. However, this is not the main difficulty encountered in trying to discover what lignin is. Even when isolated, unlike other natural polymers, lignin cannot be broken down by hydrolysis into smaller units which are easier to identify. Its molecule does not contain a relatively weak bond at periodic intervals. Polysaccharides are generally made up of one or two simple sugars that are joined up together by anhydro bonds which can be split again by hydrolysis with chemical reagents or enzymes. Proteins are made up of a larger number of different monomer units, the 18 natural amino acids, but these are all linked together by one type of bond, the peptide bond, which can again be broken by hydrolysis with acids or enzymes. It is now known that lignin is also made up of only two or three extremely similar monomers but these are joined together in such a variety of ways that efficient degradation of the molecule cannot be achieved simply by hydrolysis.

These complications proved to be the greatest deterrents to identification of the structure of lignin. Without knowledge of the make-up of lignin, it is clear that little promise could be expected from empirical studies directed at finding useful applications for it. In view of this situation, there has been a great intensification in fundamental research on the structure and properties of lignin in the hope that a better scientific foundation could be laid for future applied research.

Completely new methods of approach, especially biochemical methods, had to be adopted, but these have enjoyed a large measure of success over the past twenty years. However, the picture is still not entirely complete and rapid progress is still being made.

C. Isolation of lignin

It is extremely difficult to extract lignin from wood. The reason for this is not merely that it is so intimately mixed with cellulose or even encased in cellulose membranes like starch granules. The reverse is truer: the cellulose is embedded in a paste of lignin. The lignin is actually attached to the polysaccharides in plants by chemical bonds. Evidence for such lignin-carbohydrate bonds up to 1960 has been reviewed by *Mere-*

wether (*110*). The linkages in question may hydrogen bonds, acetal (*19*) or ester groupings, and ether linkages. Hence any method for the separation of lignin from plant polysaccharides (cellulose, hemicelluloses) must involve cleavage of these lignin-carbohydrate bonds. The greater the number of such bonds that are broken, the better the separation of the lignin and carbohydrates. Most methods that provide efficient cleavage of these bonds unfortunately cause vast alterations or degradations of the lignin and/or carbohydrates. Milder methods liberate only minute amounts of the separate components and even these fractions are already partially degraded.

In pulping processes (*26, 115, 127, 128, 138, 150, 151*), large yields of gnin-free but undegraded cellulose fibres are desired. Pulping strivesli to achieve this aim by *delignification*. The quality of the pulp and of the paper made from it – newsprint, book, tissue, or paperboard – depends to a large extent on its residual lignin content, and a comprise must be made between the amount of fiber liberation and the purity of the cellulose (*141*). The yield of pulp can constitute 30–90 % of the dry weight of the wood, and its lignin and hemicellulose content varies accordingly. Groundwood pulps are made by merely grinding white softwoods with a rotating stone and contain almost all of the lignin in the original wood. When mixed with better quality chemical pulps, these are used for newsprint because of their cheapness and suitability for fast printing. The fast absorption of printing ink, the low wet strength, and the yellowing in sunlight of newsprint are all probably results of its high lignin content. In semichemical pulping, a little lignin is removed from wood chips by the methods used for chemical pulping (see below) before the cellulose fibres are released mechanically. The resultant pulps are of slightly higher grade and can be used for boards, newsprint, magazine and tissue paper. Darker softwoods, hardwoods, and even straw can be processed in this way.

In chemical pulping, wood chips are treated in one or more stages with reagents in solution that are designed to remove some or most of the lignin from the wood, generally in an altered form. Heat and pressure accelerate the reactions involved and aid penetration of the chemicals into the wood. Most of the reactions used are unfortunately of low selectivity: the removal of lignin is incomplete and degradation of the plant fibres occurs.

Lignin contains phenolic and a few carboxylic acid groups; it therefore dissolves in sodium hydroxide. Soda pulping is based on this fact. Here, at the high temperatures used, ester bonds are hydrolyzed, and some of the carbohydrate-lignin ether bonds are split. Cold caustic soda is used for semichemical processes.

The quality and yield of pulp is improved by inclusion of certain sulfur compounds in the cooking liquors used for pulping. In the kraft process (*151*), sodium sulfide is added. This process is sometimes called sulfate pulping because sulfate is reduced to sulfide under the conditions used and produces the same effect. The sulfide helps to break ether bonds between cellulose fibers and lignin and within the lignin molecule itself. The ungrafted, partially degraded lignin molecules then dissolve more readily in the alkali. Sulfite and bisulfite pulping (*150*) involves cooking of wood chips with sulfurous acid or magnesium or calcium sulfite or bisulfite and depends upon cleavage of the same ether bonds with conversion of the liberated lignin into lignosulfonates which dissolve in water or the alkaline cooking liquor.

It is the lignin derived from these processes that presents the current problems in disposal or economical utilization that have boosted the interest in lignin. Because of the residues of lignin left in the pulps even after chemical pulping, the pulps have to be bleached, e.g. with chlorine, hypochlorite, chlorine dioxide, or hydrogen peroxide. Intermittent extraction with alkali may even remove more lignin during bleaching. Attempts have even been made to evolve pulping processes based on oxidative degradation of lignin with Cl_2, ClO_2, or HNO_3 and subsequent extraction of the wood with alkali. Despite the use of cheap nitric acid and extraction of the nitrolignin with ammonia for use as a fertilizer, even this method has had only minor economic success to date.

Even the mildest pulping process, hydrotropic extraction, i.e. repeated extraction with concentrated aqueous solutions of organophilic electrolytes such as sodium xylenesulfonate or cymenesulfonate, has failed because of an outlet for the extracted lignin, which is precipitated by mere dilution of the solution. Other methods based on hydrolysis of the wood with acids, e.g. HCl, HNO_3, $AlCl_3$, acetic acid, acetyl chloride, SO_2, or phenol, are merely of laboratory interest.

Methods for the *extraction of lignin* from wood are invariably based upon destruction of its polysaccharides and are intended to leave a residue of lignin for chemical investigation or quantitative assay. Short reviews of these methods are available (*20*, *21*, *45*, *132*). The methods that ensure complete breakdown of the cellulose, e.g. hydrolysis with 72 % sulfuric acid (Klason lignin) or cold fuming hydrochloric acid (Willstätter lignin), or oxidation with sodium periodate (Purves periodate lignin), defeat their own purpose by altering the lignin beyond recognition by self-condensation or oxidation. Even milder treatment with dioxan/HCl in the cold (Freudenberg dioxan lignin) or hot (Pepper dioxan lignin) or thioglycolic acid plus an acidic catalyst (Holmberg thioglycolic acid lignin) yield modified lignins that also contain chlorine or carboxy-

methylthio groupings. Clever attempts to remove the lignin by dissolution of the plant cellulose with cuprammonium hydroxide after hydrolysis of the hemicelluloses with dilute sulfuric acid (Freudenberg's cuproxam lignin) or to digest the cellulose with microorganisms (brown rots) which contain cellulases (Nord's enzymatically liberated lignins) also proved ineffective. Extensive degradation of the lignin occurred by oxidation owing to the presence of the copper ions or oxidases, and the removal of cellulose was incomplete. Extraction of wood with neutral organic solvents removes a small amount of lignin (Brauns' soluble lignin) together with other extraneous phenolic materials (lignans, etc.) (*46, 71*). This lignin is obviously not attached to polysaccharides and is of relatively low molecular weight (*33*); it must be a mixture of the end fractions of the polymerization process that leads to lignin, i.e. molecules that are still at an early stage in the growth process or fractions of mature molecules that have already begun to decompose by enzymatic overoxidation of the lignin (see Section J).

Much of the preliminary information on lignin structure was obtained with these preparations, so they have nevertheless been of great value in lignin research. A relatively unchanged lignin preparation has now become available, however, and has gradually ousted all of the above preparations for research purposes. When cellulose is ground in a high-speed vibrating ball-mill, its fibers are extensively degraded because the high mechanical energies cause rupture within the crystalline regions of the cellulose chains. This fact was subtly applied by *Björkman* (*17*) to release lignin from wood. Dry wood meal was ground in a non-polar medium, e.g. toluene, to prevent swelling of the fibres. Most of the cellulose is thereby degraded because it is partially crystalline, but the spongy, amorphous lignin is largely unaltered and can then be extracted with harmless solvents without catalysts, e.g. dioxan/water. Naturally some of the lignin molecules are still attached to residues of cellulose or hemicellulose: even if the milling were complete tiny non-crystalline pieces of the polysaccharide molecules would still be left adhering to the lignin. In practice, the milling is not carried to completion and only some of the lignin is extracted. It can be freed from the material still containing carbohydrate residues (so-called lignin-carbohydrate complex, LCC) by simple polymer fractionation (*71*). The sugar-free material is termed milled-wood lignin (MWL) or Björkman lignin and contains unaltered representative portions of the lignin molecule (molecular weight ~11,000) such as it occurs in wood.

D. The location of lignin in plant cells

Knowledge of the exact distribution of lignin in the xylem cells of wood is very important for a number of reasons. For instance, it is important for pulping technology in connection with questions regarding the penetration of cooking liquors and release of undegraded cellulose fibres. Again, it determines partially the physical architecture of the plant cell. Lignin distribution also affects the mechanical properties of wood, and irregularities in the normal distribution due to the formation of reaction wood are deleterious for the use of the wood for woodworking, veneering, or even pulping purposes. Tension wood has less and compression wood has more lignin than normal wood. The lack of lustre in compression wood may be due in part to its high lignin content. The number of layers in wood tracheids is also abnormal in reaction wood.

Studies of cell morphology using physical methods not only gave information on the lignin distribution in normal and reaction wood cells, but also proved that lignin is a *genuine natural product of aromatic nature* and not an artifact as claimed even comparatively recently (*134*). Several detailed reviews of work on this topic are available (*27, 30, 62, 126, 146, 148*).

The first work aimed at finding the whereabouts of lignin in plant cells was based on the ultraviolet absorption of lignin: owing to its aromatic structures lignin absorbs ultraviolet light more strongly than any other cell component, having an absorption maximum at 280 mμ. The preliminary studies of *Bailey* (*13*) led to more directed investigations by *Lange* (*99*), who set up complex distribution curves which indicated a maximum concentration of lignin (60–90 %) at the middle lamella – a layer about 0.1 mμ thick lying between the wood tracheids. The lignin concentration fell off through the S1, S2, and S3 cell layers (~15 %) to zero in the empty xylem cell lumen. The intercellular spaces also contain high concentrations of lignin. Essentially similar results were obtained by Austrian workers (*147*) who confirmed the aromatic nature of lignin in thin wood sections using infrared spectroscopy.

In more recent approaches to cell morphology, interference microscopy, x-ray diffractional analysis, and especially electron miscroscopy have been used. Improved methods of sectioning, preparation and staining, and examination of cells in wood partially degraded by chemical procedures (e.g. with HF) or partially decayed by microorganisms have thus given indications that the organization and variation in plant cells are actually much more complex than was thought from these first experiments; this applies not only from species to species or tree to tree, but even from tissue to tissue within a given plant. It would lead too far

to discuss these matters here; the interested reader is referred to more specialized publications (*30, 62, 126, 146*).

Precise studies have been carried out by Australian workers of the process of lignification in tree cells (*148, 149*). It was hoped in this way to find out more about the nature and source of the lignin precursors in the growing plant. There is an increasing gradient in the lignin content of the cells in trees from the cambium inward to the mature xylem. This may be occasioned by centripetal diffusion of lignin precursors inward from the cambium, coupled with the degree of maturity of the cells. The results of single and double ring-barking (ring-debarking) experiments suggested however that the lignin precursor can also come from the endoplasm of the cell. Perhaps both processes are in operation in the healthy, unmodified plant.

Within the individual cells, it was observed that the front of polysaccharide synthesis and the location of the oxidase enzymes responsible for lignification (see Section G) both advance inward within the cell slightly before the front of lignification, i.e. there is a time lag between the construction of the cellulose matrix and the subsequent deposition of lignin. Lignin is first deposited in the corners of the primary cell wall where several cells adjoin and then spreads out bilaterally through the intercellular layer. The lignin stops up the spaces between the cellulose microfibrils, but some penetration of the fibrils is thought to occur. Evidence for this is also given by the fact that the higher the lignin content of any layer, the lower the degree of crystallization of the cellulose microfibrils. As the cell becomes older, and formation of the secondary and tertiary walls occurs, this process of embedding the cellulose in lignin continues, but less lignin is deposited in the inner layers.

Additional information on such topics is expected from studies of plant tissue cultures (*14, 78, 80, 89, 125, 147 a*), where lignification appears to proceed almost normally. The methoxyl content of tissue culture lignins is lower than that of natural lignins from plants of the same species but the efficient utilization of lower lignin precursors and the conversion of a syringyl-type compound to a guaiacyl-type lignin (*78*) does however suggest that here too lignification proceeds by way of endoplasmic processes and not by simple centripetal diffusion of lignin precursor into the cell. It is known that the methoxyl content of the lignin in bamboo (*79*) and spruce (*71*) increases with the maturity of the plant, so plant tissue cultures are perhaps comparable with younger tissues in the immature plant. The methoxyl content of reaction wood is also abnormal (*148*). It is perhaps interesting to note in this connection that the lignin content of plant tumors (*136*) and calluses (*16*), like that of compression wood, is greater than normal.

E. The biogenesis of lignin

Since lignin contains only carbon, hydrogen and oxygen, it must be biosynthesized by the plant from carbon dioxide and water. Lignin is definitely *an end product of the plant metabolism* and cannot be remobilized for nutritive purposes. Unlike the proteins, the structural material in animal tissues, lignin is not involved in a dynamic process of degradation and regeneration in the plant organism. Once lignin has been deposited in a plant it remains there quiescent – except perhaps for very slow minor changes – as long as the plant is alive and healthy, i.e. unattacked by microbial, fungal, or insect marauders and still actively growing. Nature has provided a means for recycling the elemental components of lignin from dead vegetation via its degradation to constituents of the humic acids in the soil (see Section J).

Although the biogenesis of lignin is a unified concerted process in the plant, it is preferable for a better understanding of the mechanisms of lignification to divide up the process into portions, i.e. to consider separate stretches of the overall course.

