Lignin

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The objective of this chapter is to provide the reader with a general account of various aspects of lignin chemistry with emphasis on issues that have seen rapid growth during the past two decades. This is accomplished by describing the research efforts of over 360 literature citations, embarking from early concepts and concluding with the current views on the subject.

After a general introduction that deals with the occurrence and role of lignin in the cell wall and within woody tissue, recent advances of lignin biosynthesis are discussed commencing with a description of the metabolic pathways that determine the synthesis of the various lignin precursors. The main reactions leading to the various bonding patterns in lignin are then discussed including accumulating evidence that pertains to the connectivity of lignin to carbohydrates. The overall architecture of the lignin macromolecule is then dealt with by critically examining various aspects of the early literature and recent scientific evidence that points to the possibility of order in it. After a brief description of the methods available to isolate lignin, the chapter concludes with an outline of the various major procedures currently available for its structural determination.

1 Occurrence and Role of Lignin

Second only to cellulose, lignin is amongst the most abundant biopolymers on earth. It is estimated that the planet currently contains 3×10^{11} metric tons of lignin with an annual biosynthetic rate of approximately 2×10^{10} tons [1, 2]. Lignin constitutes approximately 30% of the dry weight of softwoods and about 20% of the weight of hardwoods [3]. Lignification is associated with the development of vascular systems in plants, providing resistance to biodegradation and environmental stresses such as changes in the balance of water and humidity [4]. Lignin is absent from primitive plants such as algae, and fungi which lack a vascular system and mechanical reinforcement. The presence of lignin within the cellulosic fibre wall, mixed with hemicelluloses, creates a naturally occurring composite material which imparts strength and rigidity to trees and plants. An additional role for lignin has recently been revealed [5–7] involving complexes of lignin phenolic acids in forage legumes and grasses. The presence of lignin phenolic acids is thought to inhibit the digestion of potentially digestible carbohydrates by ruminants.

Industrially one encounters lignin during the process of paper making, which involves the chemical or mechanical separation of the cellulosic fibres from woody or other lignified plant material. The chemical separation of lignin from cellulose has been termed "delignification" and it is one of the complex processes of the pulp and paper industry [8]. The process of delignification results in the production of vast amounts of lignin whose properties may vary depending on which delignification process has been employed and at which stage of delignification the lignin was isolated. In general, the lignin by-products resulting from the process of wood delignification are of a polymeric nature that may serve, after some modification, as additives in various formulations involving adhesives, thermosets or thermoplastics. Although chemical pulping is a long established practice, many controversial questions remain concerning the structure of native lignin (protolignin), its reactions during delignification and bleaching [9]. To expose the problem further requires an understanding of wood anatomy, lignin structure and biosynthesis and the way lignin is linked to carbohydrates in the cell wall. Since wood is the predominant raw material for the pulp and paper industry the focus of this chapter will be toward understanding wood derived lignins as opposed to those from annual plants.

1.1 Wood Structure

Figure 1 shows the detailed macroscopic composition of a softwood fibre. The cellulose microfibrils are arranged characteristically within the various layers in the cell wall, namely; the primary wall (P), the secondary wall, and the middle lamella (ML). The secondary wall is further subdivided into three sublayers (S_1, S_2, and S_3) each comprised of cellulose microfibrils in distinct orientations with reference to the main fibre axes. The S_1 and S_3 layers are both 0.1–0.2 μm in

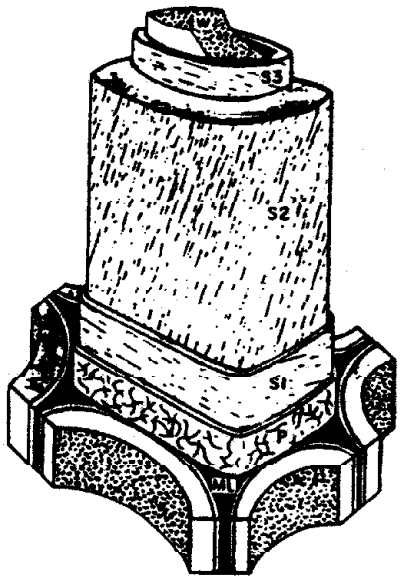
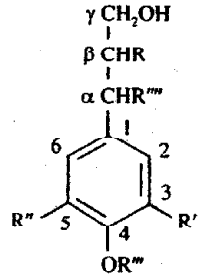


Fig. 1. Diagrammatic representation of a softwood tracheid (Reprinted with permission from Côte [12])

thickness while the S_2 layer is about 1–10 μm thick containing within it 80–95% of the cell wall material. The primary wall, (0.1 μm), contains a network of microfibrils and significantly higher lignin concentration than the secondary wall, while the middle lamella is composed predominantly of lignin [10]. Despite the fact that the concentration of lignin in the middle lamella is extremely high, it is the secondary wall that contains about 70% of the overall lignin present in wood due to its large volume [11].

Chemically, lignin is built from phenylpropane units linked together by different bonds. A description of how the different bonding patterns emerge will follow. However, at this point it is essential to mention the fundamentals of numbering the various carbon atoms in lignin. More specifically, while regular numbers are used for labelling the aromatic carbons, Greek lettering is used to label the side chain of the phenylpropanoid. The illustration of Scheme 1 may assist the reader in comprehending the meaning of the various bonding patterns described in latter parts of this chapter.



R = another phenyl propane unit

R''' = H or R

R'''' = OH or R

guaiacyl :	$R' = OCH_3$, $R'' = H$
syringyl :	$R' = R'' = OCH_3$
para-hydroxyphenyl :	$R' = R'' = H$

Scheme 1. The elementary phenylpropane building blocks of various lignins

2 Biosynthesis

Lignification is believed to occur in the intracellular layers of the cambium, where the sapwood and the bark layers of the tree merge [13]. Tracer experiments [14] and UV-microscopic observations [15] have shown that lignification is initiated within differentiating wood cells and extends to the intracellular areas, i.e. primary and secondary cell wall. Terashima [16] has shown that lignification and cellulose deposition in the plant cell wall proceeds in three distinct phases. Initially lignification occurs at the cell corner and middle lamella, after the deposition of pectins is complete and the formation of the secondary wall S_1 has been initiated. During the second phase, an extensive deposition of cellulose microfibrils, mannan and xylan in the S_2 layer takes place. Yet, the lignification process proceeds very slowly during this stage. Finally, during the third phase, lignification proceeds extensively. This occurs after the deposition of cellulose microfibrils in the S_3 layer of the secondary wall has taken place.

