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INTRODUCTION

Lignin ranks second only to cellulose as the most abundant natural product on earth. As such, it constitutes one of the largest natural resources available to man. To date its use as a chemical raw material has been limited and where it can be found in substantial quantities as an isolated product of the pulp and paper industry, it has largely been discarded.

Lignin is a complex three-dimensional aromatic polymer formed solely in the walls of living plants of the Spermatophytes, the Pteridophytes and some mosses (Roelofsen, 1956). It occurs most commonly in woody plants such as trees and shrubs but can also be found in such common plants as corn, sugar cane, bamboo and ferns. It also produces an undesirable "woody" taste in various food crops such as alfalfa, asparagus, beets, carrots and turnips (Harkin, 1969). While most of the lignin in a tree can be found in the xylem cells or wood of the trunk, it may also be found in the pith, bark, roots, branches, leaves, fruits and seeds.

Research on lignin has been carried out for over a century, starting with the observations of Anselme Payen in 1838, who described the encrusting materials which surrounded the cellulose in the woody cell wall (Pearl, 1964). These encrusting materials were later termed lignin, which is derived from *lignum*, the Latin name for wood. Research on various aspects of lignin is being carried out in many diversified fields, and it is apparent that individual scientists have a varying concept of what the term lignin means. This point has been emphasized by Pearl who refers to the "nebulous" concept of lignin (Pearl, 1967b). The voluminous results of lignin research have been the subject of numerous texts and are currently being reviewed on an annual basis in various journals (Pearl, 1967a, 1969a).

The botanist considers lignin as a normal metabolite which is characteristic of growing woody plants. Probably the major biological role of lignin is the formation, together with the cellulose and other carbohydrates, of a tissue of excellent strength and durability (Kratzl, 1965). The structure of the fully grown cell wall is stabilized by incrustation with lignin (Frey-Wyssling, Mühlethaler, 1965). While the tensile

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strength of the wall is largely due to the cellulose, lignification increases its rigidity and compressive strength. In terms of evolution, lignin first appeared in the transition from aquatic to terrestrial plants where there was a greater mechanical support requirement.

Types of Lignin

Lignin in its natural state in the plant is called protolignin. Generally, lignin can be isolated by dissolving it out of the cell wall or by dissolving the non-lignin components of the wall which surround the lignin. Many techniques have been used to isolate lignin by both methods. However, when lignin is separated from the other cell wall materials with which it is normally associated, mainly cellulose and hemicellulose, it is invariably altered. No method has yet been devised to isolate the lignin of wood in a form identical with that in its natural state, *i.e.*, protolignin (Pearl, 1964).

Each technique used to isolate lignin tends to produce similar, but slightly different materials. The isolated lignins are commonly known by the name of the treatment used for their separation. Isolation methods over the past century have resulted in various "types" of lignin. One of the oldest types is the "Klason", or "sulfuric acid" lignin which is produced by extracting wood meal with alcohol-benzene and then treating it with sulfuric acid. Other types of lignin produced later, which also involved removing the non-lignin components, include Willslätter lignin, Purves lignin and Freudenberg or cuproxam lignin. Other techniques which involve removing the lignin from the non-lignin component include Brauns or "native" lignin, and Björkman or "milled wood lignin" Björkman lignin is closer to protolignin than any other type and has become the chief reference material for fundamental studies on the nature of lignin (Kollmann, Côté, 1966).

In addition to the types of lignin based on methods of isolation, lignins may also be classified on the basis of chemical structure and origin. Not all protolignins are identical. Three well-known types are recognized: gymnosperm lignin, dicotyledonous angiosperm lignin and monocotyledonous angiosperm lignin. Some recent work has demonstrated that these general types might be further divided into subtypes (Kawamura, Bland, 1967). While softwood (gymnosperm) lignin contains guaiacyl units, hardwood (dicotyledonous angiosperm) lignin contains both guaiacyl and syringyl units. This difference allows one to distinguish between a softwood which turns brown and a hardwood which turns red with the aid of the Mäule reaction (Wise, Jahn, 1952). Work by Northcote (1958) and more recently by Sarkanen and co-

workers (1967a) has indicated that lignins may vary from one wood species to another and also within the same species.