The lower precursors of lignin are formed by the same mechanisms as are involved in the photosynthetic formation of carbohydrates (and polysaccharides) in the plant. The first stretch of lignification can thus be considered to be the formation from atmospheric carbon dioxide of the last of a series of simple sugars from which lignin is subsequently derived. The second stretch of lignification might then be considered to be the formation from this aliphatic sugar of the last of series of simple (monomeric) aromatic compounds from which the lignin polymer is made. The third stretch is then the conversion of the ultimate aromatic lignin monomers via dimers, trimers, tetramers, ... oligomers called lignols (monolignols, dilignols, etc.) into the polymer lignin.

The first stretch of lignification is actually more closely related to photosynthesis and glycolysis than to lignification and has received less attention from lignin researchers; it will therefore be considered only briefly here in combination with work on the second stretch. It is however expedient in our discussion here to treat the work done by lignin scientists on the second and third stretches of lignification separately, for the information garnered on the second stage was procured by pure biochemical techniques whereas the data on the third stretch was the outcome of more organic chemical work.

F. Biosynthesis of lignin precursors

The routes followed by plants in the biosynthesis of lignin have been widely studied using tracer techniques, principally with radiocarbon. By comparing the specific activities of the precursors fed to plants with the

specific activity of the resultant lignin or its degradation products, some idea is gained of the efficiency of the compound administered as a lignin precursor. It can then be judged whether each precursor is an obligatory intermediate [cf. (109)] of lignification or merely a possible intermediate. If the radioactive return is low, the compound tested has been degraded by the plant and the radioactivity distributed in all the plant products, not only the lignin, starting from much simpler compounds produced by

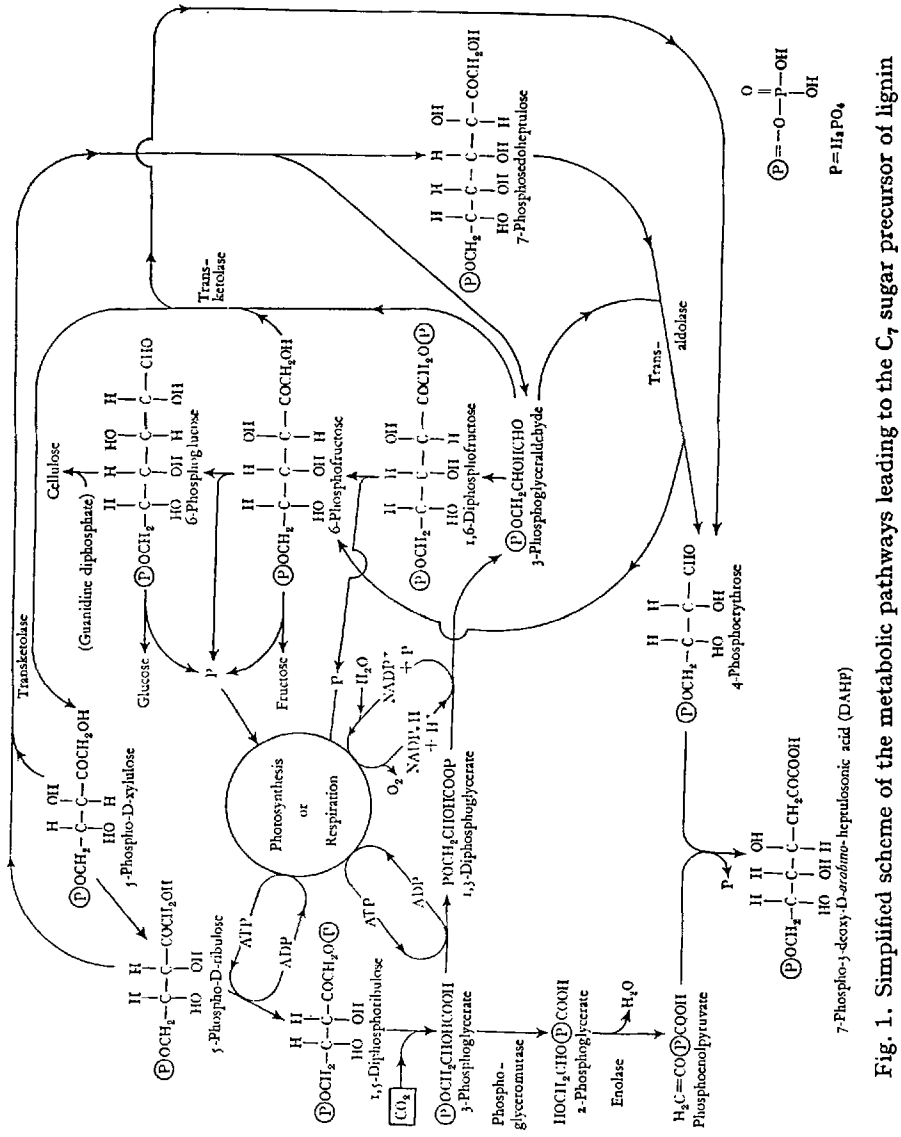
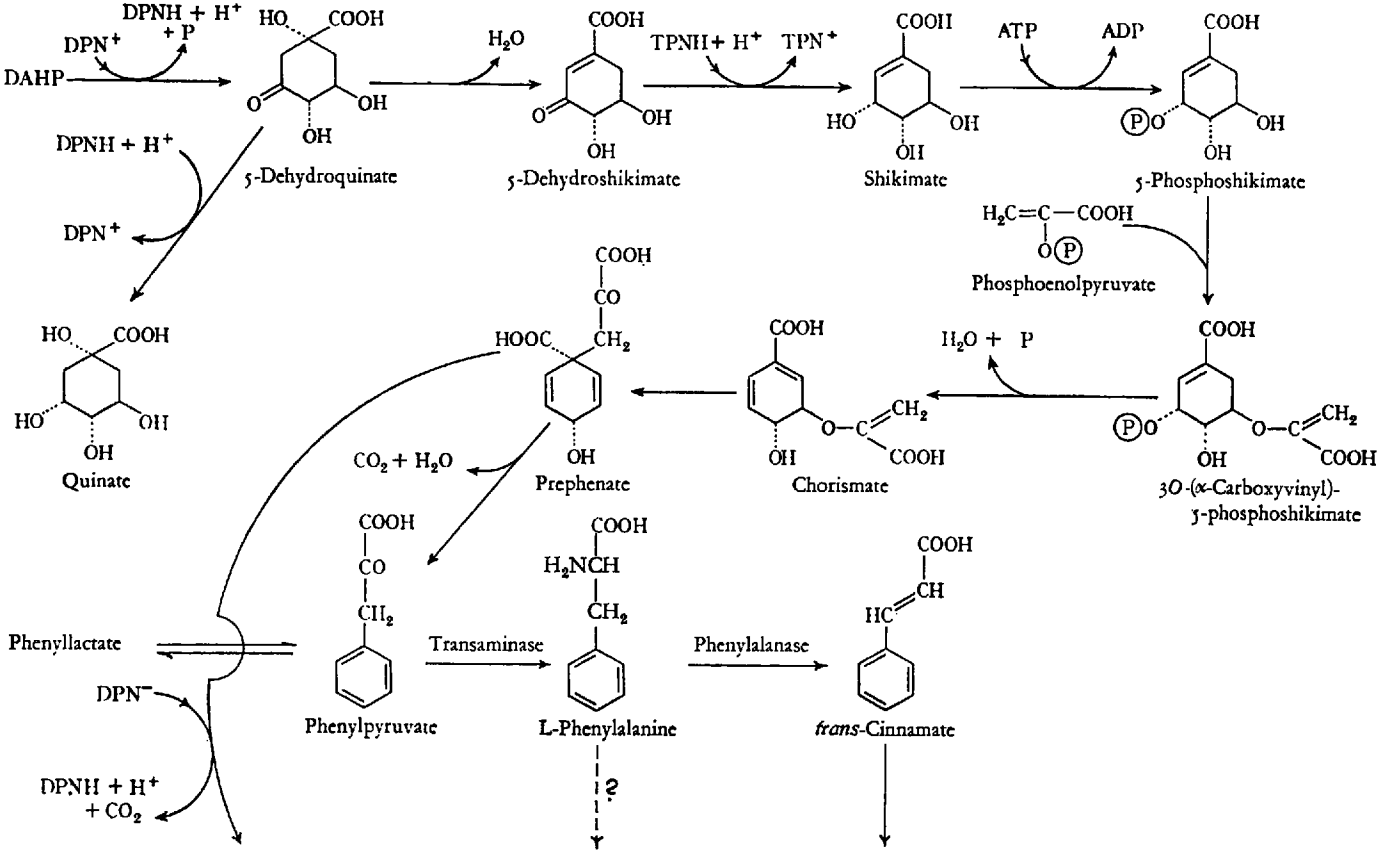


Fig. 1. Simplified scheme of the metabolic pathways leading to the C_7 sugar precursor of lignin


DPNH + H+
+ P
DPN+
DAHP
HO
COOH
O
OH
OH
5-Dehydroquinate
H2O
COOH
O
OH
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5-Dehydroshikimate
TPNH + H+
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COOH
HO
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Shikimate
ATP
ADP
COOH
PO
OH
OH
5-Phosphoshikimate
DPNH + H+
DPN+
H2C=C—COOH
O
P
Phosphoenolpyruvate
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COOH
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OH
Quinate
COOH
CO
HOOC
CH2
OH
Prephenate
CO2 + H2O
COOH
OH
O—C
CH2
COOH
Chorismate
H2O + P
COOH
PO
OH
O—C
CH2
COOH
3O-(α-Carboxyvinyl)-
5-phosphoshikimate
Phenyllactate
DPN−
DPNH + H+
+ CO2
COOH
CO
CH2
Phenylpyruvate
Transaminase
COOH
H2NCH
CH2
L-Phenylalanine
?
Phenylalanase
COOH
CH
HC
trans-Cinnamate

p-Hydroxy-phenyllactate
Transaminase
Tyrase – in grasses only
p-Hydroxyphenylpyruvate
L-Tyrosine
trans-Coumarate
trans-Caffeate
trans-Ferulate
trans-Sinapate
[S-Adenosyl-L-methionine]
[CH3]
UDP-Glucose
UDP
trans-p-Coumaryl alcohol
trans-p-Glucocoumaryl alcohol
trans-Coniferyl alcohol
D-Coniferin
trans-Sinapyl alcohol
Syringin

catabolism of the tracer. If the radioactivity yield is high, a rough clue is gained of the proximity of the precursor to the anabolic end-product lignin. Various groups have participated in work on the precursors of lignin, and again several reviews have been published (*24, 81–83, 91, 93, 112, 113, 132*). Only some of the principal points can be dealt with here.

A simplified version of the metabolic pathways that may lead from atmospheric carbon dioxide to the simple terminating sugar precursor of lignin (DAHP) is shown in Fig. 1. This primitive planar representation of some of the processes that the plant carries on in three dimensions does not of course convey any information in the complex energy or material balance or the intricate control of the processes involved in lignification. Moreover, the plant may naturally have access to more devious metabolic routes if disturbances are encountered in the more direct pathways indicated here. Some of the substances shown may be present in only extremely minute concentrations in both photosynthesizing and lignifying cells. High concentrations of any lignin precursor will only be encountered if the plant requires that particular substance as a reserve nutrient or as a convenient form for material translocation.

The second stretch of lignification, the conversion of the first non-sugar substance into the aromatic monomers ready for polymerization, has been examined more thoroughly by lignin biochemists. The pathways followed here by the plant are outline in Fig. 2. Excellent reviews of the enzymes known to be involved at each step here as well as in the polymerization at the third stretch have recently appeared (*28 a, 82*). The processes encountered in higher plants are in essence the same as those known to be in operation in the aromatization of aliphatic precursors in microorganisms following the work of *Davis* and *Sprinson* with *Escherichia coli* mutants (*32, 101*).

These preliminaries to lignification proper in the third stretch can thus be visualized somewhat as follows. The carbon dioxide absorbed in the leaves of the plant passes through the photosynthesis cycle to give glucose, fructose (and hence sucrose) and sedoheptulose. These are probably the main forms in which the plant transfers its nutrients from the zones of photosynthesis in the leaves through its vascular system to the zones of lignification in the stem, branches and trunk (*114*). In the plasma of the living cells in the regions where lignification is taking place, some of the processes shown in Fig. 1 may occur in reverse with respiration, giving rise to the C_3 and C_4 units from which the C_7 entity DAHP is formed – a change which represents entry into the one-way street of lignification. Glycolysis according to the Embdem-Meyerhof-Parnas route can give rise to phosphoenolpyruvate (C_3) directly, while erythrose phosphate (C_4) can be formed either by the pentose phosphate route via xylulose-5-

phosphate and ribulose-5-phosphate and thence to sedoheptulose-7-phosphate and glyceraldehyde phosphate or from 3-phosphoglyceraldehyde and 6-phosphofructose from glycolysis, with formation of 5-phosphoxylulose as by-product.

Some of the evidence obtained from tracer experiments that supports this scheme might be presented briefly here. In the first experiments aimed at deriving information about lignification in this way, it was found that radiocarbon dioxide was converted in part into the lignin of plants and that lignin appeared to be a metabolic end-product in the plant (*141a*). After it had been demonstrated that the shikimic acid route (Fig. 2) was in operation in higher plants (*25, 33a*) as well as in bacteria (*32, 101*), it was shown that [1-^{14}C]- and [6-^{14}C]glucose were both converted readily into lignin with little randomization of the labelled carbons (*95a, 132a*). It was recently shown that glucose, sucrose and sedoheptulose are the materials most probably translocated in the plant from the site of photosynthesis to the sites of lignification (*114*). It has even been shown that radioactive glucose is transformed into radioactive shikimic acid and thence into lignin in *Eucalyptus nitens* (*75*). Radioactive pentoses are also converted into radioactive lignin (*96a, 131*). It has even been suggested that cellulose can be remobilized to provide raw materials for pathogenetic lignification (*16*, cf. *107a*). The fact that other feasible lignin progenitors such as acetate (*33a*) or even non-phosphorylated pyruvate (*29*) are not incorporated well lends further support to the type of scheme proposed.