2.1 The Synthesis of Lignin Precursors

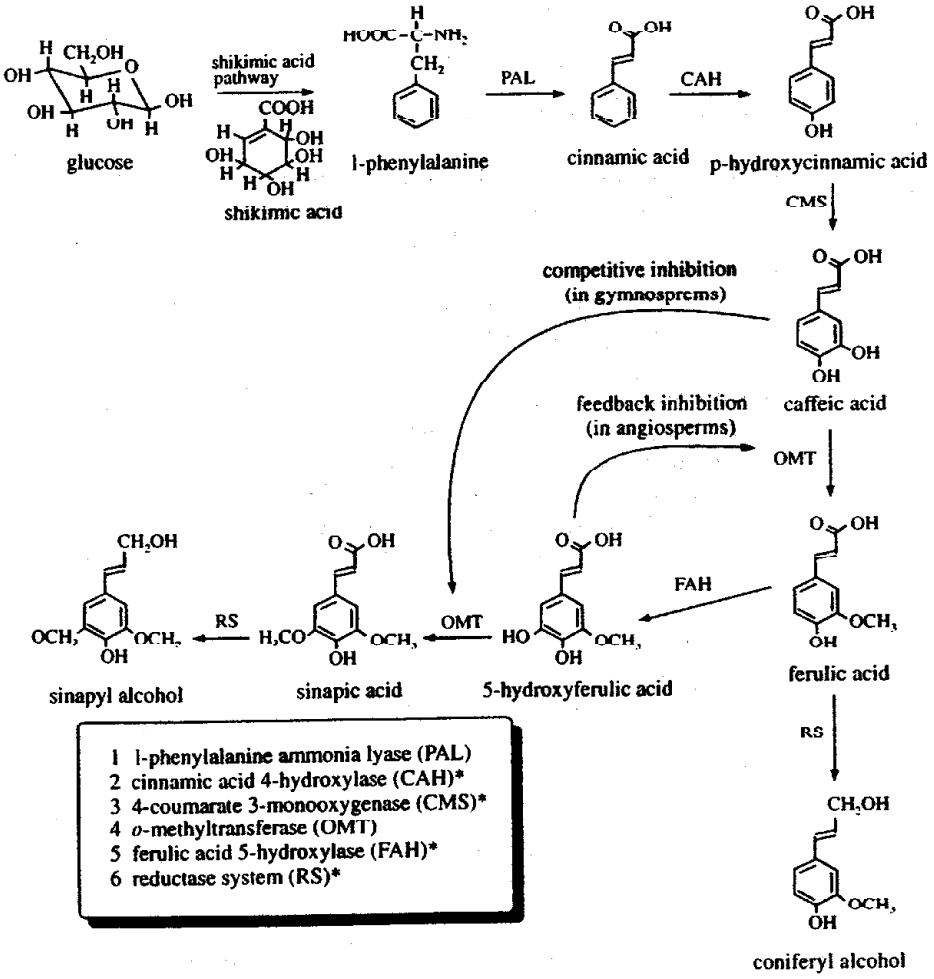
In 1971, it was demonstrated that lignin is synthesized from *l*-phenylalanine and cinnamic acids [15]. These acids are derived from carbohydrates through the shikimic and cinnamic acid pathways. Supporting evidence for this route was obtained when radioactive glucose was administered into plants, producing shikimic acid [17] and radioactive lignins [18–20]. Lignification proceeds with the conversion of *l*-phenylalanine to form *trans*-cinnamic acid (Scheme 2). This deamination process is catalyzed by *l*-phenylalanine ammonia lyase (PAL), a key enzyme found only in plants that can synthesize lignin [21, 22] and some cinnamic acid derivatives [22]. It is worth mentioning that an additional enzyme, tyrosine ammonia lyase (TAL), which catalyses the formation of *p*-coumaric acid from *l*-tyrosine, is characteristically found only in grasses [23–25]. The presence of this enzyme may account for the presence of *p*-coumaryl alcohol as an additional lignin monomer as well as esterified *p*-coumaric acid present mainly in grasses.

As lignification proceeds, cinnamic acid is hydroxylated to *p*-coumaric and caffeic acids by specific hydroxylase enzymes [26, 27]. The caffeic acid thus formed, is then methylated to ferulic acid by *O*-methyl transferase (OMT) [28–30]. Up to this point the biosynthetic pathways for softwood and hardwood lignins is believed to be common [15]. However, they seem to diverge beyond it [31, 32]. This is because OMT enzymes, of different functionality, possessing different substrate specificities were found to be present in softwoods and hardwoods [33, 34]. One of the reasons which accounts for the almost exclusive presence of guaiacyl lignin in softwoods, is that the monofunctional OMT enzyme is inhibited competitively by caffeic acid (Scheme 2). For hardwoods, the

difunctional OMT is inhibited via a feedback mechanism involving 5-hydroxyferulic acid [34, 35]. This limits the concentration of this intermediate and the production of ferulic acid, thus restricting the formation of syringyl precursors. Another key enzyme which is responsible for the differentiation of the G and G,S pathways, is the ferulic acid-5-hydroxylase.

The absence of this enzyme in gymnosperms also accounts for the near exclusive formation of guaiacyl precursors in softwoods.

The synthesis of lignin precursors is complete with the reduction of ferulic and sinapic acids to the corresponding coniferyl and sinapyl alcohols. These reactions are catalysed by three specific enzymes which constitute the reductase



* These abbreviations have been used for brevity purposes only

Scheme 2. A simplified metabolic pathway of l-phenylalanine to lignin precursors

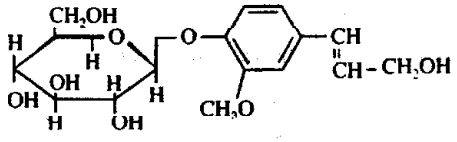
system [32, 35]. The enzymes which were recently isolated from various plants [36, 37] include the hydroxycinnamate-CoA ligase, hydroxycinnamoyl-CoA reductase and hydroxycinnamyl alcohol dehydrogenase. The ligase and reductase enzymes of angiosperms and gymnosperms respectively, were found to show pronounced differences in substrate specificities [30, 38]. In gymnosperms, both enzymes were found to be inactive with sinapic acid and sinapoyl-CoA [39, 30]. The lack of activation or reduction of sinapate in gymnosperms contributes toward the exclusive formation of guaiacyl lignins in them.

The lignin precursors formed are of low solubility and are readily oxidized; in the cell wall they are stabilized as *glucosides*. The typical glucoside for softwoods is coniferin and its structure is shown in Scheme 3.

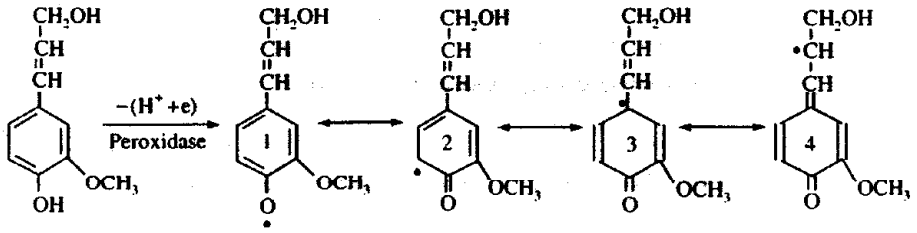
2.2 *The Dehydrogenation of the Precursors*

Early investigations [40, 41] coupled with the more recent results of Harkin and Obst [42] have shown that the dehydrogenative polymerization of lignin monomers in plants is caused by a class of enzymes called peroxidases, or the peroxidase-H_2O_2 system. These enzymes, are capable of abstracting a proton from the phenolic hydroxyl of the precursor molecules, creating resonance stabilized free radicals. Examples of such radicals, typical of softwood lignin are depicted in Scheme 4 [42, 43].