Many monocotyledonous angiosperms (grasses, sedges, rushes and palms) in addition to guaiacyl and syringyl, contain *p*-hydroxyphenyl groups (Timell, 1968).

Other work has suggested that there is a difference between the lignin obtained from juvenile and mature wood (Choulet, *et al.*, 1965) from sapwood and heartwood, from earlywood and latewood (Goring, 1964) and from inner bark and outer bark (Swan, 1966). With regard to reaction wood, lignin in compression wood, but not in tension wood, differs chemically from normal lignin to some degree (Bland, 1958, 1961; Croon, 1961).

Chemical Structure

The organic chemist views lignin as a complex polymer that challenges his fundamental interest in chemical structure (Pearl, 1964). The structure of lignin has been very difficult to determine since it is insoluble and cannot be hydrolyzed by acids, it has no regular structure or simple repeating unit, and it is difficult to remove from the cell wall without modification (Kratzl, 1965). Through the collaboration of organic chemists, biochemists, botanists and plant physiologists, numerous techniques have been used to theorize the structure of lignin (Kratzl, 1965). Some of the most recent work has been carried out by plant pathologists who have utilized the selective enzymatic action of certain fungi on model compounds closely related to lignin (Kirk, 1968).

It was felt by some that the structure of lignin is now fairly well known (Kollmann, Côté, 1966). Only carbon, hydrogen and oxygen are present in the lignin molecules, although the percentage of each varies considerably among different types of lignin. The basic building unit for all lignins is the phenyl propane unit. These units are linked together by C-O-C and C-C bonds in such a way as to form a three-dimensional polymer by cross-linking. This cross-linked polymer forms a molecular network which extends throughout the wood. Thus, one might consider all the lignin in a tree to be a single, oversize macromolecule (Goring, 1964). Some of the most extensive models of the lignin marcromolecule have been developed by Freudenberg (1966) and Adler (1961). Whether or not there is a bond between lignin and the carbohydrate fraction of the cell wall, especially the hemicelluloses, is still open to question (Kollmann, Côté, 1966).

Properties

Protolignin is a completely amorphous substance which is believed to be colorless (Timell, 1968) although the latter question has still not been completely answered (Goring, 1969b). While some believe protolignin has a much lower hygroscopicity than cellulose (Panshin, *et al.*, 1964) others have shown that lignin in wood may absorb about two-thirds as much water as cellulose (Christensen, Kelsey, 1968; Goring, 1969b). Protolignin has thermoplastic properties which are important in the bonding of fiber and particle board (Panshin, *et al.*, 1964). While some feel these properties are lost once the lignin has been separated from the wood (Panshin, *et al.*, 1964) others have shown that isolated lignins do have thermoplastic properties (Goring, 1963). The plastic properties of protolignin when hot and wet also explain, in part, why wood can be bent and formed by steaming (Harkin, 1969).

Most other known properties of lignin are those determined from isolated lignins which are usually brown or cream colored amorphous powders (Anonymous, 1964; Libby, 1962). The following properties are those of isolated lignins unless otherwise noted.

Kratzl (1965) has listed the main chemical reactions which can be used to define lignin, although Pearl (1964) indicates that most of the lignins being studied would not qualify under this somewhat rigid set of criteria. The analytical chemist usually regards lignin as a substance that remains insoluble after treating plant material with 72% sulfuric acid followed by dilution and boiling (Panshin, *et al.*, 1964). Commonly two types of chemical reactions of lignins are recognized, those involving functional groups but no change in molecular size and those which change molecular size but do not alter the functional groups. The chemical reactions of isolated lignins depend to a large extent on the method of isolation.

The problem of defining the chemical structure of lignin at one point in time was summarized by Karl Freudenberg who noted that the chemical characterization of coniferous lignin emanates from an idealized representation for which spruce lignin is the prototype (Pearl, 1964). On this basis, lignin has been found to be insoluble in water, most organic solvents and strong sulfuric acid. It is unhydrolyzable by acids, readily oxidizable, soluble in hot alkali and bisulfite solutions, and readily condenses with alcoholic, phenolic, and thio compounds. Lignin may be readily decomposed with chlorine.