The study of the second stage of lignification by introducing non-sugar precursors into plants began with the administration of shikimic acid to wheat (*25*) and sugar cane (*33a*). High conversions into lignin were found and little randomization of the labels was observed when the whereabouts of the radiocarbon was traced in products of chemical degradation of the radioactive lignin.

In the meantime, all of the higher intermediates shown in Fig. 2 have been tested and numerous comprehensive surveys of this work have appeared (*24, 81–83, 91, 93, 112, 113, 132*). Some of these simultaneously describe the formation of secondary aromatic substances in wood, i.e. lignans, tannins, flavonoids, etc., which arise by essentially similar routes coupled with acetate metabolism. A few outstanding recent developments may bear repetition here.

Various groups have found that D-coniferin is converted extremely readily into lignin (*59, 91*) although it is not necessarily an obligatory intermediate of lignification. Little is known with certainty about the purpose of the β-D-glucopyranosides of the *trans-p*-coumaryl alcohols which are encountered in certain plants [cf. (*54*)], sometimes in high

concentrations. Not much is known about their distribution in the vegetable kingdom simply because enough studies to obtain information of this kind have not yet been made, but they do not appear to be ubiquitous. The glucose may be attached to the coumaryl alcohols transiently to detoxify or protect the phenolic function for transportation in the plant or to form a reserve nutrient. Although coniferin is less soluble in water (0.53 % at 20 °C) than coniferyl alcohol (~1 %), this need not be so in cell sap. Coniferin is much more soluble in sugar solutions (*71*). It is conceivable that the coniferin present so abundantly in the cambial sap of conifers is there as "iron rations" to satisfy the immediate lignification needs of the freshly produced cells on the xylem side of the cambium during the "embryonic" stages of their development. This one substance could provide coniferyl alcohol for lignification and glucose for cellulose synthesis directly in the young cell. Later lignification may proceed from precursors derived from endoplasmic sources, e.g. glucose, by the routes outlined in Figs. 1 and 2. The facts that L-coniferin is not a good lignin precursor (*59*) and that coniferin is utilized better when applied by infusion rather than by implantation (*91*) lend support to this concept. It has been observed several times that infused radioactive L-phenylalanine gives rise to radioactive *p*-glucocoumaryl alcohol (*143*) and coniferin (*58b*, *143*) in young spruce saplings; however, the syringin found is not strongly radioactive (*143*).

It has also been observed that syringyl-type precursors are transformed in part into guaiacyl-type lignin (*78*, *90*, *91*), i.e. a demethoxylation, not merely a demethylation has taken place. It has also been found that sweet almond emulsin hydrolyses syringin only extremely slowly (*143*) and that pure peroxidase does not oxidize sinapyl alcohol well (*71*). The metabolism of the sinapyl component of lignin is perhaps not quite as simple as the analogy between its *p*-coumaryl and coniferyl components suggests.

Another very important finding was the observation of the inability of species other than Gramineae to utilize tyrosine as a lignin precursor [cf. (*24*, *112*, *113*)]. This is now known to be due to the presence or absence of the enzyme tyrase [cf. (*81*, *82*)].

The Canadian school has recently shown that the production of the higher lignin precursors, i.e. the *p*-coumaryl alcohols or their glucosides, does not proceed via the simple acids shown in Fig. 2 but actually via insoluble esters of same. The esters are probably activated esters of coenzyme A, but esters of quinic acid analogous to chlorogenic acid are also feasible in this role (*35*).

First attempts have now been made using a trapping technique to trace the formation of the more advanced intermediates of lignification,

e.g. the monolignols coniferyl alcohol and coniferaldehyde (VII) and the dilignols pinoresinol (IV) and dehydrodiconiferyl alcohol (II), i.e. intermediates encountered only at the third stage of lignification (*24a*); again satisfactory incorporations of radioactivity were detected.

Several groups have identified non-radioactive metabolic intermediates of lignification in cambial sap or sapwood extracts (*54*, *58b*, *68*). The compounds detected agree well with the schemes for the biosynthesis of lignin precursors set forth in Figs. 1 and 2 and with the data described later for the third stretch of lignification.

G. The polymerization process in lignification

This is perhaps the most complicated part of the overall process of lignification, for the changes that take place in the monomer units during their polymerization to lignin are no longer of a straightforward biochemical nature. It is now known that the polymerization is initiated by a simple enzymatic phenolic dehydrogenation that leads to a vast variety of complicated chemical structures.

Since these reactions occur simultaneously in competition with each other, the resultant polymer has a highly complex primary structure. Although originally derived from very similar monomers, namely free or methoxylated *trans-p*-coumaryl alcohols, the appearance, stereochemistry, and environment of each individual unit afterwards in lignin show much greater variety. The types of bonds linking the units together are highly diverse. In the schematic formula designed by *Freudenberg* (*42*, *43*, *43a*, *54a*) to portray a representative section of a spruce lignin molecule (see Fig. 9), only two units, viz. Nos. 2 and 4, happen to be identical and identically bonded, even though a few structures of minor importance were neglected and a recently discovered major structure [cf. (XII)] (*48*, *105*, *116*, *117*) could not be included. Since lignin has no ordered primary structure – unlike proteins it is not molded to a set pattern by genetic information – reshuffling of the same or similar monomeric residues could lead fortuitously to other portions of lignin molecules with less or more randomness of structure and bonding. *Freudenberg*'s scheme was drafted from knowledge of the mechanisms and intermediates of lignification and from analytical data on lignin (*41*). The mechanisms involved in the growth of the lignin molecule were inferred from the structures of the lignols isolated from *in vitro* experiments in which lignification was simulated using coniferyl alcohol as sole progenitor.

Nevertheless, it had to be known beforehand that lignin is in fact derived from the *p*-coumaryl alcohols and this information was not in fact accrued from the biochemical work on lignin precursors. Actually

the reverse was true. The work on the structure of lignin revealed that it must have originated from the *p*-coumaryl alcohols and hence it was the task of the biochemist to elucidate how they are formed in the plant. It is therefore perhaps worthwhile to retrace a few of the results that have brought us to this knowledge of the structure of lignin.

Knowledge of the elemental composition of conifer lignin and of the abundant occurrence of coniferin in the cambial region of spruce led *Klason* (*87*) to suggest (among many other theories) that lignin was derived from coniferyl alcohol – the aglycon of coniferin – by oxidation, for lignin has slightly less hydrogen and slightly more oxygen than coniferyl alcohol. After elucidating the structure of dehydrodiisoeugenol, a compound obtained from isoeugenol by enzymatic dehydrogenation (*31*), *Holger Erdtman* saw in this a model for lignification and correctly suggested that the process involved was not merely an oxidation, but more specifically, a dehydrogenation (*36*). It was later shown that the same enzymes did in fact produce crude lignin-like polymers from coniferyl alcohol, which at that time was available only with difficulty from natural sources (*59a*). When coniferyl alcohol became more readily accessible by synthesis using complex hydrides (*8*), it became possible to study the processes involved in this polymerization. This represented not only a major breakthrough in lignin chemistry but also a novel approach to the chemistry of natural high polymers. Previously the primary structures of natural polymers had been elucidated by degrading the natural products and separating and identifying the small degradation products obtained. The structure of lignin was gradually evolved by the reverse route, namely by identifying the oligomeric intermediates of its polymerization. This might have proved a futile task had it not been for the vast amount of empirical information about lignin that was agglomerated simultaneously by analytical and other approaches, for it might be safely said that lignin is the most complicated natural product ever encountered.

Because the methoxyl content of spruce lignin was found to be approximately 1.0 per C_9 (phenylpropanoid) unit – the isolation, purification and analysis of lignins are difficult and the limits of error correspondingly large – it was initially thought that conifer lignin was derived exclusively from coniferyl alcohol (or coniferin). Besides, because of the economic importance of conifers, softwood lignin is the lignin that has always been most widely studied.

However, degradation experiments showed that conifer lignin must contain not only coniferyl, but also *p*-coumaryl and sinapyl components as well [cf. for example (*100*)]. It has now been shown that spruce cambium contains not only coniferin, but also small amounts of

gluco-*p*-coumaryl alcohol and syringin as well (*54*). This indicates the first complication in the structure of lignin — it is not merely a homopolymer, but is a copolymer produced from either a mixture of *p*-coumaryl and coniferyl alcohols or a mixture of *p*-coumaryl, coniferyl, and sinapyl alcohols, i.e. monomers that differ only by a single or twin methoxyl group. No primitive lignin derived from *p*-coumaryl alcohol alone nor a higher lignin containing no units derived from *p*-coumaryl alcohol has ever been encountered. However, as a rule, the more primitive the plant, the lower its content of units derived from the methoxylated coumaryl alcohols.

Qualitative knowledge of the types of units contained in any lignin can be obtained by degrading it with nitrobenzene and alkali at 170 °C (*57*) and separating the mixture (*96, 100*) of aldehydes produced (*p*-hydroxybenzaldehyde, vanillin, and syringaldehyde). The yields of aldehydes do not indicate the relative proportions of the three monomers in the original lignin, for the extent of their condensation into the lignin depends upon their methoxylation pattern, and this in turn determines the amounts of aldehydes produced in the degradation. The yield of syringaldehyde reflects an exaggeratedly high content of sinapyl-type monomer in the lignin, because, owing to its two methoxyl groups, sinapyl component in lignin forms preferentially end groups or is condensed in by phenyl ether groups; these structures readily yield the simple aldehyde on degradation whereas more highly condensed structures cannot. This degradation first showed that conifer lignin is not derived merely from coniferyl alcohol (*100*) but it would go too far here to review all of the finer applications it has had since.

Strong support for the theory that lignin was derived from coniferyl alcohol liberated from coniferin was the discovery that there is a β-glucosidase in the zone of lignification in conifers [cf. (*44, 59*)]. Other observations have suggested that coniferin and the β-glucosidase are however not essential for lignification. For instance, lignification still proceeds in singly or doubly ring-debarked trees (*149*), even though the supply of coniferin is thus interrupted. The parasite mistletoe is highly lignified but contains no β-glucosidase although it appears to withdraw its lignin precursors from the host plant (*71*). Mistletoe even contains a substance that inhibits β-glucosidases (*76a*).

Since numerous degradations of lignin, e.g. ethanolysis according to *Hibbert* (*77*) or hydrogenation with a variety of catalysts (*72, 77, 119, 135*), had shown that it is in fact made up largely of phenylpropanoid units, it seemed that the theory of its origin from the three *p*-coumaryl alcohols could be considered reliable. It remained therefore to establish the nature of the processes involved in the polymerization.

The reaction is now known to be in fact a *dehydrogenative polycondensation*. The enzymes that can affect the dehydrogenation of the *p*-coumaryl alcohols are now known to be laccase (*55*, *76b*, *78*, *79*) and peroxidase. These are electron transferases (*109*); with laccase molecular oxygen, with peroxidase any hydroperoxide serves as electron acceptor. Either enzyme abstracts a single electron from the phenoxide form of the (ionized) *p*-coumaryl alcohols to form free phenoxyl radicals. It was recently shown that the free radicals derived from coniferyl alcohol are metastable, having a half-life of 45 secs in 50% aqueous dioxan (*48*). Although the alcohols are styrene derivatives, lignification does not occur by a radical-initiated styrene-type polymerization. Actually, coniferyl alcohol will inhibit free-radical polymerizations (*71*): it acts as a radical scavenger and is dehydrogenated by more active free radicals to give the metastable phenoxyl radicals which then pass on through the slow process of lignification. Only after a ligninlike polymer has been formed does the free radical polymerization start to proceed. Coniferyl alcohol can in fact be dehydrogenated by other stable, free radicals, e.g. the tri-*t*-butylphenoxyl radical (*46*, *53*); in this reaction it is a hydrogen atom and not an electron that is transferred.

The question is, therefore, what happens to the phenoxyl radicals produced from the *p*-coumaryl alcohols when they disappear.

Studies of the enzymatic dehydrogenation of coniferyl alcohol alone *in vitro* gave the answer to this poser. If coniferyl alcohol was left in contact with laccase and air for a long time, it was found that an amorphous ligninlike polymer was formed (*59a*). When the reaction medium was investigated before the high polymer had formed, it was found to contain numerous intermediates of relatively low molecular weight [cf. (*58*)]. Isolation and identification of these intermediates [cf. (*41*, *43a*, *46*)] afforded information on the principles involved in the growth of the lignin polymer. This work culminated in the publication of schematic formulae for typical portions of spruce lignin molecules (*42*, *43*, *43a*, *54a*) which were able to explain quantitatively most of the analytical data and known reactions of lignin (cf. Fig. 9).

By adapting the conditions of the dehydrogenation, e.g. by working in anhydrous solvents with inorganic oxidizing agents such as copper salts or manganese dioxide instead of enzymes, the yields of specific intermediate lignols could be increased and their isolation thus facilitated.