More specifically, the dehydrogenation reaction involves hydrogen peroxide as the electron-accepting substrate for the peroxidase [45, 46]. In addition, superoxide radicals were suggested to form via the reduction of oxygen by NAD (nicotinamide adenine dinucleotide) [47]. Recent evidence suggests that hydrogen peroxide is produced by the peroxidase enzyme itself, and may play a key role in the control mechanism [47]. An enzymatically controlled cycle for the



Scheme 3. Coniferin, a coniferyl alcohol glucoside



Scheme 4. Resonance forms of softwood lignin phenoxy radicals (43, 44)

production of hydrogen peroxide, has also been reported [42]. However, excess peroxide was found to inactivate the peroxidase by the formation of a complex of an unknown structure [48]. Recent research has also shown that the presence of laccase-like phenoloxidase can be correlated to lignin biosynthesis in *Zinnia elegans* stem tissues [49]. A review of the recent literature regarding plant laccases and selected fungal laccases and their role during lignin biosynthesis has been compiled by Dean and Eriksson [50].

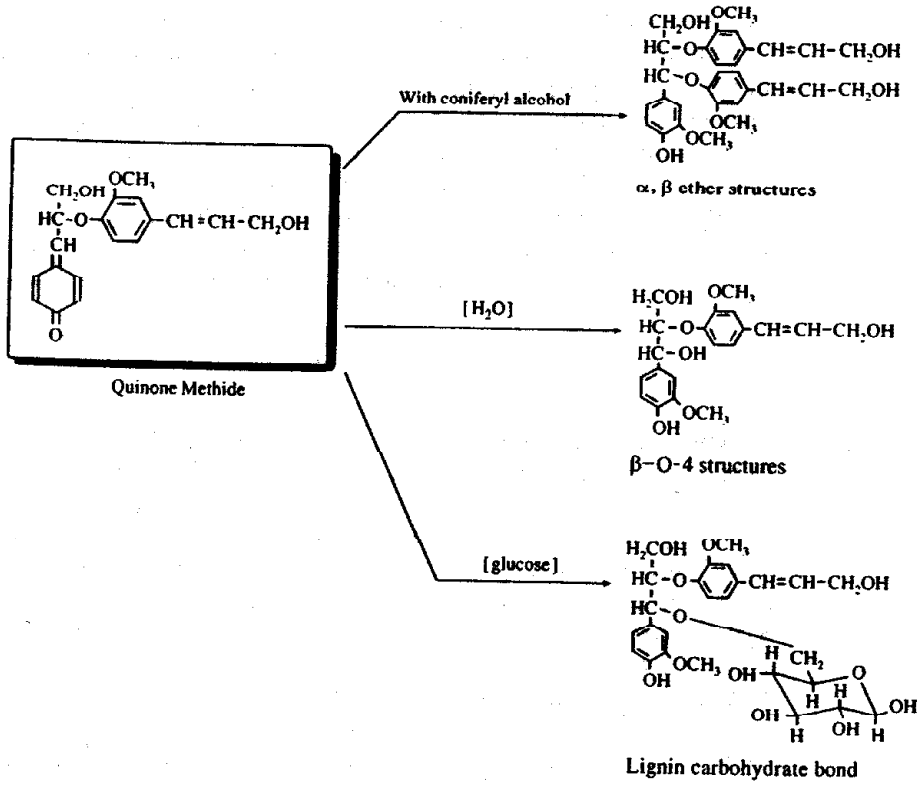
After the oxidation of the monomeric alcohols to phenoxy radicals, the reaction changes dramatically; the reactions are no longer subjected to enzymatic control, but to a random polymerization process [51, 52].

2.3 The Radical Polymerization

A major milestone in lignin chemistry was Freundenberg's success in providing experimental evidence for the enzyme-initiated dehydrogenative polymerization theory. This became possible by polymerizing coniferyl alcohol to a lignin-like dehydrogenation polymer (DHP) using liquid squeezed from the mushroom, *Psalliota campestris*, known to contain laccase and other oxidative enzymes [50]. Further attempts to synthesize lignin DHP's using the peroxidase/H_2O_2 system were successfully accomplished by Freudenderg [53, 54] and Sarkanen [55]. Yet, as Sarkanen pointed out, "these lignin polymer models", despite the fact that structurally were lignin-like, they were not identical to lignins formed in vivo [55]. When the in vitro polymerization was interrupted, structures of dimeric and oligomeric intermediates could be isolated and the lignin bonding patterns identified [56–58].

These experiments and others, involving sinapyl alcohol, support a random mechanism for the polymerization of phenoxy radicals [59–61]. The reactivity of such radicals and the probability of a specific mesomeric form to form a particular linkage depends on β electron spin density and steric considerations. Molecular orbital calculations of β electron spin densities of lignin model compounds [62] have shown that free electron spin densities are highest at specific sites within the phenylpropane unit. These reactive sites (Scheme 4) are the C_1 and C_5 positions of the phenylpropane unit, the phenolic hydrogen, and the aliphatic β-carbon. Of these reactive sites, those with groups attached at C_1 and C_3 in coniferyl alcohol and C_1, C_3 and C_5 in sinapyl alcohol, are less reactive. Alternatively, the phenoxy oxygen and the β carbon are considered as the most reactive species, readily coupling into aryl ether linkages [63, 64]. This may account for the high abundance of the β-*O*-4 inter-unit linkages in lignin, estimated to be as high as 50% in softwoods and almost 60% in hardwoods [64].

The polymerization process proceeds with the coupling of the different resonance structures. Amongst the products, highly reactive quinone methides (Scheme 5) are formed which further react by addition to various nucleophiles. Free radical exchange reactions, such as hydrogen abstraction from suitable portions of other molecules, are also possible [65, 66].

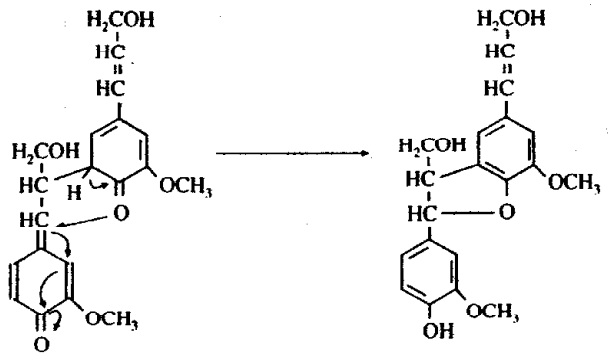


Scheme 5. Addition reactions to a quinone methide leading to the formation of the various inter-unit linkages in lignin

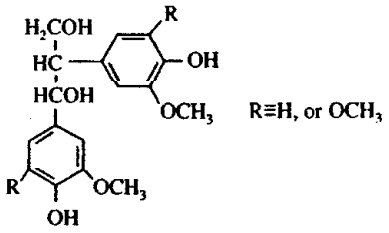
The first dimeric lignol to be isolated and identified was a phenylcoumaran structure analogous to that of dehydrodiconiferyl alcohol [67]. This structure, which comprises about 10% of the lignin units, is formed by the coupling of two free coniferyl alcohol radicals centered at C_5 and C_β positions of the phenylpropanoid (structures **2** and **4** in Scheme 4), giving rise to a dimeric quinonemethide. This coupling followed by aromatization of the ring allows the creation of an a-*O*-4 linkage (Scheme 6). The C_β-C_5 bond thus formed is actually quite stable under pulping conditions, as long as its phenolic group is etherified.

Other bonding patterns that occur in lignin are the so-called "diaryl propane" units or β-1 (Scheme 7). They are present in about 5–10% of the total phenylpropane units in lignin and they are thought to be relatively stable under alkaline pulping conditions.