Lignin may also be decomposed in nature through the chemical action of enzymes. While microbiologists and soil chemists consider lignin as a residue of decay organisms which degrade the polysaccharide fraction, lignin can be liberated from the polysaccharide fraction by

certain fungi, such as the brown rots, which utilize the lignin as a source of carbon and energy (Sopko, 1966). The degradation of lignin is important in humus production and in other areas of agricultural chemistry (Panshin, *et al.*, 1964; Kratzl, 1965). Plant pathologists are also concerned with the enzymatic alteration of lignin such as takes place by pectolytic enzymes in wood infected with *Ceratocystic ulmi* which causes Dutch elm disease (Gagnon, 1967).

An important property of lignin is its molecular weight. While the exact molecular weight of protolignin is as yet undetermined, that of Björkman lignin is 11,000 while that of isolated lignosulfonic acids ranges from 260 to 50 million (Goring, 1962). The molecular size distribution of lignin remaining in wood decayed by brown-rot fungi has recently been studied by Cowling and Brown (1968). It was found that after fungi had removed the carbohydrates, the molecular weight of the remaining lignin was higher from the more heavily decayed wood. In the most severely decayed wood, the lignin consisted of at least two components of widely different molecular weight. Results suggested that enzymatically liberated lignins are finite rather than "infinite" and are probably distributed across the cell wall with the highest molecular weight component in the compound middle lamella.

Lignin shows a maximum ultra-violet absorption at 282 millimicrons so that the lignification process of the cell may be followed quantitatively in the ultra-violet microscope with the aid of the 280 millimicron line of a mercury lamp (Frey-Wyssling, Mühlethaler, 1965). Isolated lignins have been found to absorb about 14 liters per gram–cm and lignin sulfonates about 12.5 liters (Libby, 1962).

Since isolated lignins are generally amorphous and not crystalline, no definite melting points exist for them although some lignins show definite softening points at elevated temperatures. Goring found that glass transition temperatures were between 135°C and 230°C depending on the method of isolation (Goring, 1964). The sorption of water caused a marked decrease in the softening temperature even though lignin is not plasticized by water in the same way as cellulose. Generally, since lignin is not as hygroscopic as cellulose, its presence decreases the water sorption of wood and hence increases its dimensional stability, particularly with respect to moisture content changes (Panshin, *et al.*, 1964).

The mechanical properties of wood are intimately tied to its chemical composition. The lignified cell wall has a structure comparable to that of reinforced concrete, the cellulose microfibrils being similar to the iron rods and providing tensile strength while the lignin serves as an elastic ground substance with high compressive strength (Frey-Wyssling,

Mühlethaler, 1965). Thus the lignin greatly increases the rigidity of the cell wall and makes the upright growth habit of the plant possible by stiffening the individual cells. While the lignin permeates the cell wall and is in intimate contact with the cellulose and hemicelluloses, as previously mentioned the question of a chemical bond between lignin and the holocellulose fraction is still not resolved. Such bonds would undoubtedly contribute to the overall mechanical behavior of the wall. The high concentration of lignin between cells in the region of the middle lamella serves to cement the individual cells together and facilitates the overall rigid structure of a piece of wood.

It is also possible that lignin plays an important role in the rheological properties of wood. While Eriksson (1967) found that delignification has no effect on creep, creep recovery or plastic flow of wood at a relative humidity of 70%, he found that above 80% relative humidity, creep increases. Work by Sarkanen and co-workers (1967b) concluded that the presence of varying ester groups seemed to constitute major differences in the structure of conifer lignins, both between species as well as within the same species. This work further suggested that the formation of such ester groups may serve as a natural regulatory mechanism for controlling the rheological properties of wood.