However, the first four products identified (*44*), viz. dehydrodiconiferyl alcohol (II), DL-pinoresinol (IV), guaiacylglycerol-β-coniferyl ether (VI) and coniferaldehyde (VII) (*60a*) already revealed the nature of most of the secondary reactions taking place after formation of the free phenoxyl radicals by the enzymes, although this was not immediately

realized. The *p*-coumaryl alcohols, exemplified by coniferyl alcohol in Fig. 3, have an extended π-electron system, and the unpaired electron created on the phenolic oxygen by the electron-transfer action of the phenol oxidases immediately becomes "smeared" over the whole molecule, with paticularly high electron densities on the atoms dotted in the mesomeric limiting structures R_a–R_d (Fig. 3). A high electron density

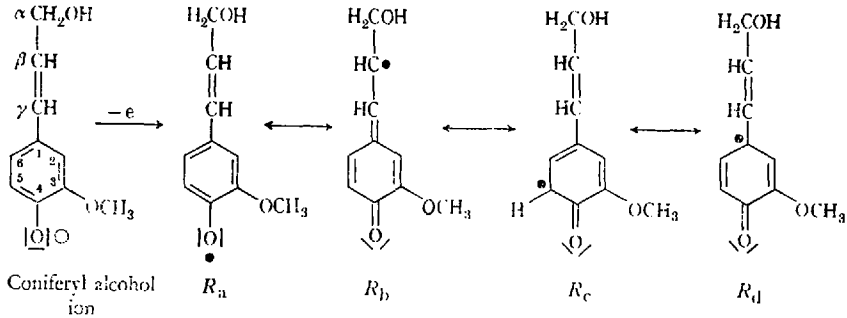
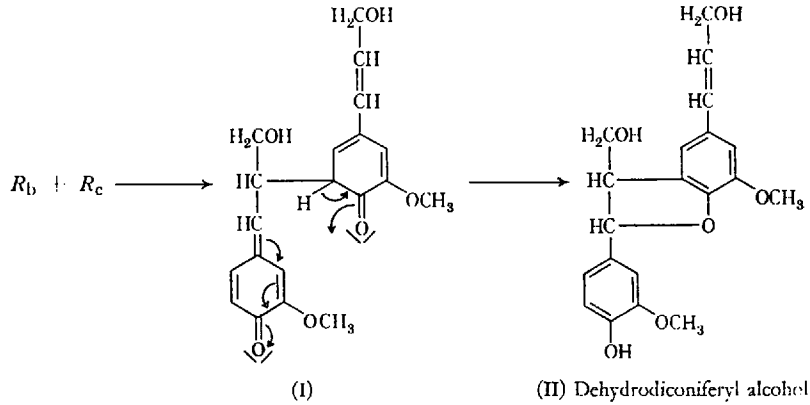


Fig. 3. Dehydrogenation of coniferyl alcohol to a highly mesomeric free radical

should also be expected by mesomerism at C-3, the point of attachment of the methoxyl group, but no evidence for the participation of a radical of this type in lignification has yet been obtained. Since no elimination of methoxyl groups is observed during degradations of lignin or lignin model compounds with laccase or peroxidase (*38*, *39*, *55*) radical reactions at C-3 seem to be impossible. No attention has been paid to stereochemistry in the formulae in Fig. 3. Although the *trans*-configuration of coniferyl alcohol may be largely retained during the subsequent reactions of the free radicals, the structures of some of the lignols, e.g. *cis*-ferulic acid (*50*) and DL-epipinoresinol (*58*), indicate that at least some inversion to *cis*-forms must occur.

The main reaction that causes disappearance of the free radicals is their pairing off in various combinations of the forms R_a–R_d. For example, coupling of a radical in the R_b form with another in the R_c form yield the labile double quinone methide (I) (see Fig. 4). Deprotonation of C-5 in the upper *o*-quinone methide moiety of (I) allows it to rearomatize to a phenoxide ion which launches a nucleophilic attack on the (benzyl) γ-carbon atom of the lower *p*-quinone methide moiety, which thereby rearomatizes to the phenoxide ion of dehydrodiconiferyl alcohol. Reprotonation at the lower phenolic oxygen then affords (II). The whole is probably a fast concerted reaction and hence the *trans*-orientation of the hydrogens on C-β and C-γ in the coniferyl alcohol is probably largely

retained in both the hydroxypropenyl side chain and the coumaran ring of (II). Exclusively *trans*-disposition of these hydrogens was found in the analogous compound dehydrodiisoeugenol prepared from *trans*-isoeugenol (*12*). It has been estimated that 18 % of the C_9-units in lignin are involved in structures of the phenylcoumaran type [cf. Units 17/18 in Fig. 9] (*5*).



(I) (II) Dehydrodiconiferyl alcohol

Fig. 4. Mechanism of the formation of dehydrodiconiferyl alcohol

In the formation of this one dimeric intermediate we can already recognize some of the typical general features involved in the formation of the lignin polymer. First we see the formation of a carbon-carbon σ-bond which is naturally non-hydrolysable; here this bond is between an alkyl and an aryl residue. The presence of such interunitary C–C bonds explains why lignin cannot be entirely degraded by hydrolysis. Second we see the formation of an alkyl aryl ether bond by nucleophilic addition – here intramolecularly – of a phenol residue onto a *p*-quinone methide; this ether is a cyclic benzyl aryl ether. Third we see that (II) contains two asymmetric carbons, namely C-2 and C-3 in the coumaran ring, but like all the other intermediates of lignification and like lignin itself, (II) is optically inactive. This means that (II) is a diracemate and that the activity of the enzymes ceases after removal of an electron from the phenoxide ion of the two coniferyl alcohol units. The oxidases do not exert any steric influence on the subsequent reactions of the free radicals they create. Fourth we see that (II) is again a phenol which can ionize and be oxidized by laccase or peroxidase, i.e. be dehydrogenated to a free phenoxyl radical which can again condense with other free radicals even though its possibilities for modification and stabilization by mesomerism are more restricted than in the case of coniferyl alcohol.

Combination of two R_b forms of coniferyl radicals (Fig. 5) gives rise to the transient double *p*-quinone methide (III). Here nucleophilic attack on the γ-carbon of the *p*-quinone methide by the hydroxyl oxygen

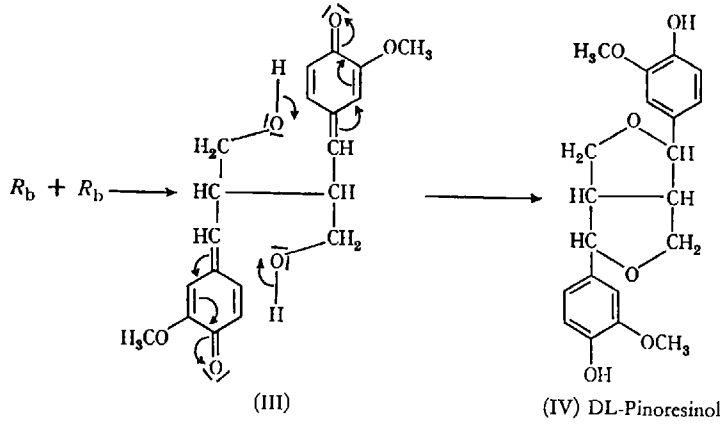


Fig. 5. Mechanism of the formation of DL-pinoresinol

of the primary alcohol group with simultaneous elimination of a proton and reformation of the phenolic aromatic system then occurs at both ends of the molecule. Reprotonation of the two phenoxide ions affords the dilignol DL-pinoresinol (IV) [cf. Units 8/9 in Fig. 9] (*44*). The two bridgehead hydrogens, i.e. those on the β-carbons, must be in *cis*-relationship for both tetrahydrofuran rings to close. The same situation is encountered in the analogous formation of DL-syringaresinol from sinapyl alcohol (*55*). Again the hydrogens on C-β and C-γ in each half of the molecule (IV), like those in coniferyl alcohol, are *trans*-disposed (*60 b*). The lignol (IV) has four asymmetric carbon atoms but again only racemates are formed during lignification. The *lignan* pinoresinol found in the resin extruded from damaged bark by spruce is optically active [cf. (*60, 60b*)], being the D-form, and must therefore be formed by a different mechanism from that involved in lignification. However, a little of a diastereomer of DL-pinoresinol, viz. DL-epipinoresinol (*60 b*) is also produced from coniferyl alcohol during simulated lignification *in vitro* (*46, 58*). Here the hydrogens on C-β and C-γ are *cis*-oriented in one half of the molecule. Further characteristics of the process of lignification can be recognized from this dimer. We see that the simple act of phenol dehydrogenation has led by subsequent reactions of the free radicals produced to two units joined by three bonds – two dialkyl ether bonds and a carbon-carbon bond, this time between two aliphatic residues. Here two phenolic groups have been regenerated for subsequent de-

hydrogenation and condensation with other free radicals. We also see that even alcohols can add onto the *p*-quinone methide intermediates of lignification to give benzyl alkyl ethers.

Combination of an R_b radical with an R_a radical yields the single *p*-quinone methide dimer (V). Here the quinone methide cannot become stabilized by an intramolecular addition reaction. Instead, nucleophilic attack of its γ-carbon atom occurs by a hydroxyl ion from the medium, for example; aromatization and protonation of the phenoxide ion thus formed give rise to guaiacylglycerol-β-coniferyl ether (VI), again in racemic form despite its two asymmetric carbon atoms. Since attack by the extraneous hydroxyl ion can occur on either side of C-γ of the *p*-quinone methide (V), complete equilibration of the specific *trans*-orientation of the hydrogens from the original coniferyl alcohol moiety in the lower half of (V) presumably occurs (see formulae on p. 131).

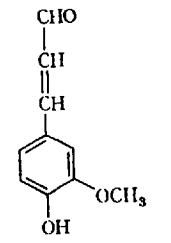
In (VI) the two units are held together by only a single alkyl aryl ether bond. This is therefore a relatively weak point for degrading the lignin molecule. This bond is in fact split readily under energetic pulping conditions (*1, 66*). In addition, this is one of the most frequent types of interunitary linkage in lignin, about 45 % of the units in lignin being held together in this way (*43a*). Cleavage of this type of bond thus explains the high delignification achieved with kraft pulping.

Another important feature of (VI) is its activated benzyl alcohol grouping. This type of group can be induced to undergo condensation reactions with phenols under acidic conditions, especially when heated. This reaction is effectively the same as the curing of phenol-formaldehyde resins by heat and acids. Condensations of this type invariably must occur when attempts are made to release lignin from wood with strong acids because of the free phenolic groups in lignin. Free *p*-hydroxybenzyl alcohol [cf. Unit 13b in Fig. 9] groupings of this kind are present in about 8 % of the units in lignin [cf. (*2, 7a*)]; the analogous etherified structures [cf. Units 6, 12 and 16] will react similarly but slower.

In this dimer, we recognize yet another important principle involved in the growth of the lignin macromolecule, namely the addition of extraneous substances from the reaction medium onto the quinone methide intermediates. This question will be discussed more fully below.

The fourth intermediate of lignification identified at an early vantage was not a dimer, but also a C_6–C_3 compound – coniferaldehyde (VII) (*60a*). Its formation from coniferyl alcohol reveals another type of reaction entailed in lignification although it was thought at first that the coniferaldehyde was actually an artifact formed by autoxidation of some coniferyl alcohol during the aeration in simulated lignification *in vitro* (*58*). In reality some radical transfers are taking place as a side

reaction to radical combinations: coniferyl radicals abstract hydrogens from the primary alcoholic group of other coniferyl alcohol molecules to form the aldehyde – possibly by disproportionation of non-mesomeric free radicals on C-α – and reform coniferyl alcohol molecules that then undergo renewed dehydrogenation. Analogous dehydrogenation of cinnamyl alcohol by tri-*t*-butylphenoxyl radicals has been observed (*53*). The coniferaldehyde can also undergo dehydrogenation to form phenoxyl radicals which condense, apparently mainly in forms analogous to R_a and R_c, with coniferyl radicals and thus also participate in lignification. Dimeric aldehydes analogous to (II) and (VI) have in fact been isolated from incubates of coniferyl alcohol with laccase (*58*). There are only 3 % such aldehydic groups in lignin (*4*) [cf. Unit 10 in Fig. 9] but these suffice to give a intense red color with phloroglucinol and concentrated hydrochloric acid, the conventional Wiesner test for lignin.



Coniferaldehyde (VII)

This end-group oxidation by the free coniferyl radicals can proceed even further to produce a little free *cis*- and *trans*-ferulic acid (*50*), which also undergoes dehydrogenative condensations via phenoxyl radicals. Some of the carboxyl and lactone groups in lignin [cf. Unit 13a in Fig. 9] doubtlessly arise in this way. Lactonic dilignols derived from coniferyl and ferulic radicals have also been isolated from lignin produced from coniferyl alcohol alone *in vitro* (*50*). The ferulic acid encountered as a predecessor of coniferyl alcohol during the biosynthesis of the latter (see Fig. 2) is in the form of an insoluble ester (*35*) and therefore cannot get directly involved in lignification like the ferulic acid later produced by hydrogen transfer reactions.

Hydrogen transfer reactions may perhaps take place to a small extent with higher oligolignols or even with lignin itself. This might lead to small amounts of "abnormal" structures, i.e. structures not formed by condensations of dehydrogenated lignols. The isolation of a compound likely to be guaiacylglycerol etherified in both its β- and γ-positions with molecules of sucrose (*53*) suggests that coniferyl radicals can dehydrogenate carbohydrate molecules as well and can then condense in the R_b form with the free carbohydrate radicals produced. The resultant C-β-to-carbohydrate bond can actually be a C—C or an ether bond, depending on whether the hydrogen abstracted from the sugar came from a carbon atom or a hydroxyl group. If such condensations actually occur between lignin and polysaccharide radicals in the plant, strong lignin-to-carbohydrate bonds that would be immune to enzymatic cellulolytic hydrolysis and resistant to chemical hydrolysis would be formed. Acetal bonds between aldehydic groups in lignin and polysaccharide hydroxyls

or ester bonds between lignin carboxyl groups and polysaccharide hydroxyls or vice versa are also feasible lignin-carbohydrate junctions (*19, 110*). However, a novel type of lignin-carbohydrate bond formed by additions of sugars onto *p*-quinone methides by an ionic mechanism is discussed below. It is the same mechanism that gives rise to the C-γ-to-sucrose ether bond in the derivative of guaiacylglycerol mentioned above (*53*).

The mixture of quinone methides initially formed by combination of the coniferyl radicals in their various mesomeric forms, i.e. (I), (III), (V), (IX) and others, can be detected by means of their characteristic spectrum with a maximum at about 312 mμ (*52*); the half-life of the mixture in 70 % aqueous dioxan is 1 hour. Those quinone methides that can rearomatize by keto-enol tautomerism, e.g. (IX), or intramolecular additions, e.g. (I) or (III) may become stabilized faster than those of type (V) which rely on addition of a foreign molecule. The quinone methides that rearomatize intramolecularly appear to react exclusively in this way, probably by a concerted mechanism that represents collapse of the activated transition state.