Ether and ester linkages are also common in lignin. With respect to ether linkages, several types have been identified to be present, namely: biaryl ethers, non-cyclic benzyl alkyl ethers, β-*O*-4 ethers and diphenyl ethers. Biaryl ethers



Scheme 6. The phenylcoumaran, (C_β-C_5) interunit linkage of lignin



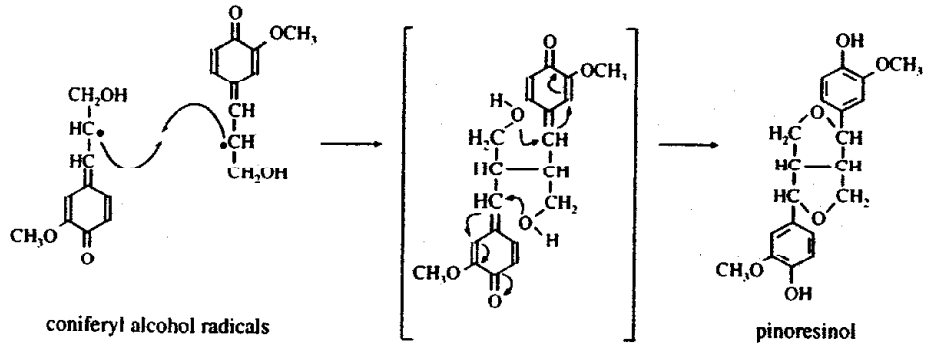
Scheme 7. The diaryl propane (β–1) interunit linkages of lignin

are generated by the combination of an *o*-methylquinonoid (structure 2 in Scheme 4) with a phenoxy radical [60, 68, 69]. They comprise only about 6% of the phenylpropanoid units in spruce lignin [60, 68]. Non-cyclic benzyl alkyl ethers, present in relatively small amounts in lignin (2–3%), have been cited for their beneficial effects during pulping [70]. However, recent attempts to detect them in milled wood lignin, using advanced NMR techniques, have failed [81]. The combination of C_β and phenoxy radicals (Scheme 5) results in the formation of β-*O*-4 linkages [68, 60, 69], linking 30–50% of the lignin units [64]. Structures which contain α, β ethers are relatively infrequent and have been estimated to account for less than 1% of the interunit linkages in lignin. However, recent efforts using advanced NMR techniques have questioned these levels [81, 364].

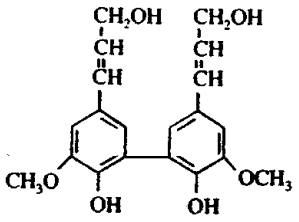
Another lignin structure, containing two ether groups is the pinoresinol (present in less than 5% of the units) [71]. This structure, is formed by the β-β coupling of two coniferyl alcohol radicals followed by double ring closure, as illustrated in Scheme 8.

Benzyl alcohol groups are also common structures in lignin. These, which account for more than 30% of the phenylpropane units, are formed by the addition of a water molecule to a quinonemethide.

Like aliphatic esters, the benzylic structures are also beneficial during alkaline pulping [72] since they facilitate cleavage of the arylglycerol-β-aryl ether residues under soda or kraft pulping conditions with the intermediate formation of epoxide or episulfide structures. 5-5′ biphenyl and diarylmethane structures which link two aromatic units either through the C_5-C_5 or Cα-C5 positions.



Scheme 8. Formation of pinoresinol linkages in lignin



Scheme 9. The biphenyl 5–5′ interunit linkage of lignin

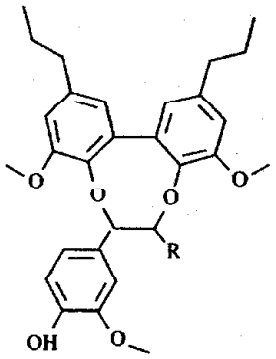
contain carbon-carbon bonds, which are stable under pulping conditions. While 5-5′ biphenyl units are present in the original wood the diarylmethane units are formed by the condensation of two aromatic rings in lignins during alkaline and/or kraft pulping (Scheme 9) [73].

As such, their importance becomes of significance in technical lignin preparations. The frequency of occurrence of such structures is obviously higher in pulped softwoods, since their lignin is composed almost exclusively of guaiacyl units which possess aromatic units that contain a free C5 position.

Recently Brunow's group announced the discovery of another bonding pattern present in softwoods lignin [74, 75]. This involves the formation of α, β ethers on the same 5-5′ biphenyl structures. The new octagonal moiety has been identified as the dibenzodioxocin of Scheme 10.

Based on these structural details, a number of lignin models have been proposed by a variety of investigators. Amongst them one may cite those of Freudenberg [68], Brauns [76], Erdtman [77], Adler [78], Forss and Fremer [79], and Glasser and Glasser [80]. Amongst the models proposed for softwood lignin, Freudenberg's is still the most widely cited [53, 68].

A very significant analytical effort has been carried out through the past several decades attempting to quantify the various bonding patterns in softwood and hardwood lignins. The results of some of these efforts are now summarized in Table 1.



R=CH_2OH, CH_3

Scheme 10. Dibenzodioxocin present in softwood lignin

Table 1. The frequency of the major linkages in softwood and hardwood lignins* [8, 55, 64, 81–84]

	% of Total Phenylpropane Units	
Type of Linkage	In Softwoods	In Hardwoods
β-Aryl ether (β-*O*-4)	45–48	60
α-Aryl ether (α-*O*-4)	6–8	6–8
Diphenyl ether (4-*O*-5)	3.5–8	6.5
α-Alkyl ether (α-*O*-γ)	small	small
Biphenyl (5-5')	9.5–17	4.5
β-1	7–10	8
Pinoresinol (β-β)	3	—
Phenylcoumaran (β-5)	9–12	6
Lignin-carbohydrate links	not determined	not determined
Dibenzodioxocin	≫	≫

* The numbering of the carbons used in the nomenclature of this table is shown in Scheme 1.

2.4 The Lignin-Carbohydrate Connectivity

Detailed studies of wood and grasses point to the likelihood that lignin is not simply deposited in the cell wall of polysaccharides but is closely linked and associated in a certain architecture with the carbohydrates [85–88]. A considerable research effort has been carried out in studying the nature of the actual chemical bonds between lignin and carbohydrates [89–91].