Removal for Paper Manufacture

Pulping is concerned with the separation of wood into its individual cellular components by the removal of lignin which acts as a cementing substance between the fibers. The lignin network is broken into fragments by the hot pulping liquor. At the same time, lignin acquires hydrophilic groups which render these fragments water soluble (Goring, 1964). In sulphite cooking, these groups form sulphonates while in alkali pulping they form carboxylic and phenolic groups. Thus to a sulfite pulp manufacturer, lignin represents a sulfonated wood component which must be washed from the pulp and which must be withheld from any waterways. To a kraft or soda mill operator, it is a material which can be precipitated from the black liquors with acid (Pearl, 1964). Lignin is further removed during the bleaching stage of the pulping processes, the major purpose of the bleaching being to increase the brightness of the pulp. Procter and co-workers (1967) have used the ultra-violet microscope to follow the pattern of lignin removal during various types of pulping.

One of the biggest problems facing the pulp industry today is that of stream and air pollution. The magnitude of this problem can be somewhat realized if one considers that most woods contain between 15 and 35% lignin (Kratzl, 1965). For more than a century, pulp mills have

released the raw effluent into streams and rivers with little or no treatment. This effluent contains not only lignin-derived compounds but also carbohydrates derived from hemicelluloses, the lower molecular weight polysaccharide fraction in wood, as well as pulping chemicals. When these products are put in a stream, they are eventually broken down by various microrganisms if given enough time and oxygen. Initially, any wood carbohydrates present are broken down, followed by the lignin-derived compounds. Any remaining compounds such as resins and tannins may or may not be broken down. Numerous studies have dealt with this problem; one, for example, determined that less than half of the lignin compounds were oxidizable in a natural stream over a 100 day period (Reabe, 1968). One potential solution to this problem is that of electrophoresis convection with which spent sulphite liquor might be fractioned into lignin, sugar and salt moieties in one operation, provided the proper membrane is chosen (Goring, 1964; Dubey, *et al.*, 1965). Air pollution and odor formation from pulp mills is also an active field of research. Some of the most recent work, for example, has been carried out by McKean and co-workers (1965), Douglass and Price (1966) and Douglass and co-workers (1969) who determined that in kraft pulping, hardwoods produce more odoriferous compounds than softwoods.

Uses

The development of by-product utilization of the pulp and paper industry has been promoted by the growing concern for water and air pollution (Pearl, 1969b). There is a strong desire on the part of the pulp manufacturers to convert pulping wastes, which are largely being discarded, into revenue-producing by-products. Lignin-derived compounds constitute a large proportion of these wastes, and there are now about 3,600 patents covering uses of lignin (Harkin, 1969). For the paper industry, the most profitable use of lignin is as a component of finished paper (Pearl, 1964). This use of lignin has increased as a result of new high-yield pulping processes combined with new refining methods (Anonymous, 1964). Additional uses for lignin-derived compounds may be divided into those obtained from sulfite pulping processes and those derived from alkaline processes.

The sulfite pulping processes yield whole spent sulfite liquor and ligno-sulfonates. The sulfite liquor has adhesive, dispersing and surface active properties which determine its use in linoleum pastes, foundry sand casting forms, emulsions, portland cement and ceramic mixes, road binders, animal feed pellet binders and the processes of soil stabilization, dust control and dye dispersion (Pearl, 1969b). Lignosul-

fonates have dispersant properties as well as the ability to combine with metallic ions, both of which make them suitable for use in controlling viscosity of oil well drilling muds, as concrete additives, as dispersing agents of dyes and pesticides, and in the process of potash extraction. Lignosulfonates are also used to produce vanilla flavoring and the overabundance of vanillin has in turn been utilized to produce vanillic acid, ethyl vanillate and other related compounds which have been used as food preservatives, as sunburn preparations and to treat certain diseases and skin fungi.

Thus far, most by-product utilization of the sulfite pulping process has been made with softwood lignin and lignosulfonates. Other uses in addition to those already mentioned have been described by Harkin (1969). The utilization of hardwood lignosulfonates is under investigation.