However, the quinone methide (V) allows of a much wider scope of variation. Not only will water (dissociation constant = 10^{-14}) add onto (V) under the normal conditions of lignification via the ionic mechanism shown in Fig. 5, but coniferyl alcohol itself or other phenolic lignin intermediates (dissociation constants of phenols $\sim 10^{-8}$–10^{-10}) will add on as well by an analogous mechanism to give guaiacylglycerol-β,γ-diaryl ethers [cf. Units 4, 7 and 11 in Fig. 9]. This reaction finds its parallel in the intramolecular nucleophilic addition of the phenolic residue encountered in the formation of dehydrodiconiferyl alcohol (II) (Fig. 4). The first adducts of this type observed during lignification *in vitro* were guaiacylglycerol-β,γ-diconiferyl ether (VIII) and guaiacylglycerol-β-coniferyl-γ-dehydroconiferyl ether (*49*). These are formed by addition of coniferyl alcohol and dehydrodiconiferyl alcohol (II), respectively, onto (V). Higher lignols incorporating analogous structures have meantime been isolated [for a survey, see (*41, 43a*)]. The presence of about 10 % of such guaiacylglycerol diethers in spruce lignin has recently been established (*56*). The formation of (VIII) explains the observation made during kinetic studies of the dehydrogenation of coniferyl alcohol by laccase that all the coniferyl alcohol has disappeared from the incubation system after only 80–90 % of it has been dehydrogenated, judging from the oxygen consumption (*55*). The addition of coniferyl alcohol onto (V) to form (VIII) thus illustrates another important principle of lignification, namely the interlinking of units without dehydrogenation through addition of lignols onto *p*-quinone methides by an ionic mechanism. This mechanism leads to non-cyclic benzyl aryl ethers

(contrast the cyclic benzyl aryl ethers in the phenylcoumarans, e.g. II or Units 17/18 in Fig. 9).

Hardwoods appear to have a much higher content of such arylglycerol-β,γ-diaryl ethers because of the more restricted possibilities for condensations in the aromatic ring of sinapyl-type units owing to their two methoxyl groups. This is reflected in the greater susceptibility of hardwood lignins to mild hydrolysis (see Section I).

The arylglycerol diethers formed by addition of phenolic lignin intermediates onto *p*-quinone methides analogous to (V) are extremely important for the properties of lignin. First, they represent branching points in lignin: there is a β,γ-dietherified arylglycerol unit in the fork of almost every branch in the lignin molecule [cf. Unit 4 in Fig. 9]. For branching to occur by the dehydrogenation/free-radical combination mechanism, a *p*-coumaryl or coniferyl unit must be dehydrogenated twice and linked to other lignin residues through C-β and an *ortho*-position before a third lignin moiety is attached at the phenolic oxygen, either by enzymatic dehydrogenation and condensation with another free radical or by addition onto a *p*-quinone methide [cf. Unit 12 in Fig. 9]; this is probably a rare occurrence. Second, the γ-aryl ether is a special type of ether, being either a *p*-hydroxybenzyl ether when in end groups [Units 11/12 in Fig. 9] or a *p*-alkoxybenzyl ether when embedded in the fully grown lignin molecule [Units 5/4/3 in Fig. 9], and is the weakest bond in lignin. Such ethers are hydrolysed with great ease by either acids or alkalies, but the comminution of the lignin molecule effected by such a hydrolysis is normally immediately offset by condensations that lead to strong carbon-carbon bonds. For example, hydrolysis with acid – even the weak acidity of the cell sap will do – leads to a benzyl carbonium ion which rapidly causes an electrophilic substitution in one of the highly activated benzene rings of another portion of the lignin molecule [cf. the bonds between Units 3/2 and 15/17 in Fig. 9]. This reaction is similar to that of the free benzyl alcohols of type (VI) with acid mentioned above and compared to the curing of phenol-formaldehyde resins. Alkaline hydrolysis of the benzyl aryl ethers probably proceeds via quinone methides which undergo nucleophilic substitutions with other phenoxide residues in the alkaline medium. The product of the condensation in either case is a diphenylmethane derivative, and like the bakelites, the condensed products are highly insoluble and intensely colored. These reactions explain the dark brittle appearance of lignins liberated from wood by hydrolysis of the polysaccharides with strong acids, e.g. Klason or Willstätter lignins. The condensations of this kind that occur naturally owing to the slightly acidic pH of the wood stabilize the branching points in the lignin by replacing the weak benzyl aryl ether bonds by strong benzylarane C–C bonds.

The intermolecular addition of alcohols analogous to the intramolecular addition encountered in the formation of DL-pinoresinol (IV) is slightly more complicated. Under ordinary lignification conditions, the only alcohols that will add onto (V) or similar *p*-quinone methides are those which are sufficiently strongly ionized. Methanol with a dissociation constant of 10^{-17} will add on (*52*), but ethanol with a dissociation constant of 10^{-20} will not (*56*). These alcohols are not encountered during lignification *in vivo* in any case. Many intermediates of lignification, on the other hand, are not only phenols but often contain free alcohol groups, e.g. the primary hydroxymethyl or cinnamyl alcohol groups of (II) or (VI) or the secondary (benzyl) alcohol group of (VI). It is conceivable that these too might add onto *p*-quinone methides of type (V).

In practice, there is little positive evidence that such an addition can occur. Addition of a benzyl alcohol moiety as an alkoxide ion to C-γ of (V) and reprotonation of the resulting phenoxide ion would produce an extremely labile bis-*p*-hydroxybenzyl ether. This is hardly likely to occur. A converse type of addition of a benzyl carbonium ion formed by abstraction of hydroxide from a benzyl alcohol residue by a proton in the slightly acidic lignification milieu onto the oxygen of the quinone methide portion of (V) is perhaps more feasible. Rearomatization would then produce a new benzyl carbonium ion at the carbon end of the quinone methide. This carbonium ion could then add on hydroxide to form a new benzyl alcohol grouping or again condense with another quinone methide. The second alternative would produce the same product as simple polymerization of the *p*-quinone methides (*61*), a reaction considered to be another possible mechanism involved in the growth of the lignin molecule (*41, 42*). The polymerization of quinone methides is accelerated by alkalies (*61*). No evidence for such a polymerization has yet been obtained from the structures of isolated lignols; if it does in fact occur during lignification, it must be to only a limited extent and the degree of polymerization will be much lower than that observed with simple *p*-quinone methide models (*61*).

On the other hand, experiments have been carried out to test the possibility of additions of primary alcoholic groups in lignols onto quinone methides. Most of the results obtained indicate that this cannot occur during lignification under physiological conditions. Lignins produced *in vitro* or *in vivo* in the presence of ^{14}C-labelled cinnamyl alcohols with no or blocked phenolic hydroxyl groups were invariably found to be radioinactive (*49a, 56*). Again, polymers were produced from coniferyl alcohol in the absence of water with MnO_2 (*49, 56*) or tri-*t*-butylphenoxyl radicals (*71*) in order to prevent the formation of (VI) but with large excess of cinnamyl or dimethoxycinnamyl alcohol present in the system in order to promote the formation of alcohol adducts with

(V). No such adduct was ever observed even when basic solvents such as anhydrous pyridine was used in attempts to catalyse the addition of the alcoholic group onto (V) (*71*). However, addition of alcohol groups onto a quinone methide model was found to be catalysed by acid (*56*). Nevertheless, it appears unlikely that any significant number of aliphatic hydroxyl groups in the lignin molecule add onto *p*-quinone methides of type (V) to give benzyl alkyl ethers within the normal physiological pH range in which lignification can occur by enzymatic dehydrogenation.

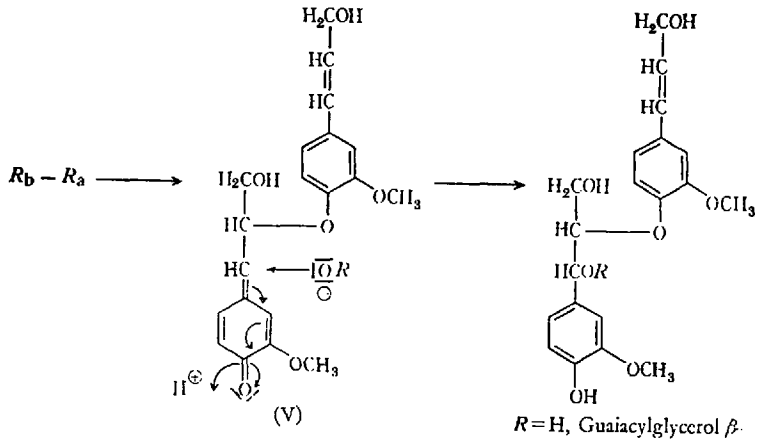
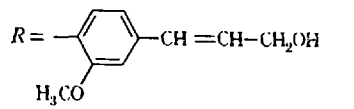


Fig. 6. Mechanism of the formation of guaiacylglycerol-β-coniferyl ether and a guaiacylglycerol-β,γ-diaryl ether [guaiacylglycerol-β,γ-diconiferyl ether]



Guaiacylglycerol-*β*,*p*-diconiferyl ether (VIII)

The carbohydrates present in plant cells where lignification is in progress also contain relatively strongly dissociated hydroxyl groups: the dissociation constants of sugars lie between 10^{-13} and 10^{-15}. It has been found that both sorbitol (*51*, *52*) and sucrose (*51*, *53*) will add onto (V) during modified lignification *in vitro* to form benzyl ethers of the sugars. These ethers are naturally *not* glycosides. Formation of similar non-glycosidic ether linkages between lignin intermediates of type (V) and dissociated hydroxyl groups of polysaccharides within the cell can be envisaged [cf. Unit 5 in Fig. 9]. Bonds of this kind and the other possible types of lignin-carbohydrate bonds already discussed result in the formation of a graft polymer between the cell-wall polysaccharides and the lignin. It therefore becomes redundant to query after the molecular weight of lignin *in situ* in wood.

In view of the importance of the dissociation constant of the hydroxyl group giving rise to the oxide ion that initiates the nucleophilic attack on the quinone methide leading to the adduct, more attention should perhaps be paid to the possibility of carboxyl groups in this role than has hitherto been the case. Carboxylic acids are more strongly ionized than phenols and will also add onto quinone methides with great ease [cf. (*52, 61*) and literature cited there]. This reaction occurs during lignifica-

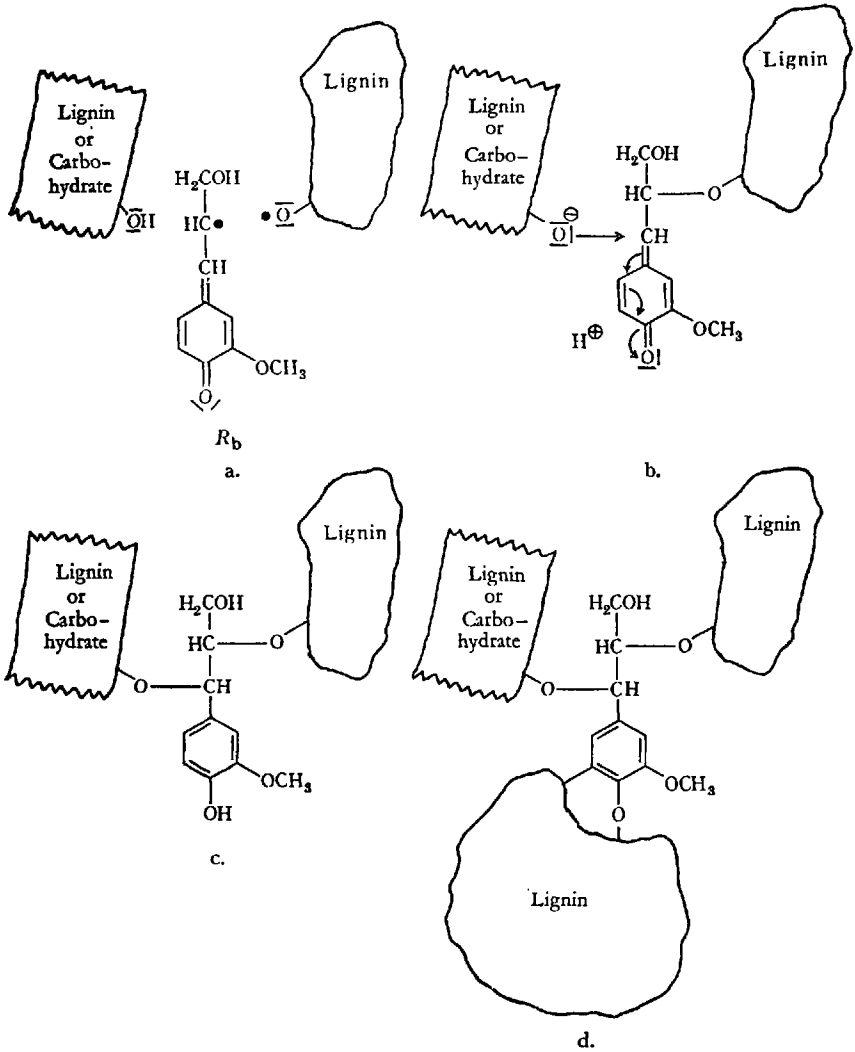


Fig. 7. Mechanism of the cross-linking of lignin macromolecules or of the grafting of lignin onto polysaccharides by single R_b-type coniferyl radicals

tion in the formation of pinoresinolide (*50*), a monolactone analogous to DL-pinoresinol (IV) [CO instead of CH_2 in one tetrahydrofuran ring]. It is feasible that any accessible carboxyl groups in hemicelluloses or the uronic acid residues in polyuronides can also add onto *p*-quinone methide intermediates of lignification. In this case a *p*-hydroxybenzyl ester bond would result instead of an ether bond. Ester bonds grafting lignin to polysaccharides could arise in this way. The evidence for such ester bonds can be found in the reviews on the lignin-carbohydrate bond (*110*).

Appreciation of these mechanisms reveals that a single free radical in the form R_b can act as a strong cross-linking agent between two large preformed pieces of lignin or between a growing lignin molecule and a preformed or growing polysaccharide molecule in the cell wall matrix. This is illustrated in Fig. 7. The preformed lump of lignin on the right in Fig. 7a once contained a free phenolic hydroxyl group that has been enzymatically dehydrogenated to give the free phenoxyl radical shown. This radical combines in its R_a form with the single R_b radical to form the *p*-quinone methide shown in Fig. 7b. Nucleophilic attack of the ionized hydroxyl group in the entity on the left in Fig. 7b, which may be derived from a phenolic hydroxyl group in another lignin molecule or from an aliphatic hydroxyl or carboxyl group in a polysaccharide molecule, leads to the cross-linked polymer shown in Fig. 7c. The weak *p*-hydroxybenzyl ether or ester bond holding the left hand portion of the cross-linked polymer in Fig. 7c becomes stabilized if the phenolic hydroxyl group of the cross-linking guaiacylglycerol unit becomes etherified by dehydrogenative condensations as lignification progresses. The stabilized cross-linked polymer is shown in Fig. 7d. Here the original R_b radical has become the centre of a branching point. Any or all of the three aggregates held together by the single coniferyl unit in Fig. 7d can continue to grow and form other cross-links. Numerous cross-linkages between lignin and polysaccharides can occur at random points in the molecules of each. The plant cell wall can therefore be a cellulose-lignin-hemicellulose-lignin-polyuronide graft polymer.