Advances in the technology of nuclear magnetic resonance spectroscopy coupled with a better understanding of the nuclear spin relaxation mechanism in solids, have provided a powerful tool for probing the molecular motion of carbohydrates and lignin in solid wood and pulp samples [92–95]. By using ferric ions as a probe, coupled to proton spin-lattice (T_1) and spin-lattice in a rotating frame ($T_{1\rho}$) relaxation time measurements, Gerasimowitz et al. [94] derived information related to the structural connectivity of carbohydrates and

lignin in wood pulp. Similar linkages were also invoked by Kolodziejski et al. who studied the ^{13}C CP/MAS NMR spectra of lodgepole pine wood [96]. By measuring the proton spin-lattice relaxation times of progressively sulphonated and methylated wood and pulp samples, Argyropoulos and Morin evaluated the molecular mobilities of lignin and carbohydrates in the presence of different counterions [95]. Lignin-carbohydrate associative interactions were invoked, once again, to rationalize for their observations. On another front, significant advances in clarifying this issue have been made by carrying out in vitro lignification experiments in the presence of model compounds. Small sugar molecules were found to undergo addition reactions resulting in the formation of ether or ester linkages [97–100]. Early work by Freudenberg demonstrated that sorbitol or sucrose can readily add to lignin, forming benzyl alkyl ether linkages [101]. These bonds were found to be further stabilized when the phenolic hydroxl group of the lignol portion was etherified. Such an etherification step is not uncommon to occur via a dehydrogenative mechanism during lignification. Such an ether linkage, which seems to be considerably more stable than those of glucosides, is considered to be one of the main reactions leading to a stable crosslink between lignin and plant polysaccharides. Another lignol-sugar that has been isolated by Harkin and Freudenberg from in vitro lignification experiments, is the guiacylglycerol-β,γ-disucrose ether [98]. The formation of this ether is believed to follow a radical mechanism which could be associated with that of lignin biosynthesis. According to this mechanism, coniferyl phenoxy radicals (catalysed by peroxidases) abstract a hydrogen from a sugar molecule to generate a sugar radical. This, quickly adds to another lignin C_β radical. The nature of the resulting bond, (C-C or ether) depends upon the formation of a carbon radical or an oxygen radical on the sugar molecule. However, the attachment of two sugar units on a phenyl propane unit is an unlikely event when one considers that during lignification a variety of competing nucleophiles are present, including water and other lignols. More recently Leary and co-workers have shown that sugars will readily add to monomeric lignin quinonemethides or benzyl alcohols forming predominantly C_β linked carbohydrate benzyl ethers or esters [102].

Ester bonds are also involved in the attachment of lignin to the plant carbohydrate moiety [103, 113]. Their importance is very significant for plants and grasses and somewhat less for wood. Ferulic acid in grasses is known to be esterified with carbohydrates, and etherified with lignin. However, the topochemistry of its attachment to lignin is not well understood [104–106]. Similarly, *p*-coumaric acid is known to be extensively esterified with lignin, but the regiochemistry of lignin acylation is still a matter of debate [107–109]. The following description of events represents the state of our knowledge as far as the biosynthetic pathways leading to such species are concerned. The α-position of quinone methides, formed during the dehydrogenative polymerization process [64], apart from being attacked by water, may also be attacked by free acids and alcohols, leading to α-esters and α-ethers. This is the case of feruloyl esters and phenolic glycosides of *p*-coumaric acid, where the free phenol or the free

carboxylic acid groups respectively, may trap quinone methides by an addition to yield α-ethers and α-esters [110, 111]. Alternatively, feruloyl esters can directly participate in the free radical polymerization process giving rise to a number of different structures [112]. Furthermore, enzymatically pre-esterified *p*-coumaric acid with *p*-hydroxycinnamyl alcohol monomers, may enable the formation of *p*-hydroxycinnamyl *p*-coumarates which could participate in the formation of the lignin macromolecule by conventional oxidative coupling reactions to yield γ-*p*-coumaroylated lignin [108].

While ether-linking ferulic acid to the α position of the lignin side chain, via "opportunistic" quinone methide trapping is still speculative, it has been reported by Ralph et al. [104] that feruloyl esters, if present in the lignifying matrix, are capable of participating in the free radical lignification process. Moreover, the identification of new ether-linked ferulic acid-coniferyl alcohol dimers in grass straws, by Jacquet et al. [106], demonstrates the occurrence of radical coupling reactions between ferulic acid and coniferyl alcohol to yield β-aryl ether structures.

As noted above, the lignin-carbohydrate bonds are possible due to the reaction of quinone methides with various lignols and carbohydrates during lignin biosynthesis. Despite this there is still controversy whether these bonds occur between lignin and cellulose or between lignin and hemicelluloses. This is certainly not an easy issue to resolve but the evidence is more in favour of a predominant lignin-hemicellulose connection. Since in hardwoods the predominant hemicellulose is xylan and considering that about 90% of this sugar can be removed by a mild alkaline treatment, ester links have been proposed to occur between the lignin and the C_6 uronic acid groups of the sugar [85, 114].

3 Lignin Architecture

Amongst the features of any delignification experiment is the observation that the molecular weights of the solubilized lignin become progressively higher as delignification proceeds [114–125]. Molecules of relatively small size become soluble early in the delignification process, while larger fragments appear in solution in latter stages. This usually occurs when lignin in wood is made soluble by chemical treatments [115, 116, 126, 127]. Szabo and Goring in 1968 recognized this effect and proposed the gel degradation theory to account for these observations. However, prior to their proposition a number of other suggestions were made. These will now be briefly discussed in order to expose some additional salient features of lignin's polymer chemistry.

The conjugated phenolic nature of the lignin precursors (Scheme 1) allows for the possibility that condensation reactions occur under delignification conditions. It is thus possible that the molecular weight of soluble lignin may increase if lignin in wood consists of finite macromolecules with reactive sites that

condense under acidic or basic [128–131] delignification conditions [132–136]. This is generally known as the "condensation theory".

With respect to the condensation theory, a point of major criticism emerges from the results of Adler et al. [137], Felicetta and McCarthy [138], and Yean and Goring [116], where a decrease in the molecular weight of soluble lignosulfonates was observed upon a second exposure to the reaction conditions. This contradicts the condensation theory because one would expect, on further reaction, either an increase or no change in the molecular weight. Nevertheless, it is now accepted that condensation reactions occur during delignification. Model compound studies have suggested that such reactions operate to some degree under both acidic [128] or basic conditions of delignification [129]. During acidolysis, the formaldehyde formed from lignin has been found to be partly consumed in condensation reactions [139]. Quantitative gel filtration studies on acidolysis products [128], however, have revealed that the fraction of "unidentified condensation products" amounts up to 14% by weight of the isolated lignin. It is thus more prudent to conclude that condensation possibly occurs during delignification [140]. Its effects, however, are only secondary contributions to the molecular weight increase phenomenon observed in sulfonic acid delignification. Evidence substantiating this suggestion has been given by Argyropoulos and Bolker [141]. In their communications, Brown et al. [142, 143] concluded that lignin in wood consisted of finite molecules, with two major components of different molecular weights. Similarly, Obiaga et al. [144] postulated that lignin in wood existed as an assembly of finite entities with a degree of polymerization equal to 18. Both conclusions were based on the apparent bimodal distributions observed when lignin samples were chromatographed on Sephadex gels. These interpretations were later proved to be incorrect by Bolker et al. [145]. The peak at the high molecular weight end of the chromatograms was shown to be an artifact, the effect of a high molecular weight tail manifesting itself at the exclusion volume of the Sephadex gel. Later results of Mbachu [146] further strengthened the findings of Bolker et al. A low exclusion volume peak has indeed been reported by Cooper [147] for high molecular weight polystyrene.

Therefore, condensation reactions most likely occur during delignification, thus producing a network polymer which must then be degraded during delignification. The effects of condensation, however, are secondary contributors to the molecular weight increase phenomena observed during wood delignification.