Alkaline pulping processes, including kraft and soda, require that the organic matter in the spent liquor be evaporated and burned for recovery of pulping chemical and heat. Although this is an economic method of handling the spent liquor, it is not the most profitable method of treating a valuable chemical raw material. Alkali lignins are utilized by industry or they may be sulfonated to produce lignosulfonates which have the many uses already described. Alkali lignins may also be used to produce aliphatic sulfur chemicals such as dimethyl sulfide and methyl mercaptan which are further converted to compounds used as solvents, anti-oxidants, agricultural chemicals and pharmaceuticals.

In addition to these major uses of lignin, numerous specialized uses have developed, some of which include: tree fertilization (Bratt, 1965), tree growth stimulation (Komissarov, *et al.*, 1965), wood varnish production (Mihailov, Gerdjokova, 1965), adhesion of wood (Tai, *et al.*, 1967) and production of plywood (Holderby, *et al.*, 1967), hardboard (Panshin, *et al.*, 1962), particle board (Nacu, Sbiera, 1966) and wall board (King, Adolphson, 1967).

Biosynthesis

The biosynthesis of lignin stands as one of the greatest achievements of wood chemistry and has involved the efforts of such noted researchers as Adler, Erdtman, Kratzl, Neish and Nord (Timell, 1968). Perhaps the most outstanding work was that of Freudenberg who prepared synthetic lignin. The determination of lignin biosynthesis has been closely allied to structural determinations and has formed the basis of a recent book by Freudenberg and Neish (1969) on the constitution and biosynthesis of lignin.

While the structure of protolignin is still inexactly known, suffi-

cient evidence is available to provide a basis for determining biosynthetic pathways. The determination of these biosynthetic pathways has taken three main approaches: (1) identification of organic compounds and possible precursors of lignin present in wood cambial tissue (2) introduction of various organic compounds and ratioactive "labeled" compounds into living wood tissues and (3) preparation of ligninlike polymers or artifical lignins from various pure organic compounds (Libby, 1962). This has involved the work of organic, bio- and physical chemists as well as botanists, plant physiologists and plant pathologists.

It is generally accepted that lignin is a three-dimensional polymer made up of oxygenated cinnamyl alcohol monomers (Pearl, 1967b). Freudenberg has proposed three monomers as the precursors of the lignin of all plants, namely coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol. If this is correct, lignin biosynthesis may be considered in two phases, the formation of these three monomers and then the conversion of these monomers into lignin. To form the monomers, the plant must first take up carbon dioxide, synthesize a benzene ring, hydroxylate it in the proper positions and add a three carbon side chain with the correct oxidation level (Brown, 1969). Final formation of the lignin involves a dehydrogenative polymerization of the monomers (Pearl, 1964). The biochemical pathways involved in these steps are still incompletely known and constitute an active field on research.

Lignification Process

In order to understand how the lignification process takes place within the individual developing wood cell, one must first understand the basic structure of the cell wall. Although there are many types of wood cells, varying in size, shape and function, their cell wall structure is similar. Individual cells are separated by a middle lamella and each contains a primary wall inside of which is a secondary wall. The secondary wall is further divided into three layers, the outer S_1, the middle S_2 and the inner S_3. In addition, a thin layer known as the warty layer is sometimes found on the inside of the S_3 next to the cell lumen.

The deposition of lignin in the cell wall is known as lignification. When a cell is formed in the cambium it has a thin primary wall and subsequently starts a phase of rapid growth. During this phase of growth and expansion the cellulosic wall is highly plastic and unlignified. Lignification begins some time after initiation of the secondary wall. It has been suggested that lignification might be a mechanism for limiting cell growth (Wardrop, 1957).

The actual process of lignification was first observed by Sanio (1873) and Sachs (1874) who thought that it began in the intercellular

layer and later extended to the secondary wall. More detailed observations were made by Wardrop (1957) and Wardrop and Bland (1959) who observed that lignification begins in the primary wall adjacent to the corner thickenings of the intercellular substance. It then proceeds to the intercellular layer and also continues throughout the primary wall. In the primary wall lignification progresses first along the tangential wall and then the radial walls. The process then continues through S_1, S_2, and S_3 layers respectively. While this sequence of the process is generally accepted as universal, it has only been observed in relatively few species.