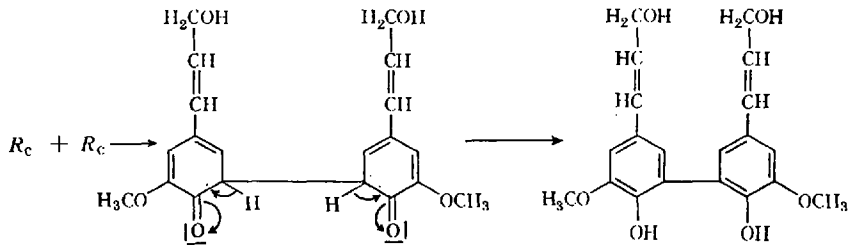


Fig. 8. Mechanism of the formation of biphenylyl-linked lignols

Let us now revert to the mechanism of growth of the pure lignin polymer. By analogy to the radical pairings already described, other types of combinations should also be expected. For instance, interlinking of two radicals in the R_c form and subsequent rearomatization by keto-enol tautomerism of the double *o*-quinone methide (IX) produced should lead to bis-5-dehydroconiferyl alcohol (X) – a biphenylyl linked dilignol [see Fig. 8]. However, since both halves of this molecule still possess an unchanged coniferyl alcohol type structure, despite its doubled molecular weight, (X) is subject to almost as rapid dehydrogenation and condensation as coniferyl alcohol itself. For this reason, it could only be shown indirectly from lignin degradations (*47*) and from dehydrogenations of di-

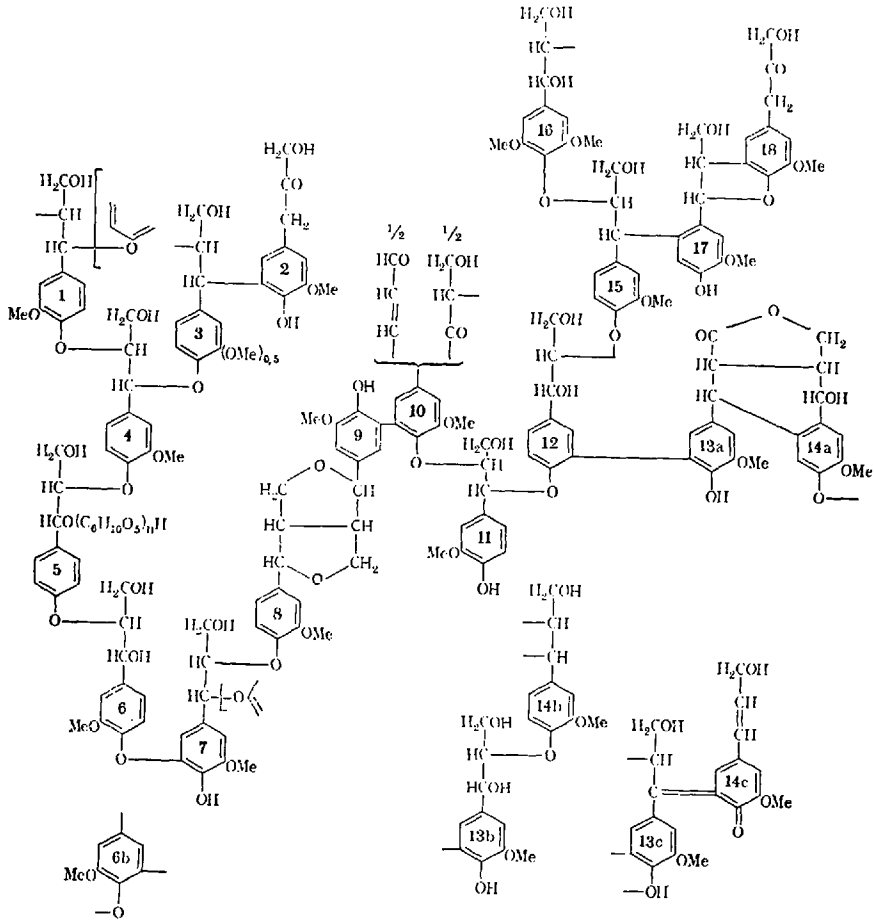
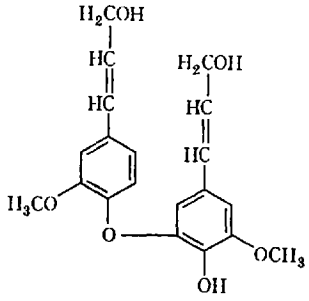


Fig. 9. Constitutional formula for spruce lignin (*43*)

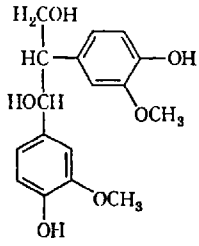
hydroconiferyl alcohol (*58a*) that (X) is in fact an intermediate of lignification. The other dilignols (II), (IV) and (VI) are dehydrogenated much more slowly than coniferyl alcohol (*71*).

Biphenylyl links between higher lignols, e.g. in the dehydrodipinoresinol obtained from pinoresinol (*60*), are easier to trace. Biphenylyl bonds may make up about 25 % of all the interunitary links in lignin (*123*) [cf. Units 9/10 and 12/13a in Fig. 9]. Again this is a strong, non-hydrolysable C–C bond.

Analogous combination of an R_a radical with an R_c radical and subsequent rearomatization by keto-enol tautomerism of the R_c portion should give rise to a diconiferyl ether (XI); again the right-hand half of this molecule has the coniferyl alcohol structure and is perhaps even activated by the coniferyl ether substituent in its 5-position. It is therefore dehydrogenated and condensed very rapidly and has consequently not yet been encountered among the intermediates of lignin made *in vitro* from coniferyl alcohol. Again indirect evidence for the presence of such diaryl ether bonds in lignin [cf. Units 6/7 in Fig. 9] has become available from oxidative degradations of lignin (*48*) and from enzymatic dehydrogenation of 4-propylguaiacol (*124*). The formation of diaryl ethers may also take place more readily with higher lignols than with coniferyl alcohol as such.



Diconiferyl ether (XI)



1,2-Diguaiacyl propane-1,3-diol (XII)

Evidence for the participation in lignification of radicals in the form R_d was not obtained from lignols extracted from simulated lignification experiments with coniferyl alcohol *in vitro*. Actually more direct proof was obtained here: structures, e.g. (XII), that must have arisen from this form of the coniferyl radical were isolated from natural lignins by mild hydrolysis (see Section I). So far only structures derived from combinations of R_d type radicals with R_b type radicals have been isolated (*47, 105, 116, 117*), but evidence for combinations of R_d radicals with R_a radicals has been secured by the detection of 4*O*-(3,4-dimethoxyphenyl)-

vanillic acid among the products of oxidative degradation of methylated lignin with $KMnO_4$ (*28, 41*). Whenever a radical in the R_d form combines with another coumaryl radical, its three-carbon side chain appears to be eliminated. The analogy to this reaction in animal organisms is the formation of the thyroid hormone thyroxine from diiodotyrosine.

The lignols described so far are all phenols and can still undergo dehydrogenation and condensation with other lignol radicals or with coniferyl, *p*-coumaryl, or sinapyl radicals. However, the ease of dehydrogenation of the lignols generally decreases as their molecular weights increase (*71*). Their substitution pattern also exerts an influence in this respect. Nonetheless, in principle, again free radical combinations, additions onto quinone methides, and hydrogen transfer reactions can occur. Many other lignols have been isolated from experiments with coniferyl alcohol alone *in vitro*, but these are all produced according to the same principles as have been outlined above. Naturally, "mixed" lignols must be produced in nature when not only coniferyl but also *p*-coumaryl and sinapyl radicals are available, but the structures of the compounds will be essentially along the same lines as those of the lignols procured from coniferyl alcohol alone. The mixture of "homolignols" plus "heterolignols" obtained when a mixture of any two or all three *p*-coumaryl alcohols is dehydrogenated *in vitro* is far too complex for there to be any reasonable chance of separating and identifying individual "mixed" lignols. A complete list of the lignols isolated and identified to date is due to be published (*41*).

The polycondensation process of lignification probably continues until the lignin molecule becomes so large and unwieldy that phenolic hydroxyl groups are no longer accessible to the oxidase enzymes or until the free phenoxyl radicals they form can no longer move around to find a partner to combine with. It has in fact been found that lignin contains stable free radicals (*54a, 140*). Here and there the oxidases may overshoot their mark and start to redegrade the lignin they have made (cf. Section J).

The oxidizing influence of the enzymes will not cease until cell dehydration or the tanning effect of the lignin causes denaturation or inactivation of the enzyme protein. Hence secondary changes may take place in the polymeric lignin molecules in the form of topochemically strongly localized reactions under the influence of the oxidases. For example, oxidations at the benzyl carbon (C-γ) may occur[cf. (*124*)]. Hydroxyl functions in this position [cf. Units 6, 12, 13b and 16 in Fig. 9] are readily oxidized to carbonyl groups, especially in biphenylyl-coupled compounds [cf. Unit 10 in Fig. 9] (*71, 123*). Moreover, over long periods of time, the slight natural acidity of wood may cause slow cleavage of benzyl aryl ethers [i. e. the γ-aryl ethers in guaiacylglycerol-β,γ-diaryl

ethers, e.g. (VIII)] and subsequent "curing" nuclear condensations (cf. p. 126) giving a more strongly condensed lignin [cf. Units 3/2 and 15/17 in Fig. 9]. These are perhaps some of the processes diffusely described as "aging" of lignin.

H. Characterization of lignins

We have seen that lignin is a highly branched amorphous polymer containing an extremely complex array of structures and that when *in situ* in the wood, it is a graft polymer with cellulose and hemicellulose to boot. Characterization of lignins thus becomes a problem.

This problem is of fundamental significance with regard to the structure of lignin. The structures of the lignols identified as intermediates of simulated lignification *in vitro* can be regarded as valid evidence for the structure of natural lignin formed *in vivo* only if it can be shown that the biosynthetically duplicated lignin made in the laboratory is the same as that produced by the plant in nature. It was fortunate that the method of extracting chemically unchanged lignins by milling pre-extracted wood under toluene (*77*) provided a means for obtaining genuine natural lignins for comparison with the products made in the laboratory. However, when comparisons are made, the special nature of the biogenesis and structures of the two products must be kept in mind. The natural product has been formed in a completely different environment from the laboratory product. The latter has had no chance to become attached to polysaccharides and has not been held in gel suspension by cell plasma. An endeavor must therefore be made to make comparisons between materials that have been treated identically after their production at least, just as would be done with other high polymers, otherwise the legitimacy of either similarities or discrepancies between the two is open to question.

Let us suppose that a comparison is to be made between spruce lignin and a biosynthetically duplicated spruce lignin (Freudenberg's dehydrogenation polymer or DHP). First, spruce milled-wood lignin must be extracted, e.g. with dioxan/water (9:1 v/v), and purified under extremely mild conditions in order to ensure that oxidations or condensations cannot occur. A narrow uniform fraction of this lignin must then be selected. The solubility in dioxan/water has already set an upper limit to the molecular weight of the lignin extracted. Material of low molecular weight can be removed by exhaustive extraction of the crude lignin in the cold with a relatively polar inert solvent, e.g. ethyl acetate (*71*). Afterwards the lignin-carbohydrate complex [cf. p. 109] must be entirely removed, but again using a physical method that will not endanger the

other lignin molecules. Here, for example, as for other high polymers fractional precipitation can be applied, e.g. by extremely slow addition of pure benzene to a highly dilute solution of the lignin in dioxan/water and removal of the carbohydrate-rich fraction that is precipitated preferentially (*71*).

The biosynthetic lignin (DHP) must of course be made from a 14:80:6 mixture of *p*-coumaryl, coniferyl and sinapyl alcohols, for these are the approximate proportions in which these three lignin precursors are available when converted into spruce lignin *in vivo* in the tree (*43*, *47*). The dehydrogenation must be continued until sufficient hydrogen has been abstracted on the average from each C_9- unit to give mainly high polymer (about 1.7–1.8 H per C_9). The dehydrogenation polymer obtained must then be purified and fractionated in exactly the same way as the spruce milled-wood lignin. Comparisons can then be made of the purified narrow polymer fractions.

Comparisons made between unpurified spruce milled-wood lignin fractions and unfractionated polymers made from coniferyl alcohol alone already revealed the great qualitative similarity between natural and biosynthetically duplicated lignins [see, for example, *K. Freudenberg* in (*94*), pp. 125–126]. When a purified lignin fraction is compared with an identical fraction made from a mixture of all three *p*-coumaryl alcohols, the resemblance is of course much greater.

The next question is which criteria are suitable to indicate unequivocally similarities or distinctions between lignin preparations. In practice, physical, analytical and chemical degradative techniques are used to characterize lignins.

Among the physical techniques, chromatography using polar solvent systems, electrophoresis in alkaline buffers or sedimentation in the ultracentrifuge can be used to test the homogeneity of samples. Simple infrared spectroscopy (in KBr) and ultraviolet spectroscopy (in dioxan/water) are of only limited value because of the large number of only slightly mutually differing chromophores or bond types in lignin. Infrared spectroscopy has been applied to establish the presence and aromaticity of lignin *in situ* in wood (*147*) and to differentiate some types of carbonyl absorption in lignin (*6*). An attempt has been made to evolve a taxonomy system based on the infrared spectra of lignins (*85*).