Another hypothesis attempting to rationalize the molecular weight increase of solubilized lignins, is the pre-sieving concept [163]. According to this hypothesis the lamellar structure of the cell wall or more specifically the pore size, determines the size of the dissolved lignin macromolecules when wood is delignified. This is particularly true during the washing of kraft pulp when high molecular weight lignins diffuse out of the fibres [148, 149]. Lignin fractions, which turned to be of the order of hundreds of thousands [150], would be too large to pass through the porous structure of the cell wall. Similarly it was found that when lignin macromolecules were spread on the surface of a non-solvent [121, 152] or deposited on a carbon coated grid for electron microscopy [153], they assumed

a thickness of 2 nm, which is considerably larger than the pore size. Only after the size of the pores becomes larger, as lignification proceeds [148] could these fractions diffuse out of the cell. This may account for the presence of small molecules early in the delignification, while larger molecules are released later, as delignification proceeds. This theory was criticized by Bolker et al. [145] on the basis of the work of Bogomolov et al. [123], who found that the molecular weights of milled wood lignins (see Sect. 5.1) increased with increasing yield. This could not have occurred if the cell wall pores were responsible for the sieving action. In milled wood there is no longer need for lignin fragments to diffuse through any pores because the wood has already fragmented to sizes much smaller than the size of individual cells.

3.1 The Gel Degradation Theory

The pioneering work of Szabo and Goring [127], which was followed by Bolker and Brenner [155], and Bolker, Rhodes and Lee [145], dealt with the gel degradation concept of delignification. In broad terms, the Szabo and Goring treatment envisages delignification as the reverse of polymerizing a trifunctional network, while the Bolker and Brenner approach is the reverse of polymerizing a network formed by the random crosslinking of monodisperse preformed chains. The difference between the two treatments arises from the assumption of Szabo and Goring that all ether bonds in lignin are of comparable reactivity [145, 156]. Bolker and Brenner, however, proposed that the sites of crosslinking were the benzyl ether groups, which are expected to be the most reactive of all the ether linkages found in lignin. They further invoked a monodispersity approximation for the primary chains of lignin. They based their argument on Hess' measurements of the molecular weight distribution of Brauns' native lignin [157]. According to the gel degradation theory, Brauns' native lignin represents the final sol fraction remaining from the gelation process of lignin. Accordingly, Bolker and Brenner argued that Brauns' native lignin mainly comprised primary uncrosslinked chains, with a monodispersity ratio (as they calculated from Hess) of $M_w/M_n = 1.04$. Yan [158], not fully convinced of the validity of the monodispersity approximation, extended the Flory-Stockmayer theory so as to include the crosslinking of primary chains with any initial size distribution. In a series of three papers Argyropoulos and Bolker undertook to experimentally test the reversibility of the Flory-Stockmayer statistics of gelation by using synthetic model networks [159–161]. The results of their efforts have provided support not only for the gel degradation theory but supplied information on the solution properties and the molecular weight species distribution expected from the random degradation of network polymers [162]. Evidence of the validity of the network structure of protolignin arrives from the viscosity, polydispersity, and molecular weight data of soluble lignins discussed previously. The random degradation of a crosslinked network is expected

(according to the gel degradation theory) to yield polymeric fragments of spherical configuration whose molecular weights, yields, and polydispersity ratios will increase as bond fragmentation intensifies within the network. The network theory of protolignin finds progressively wider acceptance among researchers in the field as the experimental evidence pointing toward its validity grows. Although there is still lack of agreement on the precise architecture of protolignin, major efforts toward solving this controversial issue are emerging from the application of the gel degradation theory and its various modifications [158, 164].

3.2 Possibility of Order in Lignin

Despite the fact that a significant compilation of scientific evidence has been accumulated over the years, pointing to lignin as an amorphous, crosslinked network polymer [165–167], formed via a random free-radical polymerization process [169–172], recently discovered aspects of lignin chemistry and details of its deposition into the cell wall have challenged the universality of this description [168].

Detailed analytical studies of synthetic (DHP's) and milled wood lignins have documented that such preparations are rather distinct as far as their bonding patterns and composition is concerned [171]. However, synthetic lignins prepared in the presence of carbohydrates, under conditions that mimic the micro environment of the cell wall, were found to resemble native lignins [173]. These observations, coupled with studies of the organization of lignin within the woody tissue [85–88], point toward the presence of an organizing influence operating during lignin synthesis and deposition [174, 175]. This dominant organising influence, according to several researchers [176–181], can be provided by strong associative interactions operating between lignin precursors and the ordered polysaccharidic matrix. Experimental evidence for these interactions has been provided by studying the adsorption of oligolignols onto cellulose [182] and by measuring the photoconductivity of wood tissue [183].

While the cellulose microfibrils are known to be deposited in the cell wall in complex ordered patterns [184, 185], the precise deposition patterns of hemicelluloses remain unknown. Evidence pointing toward the possibility of hemicelluloses being aligned to cellulose [186, 187] and linked to lignin [188] has been provided. These observations coupled with the associative interactions to be discussed later (Section 4.1), and the observation that the cellulose microfibrils are closely packed [189–192], are factors that may indeed contribute to some organization within lignin. In actual fact, Jurasek in his recent computational efforts, aimed at modelling lignin, has showed that the spatial constraints of the cell wall may indeed impose some degree of regularity in lignin [193]. Gravitis and Erins [194] have also examined lignin with various theoretical

conformational models and have arrived at the conclusion that "under some conditions quasi-ordered regions of lignin structure can be expected".

On a different front, Atalla has provided evidence indicating that the aromatic rings of lignin are aligned tangentially to the secondary wall [195, 196]. Based on these observations, he proposed a new model for the assembly of lignin [174]. The model suggests that variations in the structure of hemicelluloses, may result in corresponding systematic changes in the structure of lignin and cellulose [175, 197–199]. While cellulose provides the primary framework, hemicelluloses furnish various branches which associate themselves with specific lignin precursors. More specifically, it would be anticipated that specific monosaccharide branches are designed to organize the monolignols. while di- and trisaccharidic branches are designed to selectively bind specific di- and trilignols. As such, lignin precursors may be subjected to a certain regulatory mechanism which involves steric factors and sugar binding specificities. Perhaps the most attractive feature of this model is that it introduces a hierarchic and sequential control that occurs at multiple levels, in different phases and separate locations throughout the biosynthetic pathway. This model is different from traditional models which emphasize the compartmentation of the process [200] involving different extracellular or membrane bound enzymes [201–203].

Other evidence pointing toward lignin possessing certain orientation with respect to the cell wall has been provided by its ability to conduct electric charge as witnessed by photoconductivity measurements of wood [183, 204]. Since conductance is highly depended on the coherent orientation of the structures involved in the conduction, the ability of lignins to carry ionic charge has been taken as proof for the presence of a regular array of similar functionalities which may become the vehicle for the excitation transfer of electric charge [205–207]. A reasonable explanation for the observed photoconductivity in wood is that the regularity of the surfaces in the polysaccharidic matrix probably imparts to the lignin a coherence of spatial organization that is sufficient to facilitate some interactions or coupling between its lowest unoccupied molecular orbitals

In 1994 a report of "visual encounter" of order in lignin appeared [208]. Using a scanning tunnelling microscope (STM) images of building units or modules assembled into larger assemblies were claimed to have been recorded. During the same year Faulon and Hatcher [209] presented their calculations that suggested the biopolymer to have a helical structure, characteristic of many naturally occurring macromolecules.