The relationship between the lignification sequence and the exact location of protolignin formation is still unknown. It is presently open to question whether the lignin precursors arise in the cambial zone (Freudenberg, 1952), within the cell (Timell, 1968), within the intercellular material (Wardrop, Bland, 1959) or a combination of these (Barskaya, 1962). Assuming these precursors do arise in the cytoplasm, one must resolve just which cytoplasmic organelles are involved in precursor production.

The seasonal variation in the pattern of lignification has been studied (Wardrop, 1957) as well as the time required for the process to take place (Necesany, *et al.*, 1965). Some observations have been made on the process in normal versus compression wood in balsam fir (Kutscha, 1968).

Distribution in the Cell Wall

The chemical use of wood depends on knowing how much of each chemical constituent is present and where it is located in the cell wall. As previously mentioned, lignified tissue may contain between 15 and 35% lignin (Kratzl, 1965). This lignin may be found in all layers of the cell wall including the middle lamella, primary wall, secondary wall (S_1, S_2, and S_3) and warty layer (Esau, 1965; Sachs, 1965). The concentration of lignin, however, varies within each layer. Generally the concentration of lignin decreases as one moves from the middle lamella towards the lumen although some work indicates that the S_3 layer may be more heavily lignified than the S_2 (Sachs, *et al.*, 1962; Côté, *et al.*, 1966). While previously it was felt that most of the lignin in wood was located in the middle lamella and primary wall (compound middle lamella), Berlyn and Mark (1965) indicate that somewhat less than 40% of the lignin in wood is located in this region. It was emphasized that while most of the compound middle lamella is lignin, most of the lignin of the cell wall as a whole is not in this region, but rather in the secondary wall. This has recently been confirmed by Fergus and co-

workers (1969) who determined that, for spruce, the lignin concentration in the compound middle lamella is about twice that in the secondary wall. However, the much larger volume of the secondary wall results in some 72 to 82% of the total lignin in wood being located in this layer. In addition, it was determined that the lignin concentration in the middle lamella was found to be equal to that in the cell corner middle lamella, the concentration in the cell corner middle lamella was nearly four times that in the secondary wall and the concentration in the primary wall, about twice that in the secondary wall. Further information on distribution in the wall may be found in a review by Milyutina and Sergeeva (1964).

The distribution of lignin across the cell wall is not the same for all species or types of wood within species. Softwoods, for example, have a larger proportion of cell wall lignin located in their secondary walls than do hardwoods (Sachs, *et al.*, 1963; Meier, 1955). The distribution of lignin in growth increments has been studied (Wu, Wilson, 1967), as well as the distribution in early and latewood cells (Fergus, *et al.*, 1969). Various cell types have been studied such as tracheids and ray cells (Côté, *et al.*, 1968), and vessels and fibers (Fergus, Goring, 1968). In compression wood tracheids there is a high concentration of lignin in the region between the S_1 and S_2 layers (Bailey, Kerr, 1937; Côté, *et al.*, 1968) while in tension wood fibers the gelatinous layer is essentially free of lignin (Wardrop, Dadswell, 1948, 1955; Bentum, *et al.*, 1969).

Effects of Lignin on Wall Properties

Due to the specific properties of lignin already discussed, one might imagine that lignification results in a marked change in cell wall properties. As lignin replaces the highly plastic matrix substance, the now fully-grown wall becomes stabilized or rigid and hard. Lignin inhibits biological attack of the wood, particularly by fungi, and decreases the wood's permeability to gases and liquids (Sachs, *et al.*, 1962). Since the lignin decreases the water sorption of the wall it therefore increases the wall's dimensional stability with changes in moisture content. Mechanically, the lignin greatly increases the compressive strength of the wood.

Techniques for Studying the Lignification Process

Techniques which can be used to visualize the lignification process include the use of chemicals and stains, the use of ultra-violet and infrared light and the use of electron microscopy.