In the ultraviolet range, the peak at about 280 mμ shown by all lignins is very uncharacteristic: many lignols, some lignin precursors, degraded lignins, lignin model compounds, lignans, tannins, etc. all exhibit a peak in this region. More reliance can be placed on the ionization difference ($\Delta\varepsilon$) spectra of lignins (*11*). The curve obtained by subtracting the ultraviolet molar extinction of the lignin in neutral solution

from that in alkaline solution in the same solvent is characteristic for the number and type of phenolic hydroxyl groups in the lignin. The extinction should preferably be based on the molecular weight of the average C_9 unit in the copolymer (*46, 54a, 71*) and not simply on the methoxyl content of the sample as originally done. Ionization difference spectra of the reduced lignin ($\Delta\varepsilon_H$-spectra) give additional information or characteristics (*6, 7a*). The extinction in the trough at about 250 mμ in lignin spectra was used to determine the biphenyl content of lignin (*123*). The nuclear magnetic resonance spectra of lignins or their methylated or acetylated derivatives also provide highly characteristic data (*15, 103*). Electron paramagnetic spin resonance spectra indicate unpaired electrons in lignins (*54a, 140*).

A criterion for regarding a substance as a true lignin on the basis of its elemental analysis has been set up by *Freudenberg* and *Harkin* (*46, 54a*). In accordance with its origin from mixtures of the lower two or all three *p*-coumaryl alcohols and with the mechanisms of its formation, a lignin should have an elemental composition which when recalculated on the empirical basis of a single average phenylpropanoid unit gives the formula

$$C_9H_{\sim 8-x}O_2[H_2O]_{<1.0}[OCH_3]_x$$

where x is the mean methoxyl content per phenylpropanoid unit in the lignin and is less than 1.50. Mathematical expressions for recalculating lignin elemental analyses in this manner have been published (*46, 54a*).

The C_9-based formula represents the mean composition of all the units in the lignin copolymer. The C_9 basis derives from the propenylbenzene skeleton of the *p*-coumaryl alcohols, the O_2 term from their terminal phenolic and alcoholic oxygens which remain attached to the skeleton no matter what changes the lignin monomers undergo during polymerization (the elimination of the side chain of R_α radicals forms an insignificant exception – see below). The water term reflects the water added onto *p*-quinone methides of type (V) to give benzyl alcoholic groupings. This must be less than 1.0 molecule on the average per C_9, for this value represents the maximum that can be attained if every unit in lignin were a benzyl alcohol produced in this way. If the maximum value of 1.0 were in fact encountered, this would imply that lignin is a polyether derived exclusively from *p*-hydroxyphenylglycerol and its methoxylated derivatives, each interunitary ether linkage being between C-β of one molecule and the phenolic oxygen of the next [cf. (*40*)]. This is of course not normally the case, as innumerable elemental and functional-group analyses of non-sulfonated lignins have shown. A polyether of this kind would be far more susceptible to degradation reactions than lignin is found to be.

The hydrogen balance is the trickiest part of this formula. *p*-Coumaryl alcohol contains 10 hydrogens, coniferyl alcohol 9 and 1 methoxyl group, sinapyl alcohol 8 and 2 methoxyl groups. The alcohol mixture from which lignin is derived therefore contains 10-x hydrogens and x methoxyl groups, where x is again the mean methoxyl content per alcohol (C_9) molecule. The figure of ~8-x therefore indicates that on the average almost 2 atoms of hydrogen have been removed from each phenylpropanoid skeleton during the polymerization process leading to lignin. Loss of hydrogen from the coumaryl alcohols is promoted by repeated dehydrogenation of phenolic groups, first in the monomers and then in the lignols, and by oxidation of the terminal primary alcoholic and secondary (benzyl) alcoholic groups to the corresponding carbonyl functions [cf. the side-chains of Units 10 and 13a in Fig. 9] by radical transfer and disproportionation reactions. Oxidation of some guaiacylglycerol-β-aryl ether moieties may introduce some unconjugated carbonyl groups at C-β [cf. (*6*) and the side-chains of Units 2 and 18 in Fig. 9] into lignin and thereby slightly depress its hydrogen content; however, no mechanism for this reaction has yet been conceived.

Disregarding for the moment any hydrogen losses incurred by reactions leading to carbonyl groups in lignin, if the only polymerization process occurring during lignification were dehydrogenative condensation, each monomer would have to lose 2 atoms of hydrogen in order to condense bilaterally to give a linear high polymer. Some *p*-coumaryl or coniferyl units would have to lose 3 atoms of hydrogen in order to give rise to branching, but owing to the statistical nature of the free radical combinations, branching of the lignin molecule could occur only seldom by this mechanism. The addition of phenols (lignols) onto *p*-quinone methides of type (V) gives rise to branching and increases the molecular size without dehydrogenations, so loss of less than 2 atoms of hydrogen per C_9 unit should actually be expected. However, oxidation of some primary alcoholic groups to aldehydes (*58*, *60a*) and acids (*50*) or of benzyl alcoholic groups to ketones (*71*, *123*, *124*) again increases the hydrogen loss to almost 2 atoms per C_9 again. Elimination of the hydroxypropenyl side-chains from radicals of type R_d does not distort the lignin formula very much, for it simulates only a trivially higher hydrogen loss and a slightly lower addition of water. Moreover, there are probably not many groups in lignins derived from R_d radicals, and their effect on the overall picture of the analytical composition is therefore negligible. In a mechanism proposed to explain the condensation of coniferyl radicals in the form R_b with free radical forms analogous to R_d of higher lignols with a guaiacylglycerol end-unit to give rise to 1,2-diguaiacylpropane-1,3-diol (XII), no loss of the C_3 side-chain occurs at all, for it remains adhering to the oligolignol residue in the form of an aldehyde (*105*). Beechwood lignin,

in which most of the lignols derived from R_d type radicals have been found (*116*), conforms well to the above formula. The formula is also well satisfied by carbohydrate-free milled-wood lignins isolated from various species of plants (*42, 54a*) and even from a lignite (*71*),[4] but not by lignin-carbohydrate complexes (*71*). Here the relatively high oxygen content of carbohydrates pushes the hydrogen content in the C_9 formula down to a value irreconcilable with that of the original *p*-coumaryl alcohol mixture, and the water apparently added is consequently greater than 1 mole per C_9 unit.

When only small amounts of milled-wood lignin are available, its carbohydrate impurities can be removed by acidic hydrolysis (e.g. with sulfuric acid according to *Klason*); the accompanying condensations in the lignin are unimportant because the lignin is intended only for elemental analysis by combustion. Since thioglycolic acid is a convenient reagent for hydrolysing lignin-carbohydrate bonds, formulae for recalculating thioglycolic acid lignins on a C_9-basis have also been published (*54a*).

Since lignin is not a uniform entity, chemical criteria for its characterization have centred around analytical determinations of its functional groups, e.g. total hydroxyl content, phenolic hydroxyls (*56*), methoxyl and other ether groups, benzyl alcohol groups (*7a*), carbonyl groups (*6*), etc., and estimations of its content of special structural features, e.g. phenylcoumaran units (*5*), biphenylyl linkages (*123*), etc.

In accordance with the random and polygenous nature of lignin molecules, a relatively large limit of error is incurred in any analytical assay on lignin, and values finally published are often averages of a vast number of widely varying estimations. Reactions that work well with simple lignin models are frequently disappointing when applied to lignin because of the greater condensation of the types of groupings being analysed and because of interferences from alien groups.

It would take too much space here to review in detail the numerous methods that have been applied to assay all the functions in lignin; this is almost a specialized subject in itself. The prominent work of the Swedish schools in this connection has been well reviewed by *Adler* (*2, 7a*). References to the results of analytical work and how they are accurately reflected by his constitutional scheme for spruce lignin are given by *Freudenberg* (*41, 42, 43, 43a*).

Further chemical criteria for lignin take the form of the degradation reactions dealt with in the next section. Here again the results are of only qualitative or heuristic value.

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I. Chemical degradations of lignin

It is the primary aim of degradations of lignin either to provide information on the structure of lignin or to afford useful products for commercial exploitation. Frequently these aims overlap, but in practice, neither intention is entirely fulfilled. The root of the evil is to be found in the abundant carbon-carbon interunitary linkages in lignin. These prevent thorough degradations to high yields of low molecular-weight materials.

The approaches adopted in attacking the lignin molecule can be divided into three categories: oxidative, reductive and hydrolytic degradations.

Oxidative degradations of lignins do yield useful products with limited commercial markets. The production of aromatic aldehydes from softwood lignosulfonates is a well established industrial process for the production of vanillin as a flavoring agent [cf. (*9*)]. Hardwood lignins give too much syringaldehyde to be of use for vanillin production. The oxidation of lignin to the aldehydes is carried out in alkaline solution with nitrobenzene as oxidant in the laboratory (*57, 77, 96, 100*) or simply with air in industrial setups; copper catalysts are sometimes used [cf. (*35, 145*)]. It is the by-products of vanillin that are of interest for the structure of lignin. The *p*-hydroxybenzaldehyde and syringaldehyde accompanying vanillin even from conifer lignins first showed that lignin is a copolymer [cf. (*100*)]. Other lesser by-products such as dehydrodivanillin indicated the presence of certain bonding types in lignin (here biphenylyl links). The reaction is also of taxonomic or phylogenetic value [cf. (*145*)]; the higher the plant, the greater the amount of vanillin or syringaldehyde it gives. This degradation has found very extensive applications by lignin scientists; it would divert too much from the main theme to discuss this matter here. One of the latest developments was the use of thin-layer chromatography to separate the aldehyde mixture (*96*).

Oxidation of lignin to carboxylic acids, although intensely investigated, shows little commercial promise, even though cheaper oxidation methods can be used, e.g. aeration in alkaline media. The price of acids from other sources is too competitive and the mixture produced from lignin too complex for convenient separation and purification of single substances. On the other hand, it is the complexity of the mixture that makes this degradation so valuable for studies of the structure of lignin (*46, 47*). For structural work, the phenolic groups in the lignin are methylated before degrading the material in order to preserve as many aromatic rings as possible. Stronger, faster oxidizing agents such as permanganate can then be used. The aliphatic portions of lignin are

largely degraded leaving residual carboxyl groups adhering to the aromatic moieties. Separation and identification of the acids produced gives abundant information on the types of bonding present or on the substitution pattern of the aromatic rings in the original lignin (*46, 47, 56a*).

If the lignin degraded in this manner has been specifically labelled by introducing a radioactive lignin precursor beforehand to the plant, e.g. L-phenylalanine or D-coniferin, additional data can be secured by measuring the radioactivities of certain acids or specific carboxyl groups therein (*41, 48, 56a*). Conclusions can then be drawn concerning the substitution of even some of the aliphatic portions of the lignin polymer.

The similarities in the structure and yield of the acids obtained by this degradation from natural spruce lignin and a biosynthetic "spruce lignin" copolymer (mixed DHP) made *in vitro* from a mixture of the three *p*-coumaryl alcohols established the general identity of the two preparations (*46, 47*).

The structures of the degradation acids give strong support to the theory of lignin growth outlined in previous sections and to the formula for spruce lignin shown in Fig. 9 (*41, 42, 46, 43a*). They also indicated that other minor types of bonding occur in lignin that have not been mentioned above. Some condensations are observed in the 2- and 6-positions in aryl rings [cf. Unit 17 in Fig. 9]. Units condensed in this way may arise to a limited extent by condensation of radicals produced by radical transfers or through minor limiting forms of the ordinary phenoxyl radicals, or via nucleophilic condensative rearrangements of *p*-hydroxybenzyl aryl ethers owing to the slight acidity of the lignification medium. These findings are borne out by experiments *in vitro* with deuterated coniferyl alcohols (*56b*). This work also gave proof of the occurrence of diaryl ethers in lignin produced by condensations of R_a and R_c type radicals or R_a and R_d type radicals with subsequent elimination of the side chain (*28, 41*).

Since the first experiments on catalytic hydrogenation of lignin were carried out in 1938 (*72*), reductive degradations of lignins have been attempted using a wide variety of catalysts and conditions [see for example (*67, 77, 119, 135*)]. Although the propylcyclohexanols and propylphenols to be expected from this reaction would be valuable products for commercial applications, again the yields obtained in practice are very low and the products are too variegated for convenient separation. The carbon-carbon bonds in the lignin are again the villains responsible for the lack of success here. However, the products isolated and identified once more support the above theories of the nature of lignin and its derivation from phenylpropanoid precursors. The literature on this work can be traced back from the recent attempts to separate hydrogenolysis prod-

ucts by gas chromatography (*119*). After thorough testing in industry, even the currently most effective hydrogenolytic process, the Japanese Noguchi process (*93a*), has been practically abandoned because it is uneconomical (*67*). Alkali metals in liquid ammonia afford essentially the same results (*135*) as catalytic hydrogenation.

Chemical hydrolyses of lignins are of no commercial value, for the drastic conditions required to attain reasonable rates of hydrolysis of the stable ether bonds in lignin simultaneously cause intermolecular condensations of the molecule resulting in diphenylmethane-type products of higher rather than lower molecular weight and dark, brittle, bakelite-like consistency. The condensing molecules are the free or etherified benzyl alcohol groupings in lignin and, in part, formaldehyde eliminated from the hydroxymethyl end groups of a few other units. Moreover, once again too many units remain interlinked by C–C bonds, so only partial separation of units occurs.

Hydrolyses of lignin have however proved to be of immense value in helping to elucidate and consolidate the structure of lignin. The first effective hydrolysis of lignin was achieved by *Hibbert* and his coworkers by treating wood with ethanol and hydrochloric acid (*77*). A series of C_6–C_3 ketones were obtained which again affirmed the mainly coniferyl nature of softwood lignins and the predominance of coniferyl and sinapyl residues in hardwood lignins. It was later established that the ketones originated from guaiacylglycerol ethers, e.g. of type (VI) or (VIII), in the lignin [cf. (*94*)]. This simple degradation forms an excellent criterion for establishing the coniferyl or sinapyl nature of lignins. Since it characterizes lignins so well and since the phenylpropanoid skeleton remains intact, it has been used frequently in tracer work [cf. (*90, 91, 94*)]. The most recent improvement of the method is the separation of the ketones by gas chromatography (*95*).