When one considers most of the evidence presented above, it becomes apparent that a better paradigm for lignin needs to be developed. As Goring concluded [210], one should distinguish between lignin in the middle lamella and that of the secondary wall. Until the lignification process has been fully understood. the traditional concept of lignin being a uniform. amorphous, three dimensional network polymer, is probably too simple to reconcile with all the recent scientific data relating to its native structure and properties.

4 Solution Properties of Lignin

Early attempts to study the properties of lignin in solution commenced with the observation that native lignin is insoluble in all good solvents [211]. In 1956, Bjorkman [212] discovered that when spruce wood is milled thoroughly, up to 50% of the lignin could be degraded and extracted in aqueous dioxane. During the milling process, covalent bonds were found to rupture [213], and low molecular weight lignin fractions solubilize. This behaviour was also described by Lindberg [214] and Schuerch [211]. In 1960, Gupta et al. [215, 217] pointed out that by invoking a three dimensional polymer network architecture for lignin, the molecular weight distributions of isolated solubilized lignins could be accounted for (Section 3). The soluble macromolecules believed to reflect the properties of the network from which they emerged.

Amongst the most significant of the early observations were the predominant physicochemical characteristics of lignin molecules in solution. These include the intrinsic viscosity, branching parameter, and the degree of polydispersity. Their determination provided useful structural information in relation to the overall architecture of protolignin. The intrinsic viscosities [216], at comparable molecular weights, were found to be 1/40 of those of polysaccharides and about 1/40 of other synthetic polymers [11, 215, 217]. The low intrinsic viscosities of dioxane [218], kraft [219], lignosulfonate [220], and alkali [215, 217] lignins, in various solvents, led Goring to conclude that these molecules in solution are compact spherical microgel particles [11, 218]. He also reported that the values of the Mark-Houwink exponent (α), ranged between 0 and 0.5. This constituted further confirmation that lignins in solution behave as molecules whose solvated shape is something between an Einstein sphere (a "ball" impermeable to solvent) and a non free-draining random coil. Similar conclusions were derived when other parameters, such as sedimentation coefficients and diffusion constants, were measured [221, 222]. These measurements showed that soluble lignins are rather compact molecules in solution, though not as compact as simple solid spheres. In general the chains of the lignin macromolecules in solution are more densely packed than those of linear flexible polymers such as polystyrene. The branching parameter, or contraction factor (g) introduced by Zimm and Stockmayer [223], when measured on various alkali lignin fractions, was found to decrease with increasing molecular weight [217], as expected for such molecular configurations. Independently, Alekseev et al. [224] calculated the value of the Mark-Houwink exponent (α) for lignin solutions using viscosity, sedimentation, and diffusion data. His calculations supplied further support to the idea that the lignin macromolecules in solution are of a rigid spherical configuration [221, 222]. More recent results of Pla and Robert [225] showed α to be 0.5 for dioxane lignin solutions. They, too, interpreted their results in terms of the branched or crosslinked nature of the dissolved lignin macromolecules. Low Mark-Houwink exponents are not unusual for truly branched macromolecules in solution. A good example of this can be found in the work of Argyropoulos

and Bolker who have shown that the soluble fragments isolated from a series of polyester network polymers beyond the gel point showed an α value of only 0.15 [226].

4.1 Lignin Associative Interactions

Several authors have raised the possibility that lignin in wood is composed of low molecular weight molecules which on becoming soluble tend to associate by non-covalent linkages into complexes of higher molecular weight [227–232]. It was shown by S. Sarkanen [228, 232] that this process seems to be reversible and solvent dependent. Low molecular weight kraft lignin fractions generated higher molecular weight complexes in certain solvents. Such associative interactions among lignin components have been found to occur during gel permeation chromatography in different mobile phases. Non-aqueous solvents exhibited multimodal chromatograms, while alkaline aqueous eluents gave rise to broad chromatographic envelopes with sufficient resolution only in the low molecular weight region [233, 234]. Connors, Sarkanen and McCarthy [228] have discovered that in non-aqueous solvents, association complexes of kraft lignins gave rise to apparent molecular weights (as determined by GPC) as much as three orders of magnitude larger than their constituents. These effects were eliminated when LiCl was added to the DMF used as the mobile phase. Accounts by Ekman and Lindberg [235] and Lindberg [236] show very major changes occurring in the solubility, hydrogen bond behaviour, and size exclusion chromatograms of lignins after they have been methylated or acetylated. Conclusions on extensive hydrogen bond formation between free hydroxyl groups in dioxane lignins have also been reported by Hatakeyama et al. [237] and Bogomolov et al. [238]. Obviously, any association between lignin molecules in solution would cause an increase in their apparent molecular weight values.

4.2 Lignin Polydispersity

A considerable amount of scientific data exists supporting the fact that soluble lignins and derivatives are of high polydispersity [239–246]. The weight to number average molecular weight ratios for different lignins may vary from about 3 to over 10–11 [270, 271], indicating that lignins are extremely polydisperse materials. Fractionation studies on lignosulfonates [114] and alkali lignins [241, 217] have shown that they contain molecular weights ranging from 1000 to greater than a million (g mol^{-1}) [247]. The molecular weight distribution curves for lignins are expected to be unimodal. However, a number of authors have reported bimodal distributions for soluble lignin [227, 248–249]. Such behaviour was also observed when lignin was isolated from the pulping liquors of wood [227, 250]. It was only after improvements in chromatographic techniques that these bimodal distributions could be to resolved into

distributions with several maxima [251–253]. More information on molecular weight distribution within the soluble phase beyond the gel point, which according to the gel degradation theory should resemble those obtained during lignin degradation, can be found in the work of Argyropoulos and Bolker [162]. Recently, Glasser has produced a universal plot of polydispersity versus weight average molecular weight for a series of prototype lignins supplying a linear expression with some predictive power [270, 271].

5 Lignin Preparations

Generally, there is a lack of agreement on the ultimate structure of lignin and this may be because, as Pearl wrote [254] "*It is practically impossible to isolate two lignin preparations with identical properties, even by the same procedure*". Although many methods are available for the isolation and purification of lignin, none provides 100% yield and structural authenticity. To isolate lignin which closely represents the native material, reactive chemicals and elevated temperatures must be avoided.

5.1 Laboratory Lignin Preparations

Milled wood lignin (MWL) [255] and cellulase enzyme lignin (CEL) [256] are good examples of preparations closely resembling the native material. Yet, these preparations are never free of even minor chemical modifications. For example several secondary reactions may occur during the milling process, as a result of free radicals produced during the process [257]. Moreover, CEL preparations are known to contain protein impurities introduced during the enzymatic treatment. Despite these shortcomings, MWL and CEL preparations show minimal structural modification with yields ranging between 25%–66% of the total lignin and with carbohydrate contents ranging between 2–10%. The molecular weights (M_w) of these lignin preparations range between 15,000–20,000 and predominantly consist of alkyl-aryl ether linkages [258].

Other techniques for isolating lignin involve treatment of the wood with organic solvents such as dioxane [294] or ethanol [260, 261], sometimes in the presence of catalytic amounts of mineral acids (H_2SO_4), or inorganic Lewis acids, at elevated temperatures and pressures [262]. The combination of acids and organic solvents causes the hydrolysis of the ether bonds in lignin and those between lignin and carbohydrates. Such products are relatively free of carbohydrates, and of low molecular weight fragments [263]. Another technique involves the degradation of the cellulosic constituents of wood by sodium paraperiodate [259, 260] or their solubilization by complexation with cuprammonium hydroxide [261] (cuoxam lignin). The latter gives lignin in high yields

which is known to retain the morphological features of wood [264, 265] and is totally insoluble in organic solvents. Amongst various lignin preparations, Fleming and Bolker found cuoxam lignin to be the most suitable for model delignification experiments [266]. For additional methods of lignin isolation procedures the reader is referred to more specialized texts [11, 255, 263, 267].