Lignified plant tissue gives characteristic color reactions with numerous organic and inorganic reagents. In the early nineteenth cen-

tury Runge (1834) treated sprucewood with phenol and hydrochloric acid which gave a greenish-blue color and with aniline sulfate which gave a yellow color. Since then, a large number of color reactions which are characteristic of lignified material was determined, most of which may be found in the references listed by Pearl (1967b). The major reactions have been classified by Browning (1967). Since the lignin polymer maintains some of the phenolic properties of the monomer it is slightly acid and therefore stains with basic dyes. Its aromatic structure accounts for certain colorations such as the cherry red produced with phloroglucinol and hydrochloric acid. The phloroglucinal reaction, while reasonably specific but not too sensitive, is probably the most commonly used lignin reagent among botanists and may be used on fresh, fixed, freeze-substituted or freeze-dried tissue (Jensen, 1962). The reaction should be used with caution, however, since in addition to lignin, phloroglucinal has also been known to stain wound gum (Esau, 1965). Other compounds recommended by Jensen (1962) as indicating lignin include Azure B, Schiff's reagent and the chlorine-sulfite test. Another common test is the Mäule reaction which allows one to distinguish between hardwood and softwood lignins (Gibbs, 1957). While new reagents are continually being found which appear to indicate the presence of lignin it should be emphasized that when using stains for lignin, a negative reaction does not necessarily indicate that lignin is absent (Jensen, 1962). Also, since the basis for most of the reactions is not completely understood care must be taken in their utilization.

Ultra-violet light has been used to study lignin with the fluorescence microscope and with the ultra-violet microscope.

The fluorescence microscope utilizes the principle whereby a substance is said to fluoresce if it is capable of absorbing invisible ultra-violet light which stimulates the emission of visible light. The color and intensity of the visible light is largely dependent on chemical composition, temperature, pH and density of the absorbing material. Usually the near ultra-violet part of the spectrum, from 300 to 400 millimicrons, is used since this is most efficient and includes the very intense 365 millimicron line (Needham, 1958). Lignified cell walls emit a striking blue light (Frey-Wyssling, 1964) and this phenomenon has been used to study the presence of lignin in the wall (Eichler, 1935; Barskii, Bardinskaya, 1959).

One problem which occurs when using this technique is that the lignin is degraded by the ultra-violet light to such an extent that the fluorescent image decays with time (Scott, Goring, 1969). Work by Leary (1968) has suggested that the yellowing of lignified materials, such as wood and newspaper, normally begins by oxidation initiated by

hydrogen removal from the phenols after excitation of a substance absorbing light near 365 millimicrons. This phenomenon then might also explain the decay in the fluorescent image.

The use of the ultra-violet microscope in studying lignin in the cell wall is based on the principle that lignin possesses a characteristic ultraviolet absorption spectrum with absorption maxima around 212 and 280 millimicrons (Brauns, 1952). Since no other components in the cell wall possess this characteristic, this method has been used to study the lignification process (Wardrop, Bland, 1959), to localize lignin in the wall (Lange, 1945) and to follow the removal of lignin from the wall by various pulping processes (Jayme, Torgersen, 1967; Goring, 1969a). While the method is an excellent one, and has recently been described by Scott, *et al.*, (1969) the apparatus is costly and its use is relatively complex (Jensen, 1962).

In addition to ultra-violet light, infrared absorption has been used to determine functional groups and linkages in certain lignin preparations (Pearl, 1967b). Infrared methods for detecting lignin have also been applied to wood sections (Tschammler, *et al.*, 1953) as well as to pulp and paper samples (Marton, 1967). Whether this technique is suitable for studying the lignification process in developing tissue is open to question.

The problem of using electron microscopy to study lignification arises from the fact that lignin cannot be distinguished from cellulose because their powers to diffract electrons are equal (Frey-Wyssling, Mühlethaler, 1965). Therefore both the unlignified and lignified wall appears homogeneous. This problem has been counterbalanced by the development of various techniques in an attempt to make the lignin visible. Techniques used to study the distribution of lignin in the cell wall have included the use of high molecular weight lignin indicators such as *p*-(acetoxy-mercuri) aniline or the removal of the polysaccharide component from the cell wall by acids and enzymes derived from fungi (Sachs, *et al.*, 1962; Côté, *et al.*, 1966; Côté, *et al.*, 1968). Another lignin indicator which has been used to study the lignification process is potassium permanganate (Wardrop, 1965; Kutscha, 1968). The removal of the polysaccharide component from developing tissue to study lignification has apparently not been used as a technique.