Lately it has been realized that if the *p*-hydroxy- or *p*-alkoxybenzyl aryl ethers that occur in lignin (*56*) are hydrolysed carefully under extremely mild conditions, concurrent condensations can be largely avoided, and minute amounts of largely or completely unaltered lignin fractions can be isolated. Because of the statistical distribution of the benzyl aryl ethers throughout the lignin molecule, mono-, di-, tri-, tetra- . . . oligolignols are released. Isolation and identification of some of these degradation products established affirmatively that the lignols found during simulated lignification *in vitro* are really incorporated into the lignin molecule.

The first evidence of this kind was supplied by the Göteborg school [cf. (*1*)]; here 0.2 N HCl in 90 % aqueous dioxan was used for the hydrolysis. The slightly modified lignol products secured proved the presence of

structures of types (II) and (VI) in spruce lignin (*104*). Evidence was given later (*105*) for the participation of R_d type radicals in lignification with subsequent loss of their side chains leading to 1,2-guaiacylpropane-1,3-diol (XII) structures. Similar structures derived from sinapyl alcohol were recently also isolated from beechwood after hydrolysis with dilute acetic acid or simply hot water (*43, 48, 116*). Other unchanged mono- and dilignols, e.g. coniferyl and sinapyl alcohols and the corresponding aldehydes, dehydrodiconiferyl alcohol (II), DL-pinoresinol (IV) and its 5,5'-dimethoxy derivative syringaresinol, and a derivative of guaiacylglycerol-β-coniferyl ether (VI) were also isolated at almost the same time (*42, 48*); dilute acetic acid or 0.5 % HCl in anhydrous methanol was used as hydrolysing agent. Lately tri- and tetralignols containing units derived from R_d-type radicals have been isolated from beechwood hydrolysates (*41, 117*).

The yields of lignols liberated from beech are higher than those of the lignols obtained from spruce because hardwood lignins contain more arylglycerol-β,γ-diaryl ethers owing to their higher content of sinapyl type units. The higher content of sinapyl residues probably also results in a larger number of units derived from R_d type sinapyl radicals.

J. Biological degradations of lignins

So far no single enzyme is known that will effect rapid decomposition of lignin. Nevertheless, lignin can be degraded slowly by certain microorganisms and by soil bacteria [cf. (*39, 64, 84, 86, 132, 142*)]. When healthy wood is attacked by such microorganisms, its degradation is an undesirable pathological symptom and is thus important for forestry economics. Studies of the biological degradation of lignins have generally been prompted by only this latter consideration.

The microorganisms that will attack wood in this way are normally basidiomycetes species and are called white or brown rots depending on the color of the degraded wood residues remaining after their attack. Brown rots degrade preferentially the polysaccharides in the wood, but simultaneously demethylate, oxidise and degrade the lignin; hence the appearence of the brown color. White rots degrade lignin better and leave more cellulose, hence the brightening of the wood. No microorganism is yet known that will attack exclusively either the lignin or the polysaccharides in wood.

Attempts to isolate lignin by degrading wood with brown rot fungi afforded only degraded lignins of low molecular weight (<1000) [cf. (*33, 94, 132*)] similar to *Brauns'* soluble lignin. This is probably due

largely to the oxidative degradation of the lignin by small amounts of oxidases secreted by the brown rots.

Ironically, one of the main enzymes thought to be involved in the degradation of lignin is laccase (*38, 39, 94*), one of the two oxidases that may be involved in its synthesis (*55, 76b*). The laccase used to duplicate lignification *in vitro* is in fact derived from the edible mushroom *Agaricus* (*Psalliota*) *campestris* (*55, 82*) or the brown rot *Polyporus versicolor* (*37*). The latter was one of the species used to make enzymatically liberated lignin (*33*), so it is therefore not surprising that the lignin was degraded.

In nature, the microorganisms or the mushroom mycelia produce the oxidases laccase and peroxidase as exoenzymes, i.e. they secrete the enzymes into their environment in order to digest the lignin there extracellularly to molecules that are small enough to be resorbed for nutritive purposes. The toxic plant phenols are thereby conveniently converted into non-toxic quinones. Pentachlorophenol cannot be oxidised in this way and this is perhaps why it is such an effective fungistatic and fungicide. Lignin degradations of this type by purified laccase have been observed *in vitro* (*55*). Prolonged incubation of spruce milled-wood lignin with the enzyme led to separation of some benzene rings from their aliphatic attachment in the form of *p*-benzoquinones. Dehydrogenative attack of phenolic groups that does not lead to subsequent condensations can apparently lead to cleavage between the aromatic and aliphatic portions of lignin. Syringaresinol – the 5,5′-dimethoxy derivative of (IV) – is a sinapyl alcohol dilignol which cannot undergo condensations with phenoxyl radicals of its own species; its degradation by laccase is therefore particularly pronounced (*55*), only dimethoxy-*p*-benzoquine being isolable. Enzymatic dehydrogenation of sinapyl alcohol alone leads exclusively to syringaresinol and then causes degradation of the latter; this explains why no ligninlike polymer can be produced from sinapyl alcohol alone (*55*).

In recent work, lignin has been subjected to attack by complete white rot organisms that had been adapted to live on lignin as sole source of carbon (*64, 84*). Here the lignin is subjected to attack by a whole series of enzymes at once. Degradation does take place and some degradation products have been identified on chromatograms or by their ultraviolet spectra (*64, 84*). Mechanisms for the degradation have been published (*64, 84, 132, 144*). It is thought that an etherase which splits guaiacylglycerol-β-aryl ethers [cf. (VI)] is in operation during the degradation of lignin by white rots. This hypothesis is supported by the observation that some white rots degrade lignin but do not appear to produce extracellular oxidases (*86*). These species do not give the Bavendamm reaction, the standard test for white rots that is caused by their extra-

cellular oxidases [see, for example (*106*)]. However, no anaerobic degradation of lignin has ever been observed. Endeavors have been made to clarify the mechanisms of lignin degradation by white rots by feeding model compounds to the organisms, and isolation of the degrading enzymes has been attempted. Although the white rot *Polystictus versicolor* can metabolize guaiacylglycol- and guaiacylglycerol-β-guaiacyl ethers, it does not attack the corresponding veratryl compounds (*126 a*). Another white rot, *Fomes fomentarius*, demethylates veratrylglycerol-β-guaiacyl ether and other methylated phenols; it then degrades the guaiacylglycerol-β-guaiacyl ether formed to vanillin, vanillic acid and other products (*84*). This may suggest that the β-aryl ether bond is split by the action of a phenolase rather than by direct participation of an etherase. However, lately an enzyme has been secured from another fungus, *Poria subacida*, which releases guaiacol from veratrylglycerol-β-coniferyl ether although no other phenolic degradation product could be detected (*64 a*). This enzyme may be a true alkyl aryl etherase.

Naturally lignins that have been modified by chemical reactions, e.g. condensed or sulfonated by pulping, are far less susceptible to biological decay than natural lignins, because the weak, readily hydrolyzable benzyl aryl ether bonds or the readily oxidisable benzyl alcohols of guaiacylglycerol units have been sulfonated or transformed by condensation reactions into strong carbon-carbon bonds.

The fact that the lignin of hardwoods, which are at the highest level of evolution on the phylogenetic scale, contains the most sinapyl-type elements is perhaps understandable in terms of its biodegradability. The higher methoxyl content of the sinapyl units reduces the possibilities for carbon-carbon interunitary linkages in hardwood lignin compared with conifer lignin. That hardwood lignin is a weaker lignin than conifer lignin is revealed by its greater susceptibility to mild chemical hydrolysis (cf. Section I). Nature may prefer this weaker lignin in order to be able to recycle by biological decay the carbon from lignins that have outserved their purpose. The trees compensate for this weakening in their lignin by adapting the structure of their xylem cells from the long narrow tracheids of conifers to the thicker stunted hardwood cells.

Biological degradation of lignins in the soil proceeds by similar but more complex mechanisms that lead in time to the formation of humic acids. Again laccase appears to play a major initial role in this process (*39, 142*) but the breakdown of the lignin molecule is much more radical, for demethylations and opening of the aromatic rings occur (*39, 144*). Later condensations with amino acids take place. This topic leads away from that of lignin, so it will not be discussed at length here, especially since several reviews of the current knowledge on humic acids are available (*38, 139*).

K. Pulping research

The pulping processes in use at present are all relatively old and have generally been evolved from empirical experimentation. Excellent recent books are available on the chemical technology of pulping (*22, 26, 102, 115, 127, 128, 138*) and the two major chemical pulping processes have been thoroughly reviewed (*150, 151*).

Clarification of the chemical structure and reactivity of lignin has initiated much basic research on the nature of the processes occurring during the established pulping reactions and aftertreatment, e.g. bleaching, of pulps. Many of the vaguer ideas of the past as to what changes take place in the lignin molecules during pulping have been brought into clearer focus by studies on apt lignin models under pulping conditions. The Scandinavian schools have been prominent in this work (*1, 7, 66, 129*). The mechanisms of kraft pulping have thus been largely clarified. Here the lignin is solubilized mainly by reduction in its molecular size owing to extensive cleavage of its guaiacylglycerol-β-aryl ether components [cf. (VI) and Units 4/1, 5/4, 6/5, 7/8, 11/10, 12/15, 15/16, 13b/14b in Fig. 9]. Hydrosulfide ions catalyse this reaction and block reactive benzyl carbonium ion intermediates by nucleophilic addition; this prevents recondensation of the lignin fractions (*1, 66*).

In industrial research, attempts are being made to increase the yields or throughputs in existing methods (*73*), e.g. by the selection of optimum operating conditions, by using continuous digestion techniques, or by using promoter additives such as sodium borohydride (*74*) or polysulfides (*122*). Efforts are still being made to discover or evolve fundamentally novel pulping processes but so far no outstanding success has been achieved in such projects.

Interesting studies on the course of delignification during chemical pulping have recently been reported (*18, 88*). Periods of rapid and slow delignification can be differentiated and the release of lignin is thought to proceed via a free-radical mechanism (*88*).

Some of the observations made in pulping studies can perhaps now be interpreted better in terms of the readily hydrolysable benzyl aryl ethers [cf. structure (VIII) and Units 4/3, and 11/12 in Fig. 9] recently discovered in lignin (*56*). Cleavage of these weak ethers by alkali is almost instantaneous and the phenolic group liberated increases the alkali solubility of the lignin. The fast initial (bulk) delignification (*88*) might be due to cleavage of such ethers. As a reducing agent, borohydride prevents recondensation of hydrolysed lignin moieties by instantaneous reduction of the active benzyl carbonium ions produced when the benzyl aryl ethers are hydrolysed. Polysulfide also suppresses condensations of

the lignin released during pulping by oxidizing free benzyl alcohol groupings [cf. structure (VI) and Units 6, 12, 13b and 16 in Fig. 9] to ketones (*111*). The extra alkali used in combination with sodium borohydride retards reduction of such aryl ketones already present in lignin [cf. Unit 10] to condensable benzyl alcohols.

Knowledge of the structure and reactions of lignin has also helped to instigate searches for the types of structures that give rise to the chromophores that cause unwanted coloration in pulps (*7*). Initial attempts have been made to discover how these chromophores are destroyed during bleaching of pulps by oxidizing agents (*65, 129, 130*).

L. Utilization of lignin

Thousands of patents have been issued covering potential applications of lignins from pulping wastes or the production of useful lignin degradation products, but so far the real eureka has not yet been found. Several short reviews on the utilization of lignin have recently appeared (*9, 34, 92, 93a, 120, 121*). Here too attempts are being made to adopt a more fundamental approach to the problem by agglomerating more basic information on the structures of the lignin products that are to be utilized. Some of the methods that were applied in studying the structure of natural lignin have now been used to examine pulped lignins (*108*).

Some remarkable applications of lignosulfonates have been evolved of late that are based on their ability to act as sequestering or complexing agents for inorganic ions [cf. (*69*)]. These include the addition of lignosulfonates as a viscosity-controlling agent to oil-well drilling muds and as a dispersant in the purification of ores by flotation, in the preparation of ready-mixed concrete, and in the production of clay slips for making porcelain or ceramics. Low molecular-weight lignosulfonates can replace polyhydric alcohols as humectants in printing inks and dyeing pastes. The chelating properties of lignosulfonates also explain their use as a boiler-scale preventive and as a trace-element retainer in soil-improving agents. The long established use of waste liquors as a road-binder in non-asphalt roads and as a dust controlling agent in soft road shoulders is understandable in view of these properties of lignosulfonates.

Lignosulfonates have recently been tried as a filler for rubber but are slightly less efficient than carbon black, the cheap conventional filler with which it must compete. However, it is conceivable that lignin could increase the stability of rubber to ozone, the natural reagent which causes vulcanized rubber to "perish."

The production of vanillin for flavoring purposes still provides a major useful outlet for softwood lignins, but the lignosulfonates from a few mills suffice to saturate the market, even though the yield of vanillin is low.

Some lignosulfonic acids are used as a tanning auxiliary. The tanning effect is due to the sulfonic acid groups and not to the phenolic hydroxyls in the product: there is only about one free phenol group in every third C_9 unit in lignin and many of these may be sterically inaccessible. Leather tanned with lignosulfonates is therefore not water-proof.

Lignins from waste liquors are also used as extenders for phenoplasts and in adhesives for laminated paper and board, linoleum pastes, animal feedstuff pellets, etc.

A reaction that leads to undesirable by-products during kraft pulping has recently been subtly applied to produce a useful lignin degradation product. The inorganic sulfur compounds used to promote lignin solubilization during kraft pulping inavoidably cause some demethylation of its methoxylated aryl moieties giving rise to methyl thiol and dimethyl sulfide, which produce the obnoxious odors associated with such pulping installations. Now intentional demethylation of the lignin in spent liquors is carried out with sulfur compounds (*76*) in order to obtain dimethyl sulfide, which is then oxidised, e.g. with hydrogen peroxide, to dimethyl sulfoxide, a compound that has gained considerable prominence of late as a powerful solvent and a tissue-penetrating antirheumatic.

The lignin consumption in some of these applications runs into millions of pounds, but the current lignin production runs into millions of tons. It is with this thought that the cycle of the present discussion must close. The lack of success in finding economical applications or greater markets for more substantial amounts of lignin products is thus still motivating the extensive research that is currently being conducted in the field of lignin chemistry.

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