Factors Affecting Lignification Process

Lignification depends not only on the supply of a suitable precursor but also on the nature of the cells and the cell wall to which the precursor is available (Siegel, 1956). The search for precursors has led to tissue

culture work in which artificial lignification was produced (Wardrop, Davies, 1959) as well as the labeling of compounds which were introduced into the cambium and subsequently incorporated into the lignin of the mature wood (Kratzl, Billek, 1957). However, as previously pointed out, the exact origin of the precursors is still unknown and therefore their control in the living tree is still not possible. Work by Siegel (1956) has shown that the amount of lignin deposited in the cell wall depends on the chemical nature of the cellulose in the wall. Wardrop (1957) suggests that the selective deposition in the initial lignification of the primary wall may be related to the progressive withdrawal of cytoplasm from the wall.

Lignification may be affected by mechanical treatment or by environmental factors. For example, the removal of a ring of bark from the growing tree can cause changes in the normal lignification sequence in both softwoods and hardwoods at the cellular level (Wardrop, 1957). Evidence for environmental factors effecting the process is controversial. While the pattern of lignification remains substantially constant irrespective of growth conditions throughout the annual growth cycle, the ease of recognizing the different stages varies considerably (Wardrop, 1957). With respect to light condition, Kratzl (1948) has shown that lignification proceeds in the absence of light in potato plants while Phillips (1954) reported that shading of leaves in *Fraxinus* resulted in reduced lignification, and Necesany (1969) has shown that light deficiency more or less inhibits lignification of cell walls. The inhibition of lignification with light deficiency has been explained by Loomis (1953) in terms of a growth-differentation balance in which any factor, such as high light intensity or deficiency of water or nitrogen, which checks growth without reducing photosynthesis tends to increase differentation responses such as lignification. This balance is manipulated in the production of tobacco for cigar wrappers. In this case shaded conditions, which result in high humidity and lower light intensity, produce stimulated growth in the form of broad thin leaves and reduced differentation which gives a more pliable leaf of low lignin content (Wilson, Loomis, 1967). However, in still another study, a number of environmental factors were modified to see if lignification of seedlings was affected by, for example, several days in complete darkness, drastic reduction in nutrient supply, low temperatures, high temperatures, as well as defoliation and girdling (Anonymous, 1963). Surprisingly, none of the treatments appeared to affect lignification significantly in the latter study.

In Summary

Lignin constitutes a vast natural resource. It can be found in various forms depending on where it is obtained and how it is isolated. While its chemical structure is complex, it is now fairly well understood as are the basic chemical properties of lignin.

Wood can be broken down into its individual cellular components by various processes which remove the lignin. Most of these processes result in undesirable stream and air pollution and the largest percentage of the lignin removed is discarded. While numerous uses have been developed for lignin, many additional uses are still possible and desirable.

The biochemical formation of lignin and the anatomical aspects of the lignification process are still incompletely known. Neither have the distribution of the lignin in the cell wall and its affect on cell wall properties been completely established. While various microscopical techniques are currently available for visualizing the lignification processes, undoubtedly additional techniques will be developed in the future. Those genetic and environmental factors affecting the lignification process are understood very little at present.

In order to be able to control the production of lignin in nature we must thoroughly understand the lignification process including how lignin is metabolized, how it is layed down in the cell wall and where it is located in the wall. In order to most easily isolate lignin we must understand more thoroughly its chemical structure and how it is bound to the other wall components. To be able to use lignin more effectively as well as develop new uses for it we must understand its properties thoroughly in both the natural and isolated state. Likewise, the most effective use of lignin-containing materials such as wood and wood products depends on understanding how the properties of these materials are affected by the properties of lignin.

Thus one can see that lignin and its formation constitutes an active area of research and should continue to be such in order to realize the full potential of this untapped natural resource.

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