Due to the variety of techniques available for isolating lignin, and the structural variations introduced during the isolation, each lignin preparation is usually identified by the wood species and the isolation technique, e.g., spruce-dioxane lignin, or birch-cuoxam lignin. It is important to distinguish that all these preparations are distinct from protolignin, which is a term used for the material as it occurs in the plant tissue [268, 269].

5.2 Commercially Produced Lignins

Commercial lignins from hardwood and softwood trees are available from the two major pulping processes, sulfite pulping and kraft pulping, which uses sodium sulfide and sodium hydroxide. In addition, pilot plant scale procedures exist which employ either organic solvents (organosolv pulping) or high pressure steam followed by alkaline extraction (steam explosion pulping). While the sulfite pulping process generates water-soluble lignosulfonates as their sodium, magnesium, or calcium salts, the kraft process produces lignins that are soluble only in aqueous alkali.

In addition to differences in structure and botanic origin, commercial lignins also vary in molecular weight. Most such lignins are of weight average molecular weights (M_w) ranging between 3000 and 20,000 and polydispersity ratios (M_w/M_n) between 2 and 12 [270, 271].

6 Methods of Lignin Analysis

6.1 Degradative Methods

The methods described in this section require the sample to be exposed to certain reagents and specified conditions that degrade the lignin in a prescribed manner, in accordance with documented reaction sequences. The products are then quantitatively analysed by chromatographic techniques yielding structural information about the building blocks of the lignin sample [272]. Such techniques suffer from being laborious, involving many steps and complex chemical manipulations, often subjecting the derived quantitative information to large errors and diminished reproducibility. Despite these limitations these methods have offered significant advances to our knowledge of lignin structure and reactivity.

Lignins can be selectively oxidized in alkaline nitrobenzene or permanganate solutions. The alkaline nitrobenzene oxidation of lignin was first introduced in

1939 by Freudenberg and co-workers [273, 274] and was further optimized by Leopold [275–277]. Traditionally, nitrobenzene oxidation is used for the classification of wood or plant tissue and of lignin according to its botanical origin. During this procedure the lignin or the fibrous sample is oxidized in sodium hydroxide (2N) in the presence of nitrobenzene at 170–180 °C for 3–4 hours. The products, mainly aldehydes, originating from the guaiacyl, syringyl and *p*-hydroxyphenyl structures in lignin are then subjected to quantitative determination.

The oxidation of lignin in pulps by standard permanganate solutions has been the basis of the permanganate and kappa number tests, extensively used by the pulp and paper industry as a means of rapidly determining the lignin content of pulps. These techniques are based on the fact that potassium permanganate readily and selectively oxidizes lignin in the presence of carbohydrates. The permanganate number is conventionally reported to be the number of millilitres of 0.1 N $KMnO_4$ consumed by 1 g of oven-dry pulp. However, since the size of the pulp sample and the amount of permanganate applied affected the results it was modified by Tasman and Berzins [278, 279] who proposed the kappa number. As such, the sample size was adjusted to ensure that approximately half of the applied permanganate is consumed. This test has been of extreme significance to the pulp and paper industry in determining the degree of delignification of a fibrous feedstock during pulping and bleaching operations. Recent research, however, has brought to light that hexeneuronic acids, formed during pulping, consume permanganate during the kappa number test leading to significant errors specially when hardwoods are examined [280].

Analytical protocols involving alkaline permanganate oxidations of fibrous and lignin samples may provide similar information to that obtained by nitrobenzene oxidation. Moreover, these techniques offer structural details about the frequency of the various bonding patterns in lignin [281]. Despite the many steps involved and the fact that the obtained information is confined to units possessing free phenolic hydroxyl groups, alkaline permanganate oxidations have been extremely important in many aspects of lignin chemistry. The significance of the technique coupled with the complex nature of the various chemical manipulations have caused the development of a four step standardized procedure [53]. The procedure commences with an alkaline CuO predegradation step, followed by a methylation step, and ends with two oxidation steps involving permanganate and hydrogen peroxide [282]. The structure of lignins present in neutral sulfite [283–285] and in kraft pulps [286–288] has been elucidated using such techniques.

Another procedure which has significantly contributed to our understanding of the lignin structure is that of hydrogenolysis [289–291]. The procedure consists of hydrolytic and thermolytic reactions which cause the partial breakdown and solubilization of lignin. The lignin fragments, diffuse into the liquid phase where they are rapidly stabilised against condensation reactions by in situ generated hydrogen atoms on the surface of a catalyst [292]. Despite the fact that the procedure is not in wide use today the principle may be applicable to converting lignin into valuable low molecular weight products [293].

Pepper et al. were the first to propose the principle of acidolysis as a procedure for isolating lignin from plant material [294] and recently from kraft pulps [280]. Later the same principle was used as an analytical tool for determining the occurrence of β-*O*-4 and β-5 structures in spruce lignins [295]. The procedure calls for refluxing the lignocellulosic material in 0.2 N HCl in a dioxane: water mixture (9 : 1, v/v). These conditions have been shown to cleave both α and β aryl ethers linkages in lignin. Such studies have provided evidence for the existence of additional structural elements in lignin such as β-β, β-1, and quinonoid [69, 296–299]. Acidolysis protocols in the presence of catalysts, other than hydrogen chloride, have been proposed by Yashuda [300] and Karlsson [301] and reductive cleavage protocols by Shevchenko et al. [302]. The main critique of all methods involving acidolysis has been the fact that almost invariably the product yields are rather low as a result of condensation reactions taking place in acidic media [168]. Consequently efforts have been made to address these limitations. Since the combination of boron trifluoride and thioethanol in anhydrous media is known to convert benzylic cation intermediates to thio-benzyl derivatives, Lapierre et al. have used this principle to develop the technique of thioacidolysis [303]. This combination has been shown to quantitatively and selectively cleave the arylglycerol-β-aryl ether linkages in lignin [303–305]. Recent developments of the technique involving the desulfurization of the products using Raney nickel have been claimed to offer improvements over earlier protocols [306].

6.2 Non Degradative Methods

Non-degradative methods, involve mostly modern spectroscopic and other non evasive techniques, yielding structural information about the sample without the need of subjecting it to harsh chemical environments. Characteristic π-π^* transitions of the aromatic nuclei in lignin make it a strong absorber of UV light. As such, lignin absorbs much more strongly than carbohydrates with an absorption pattern which has a maximum at approximately 280 nm. Based on detailed measurements of the UV absorption spectra of model compounds involving guaiacyl and syringyl moieties [307], Fergus and Goring pioneered the use of UV microscopy for studying the distribution of lignin across the cell wall, in different morphological regions within woody tissue [308, 309] and during delignification in kraft and neutral sulphite pulping [310]. Similar efforts by Boutelje and Eriksson [311] and Yang and Goring [312] firmly established the use of UV microscopy for such endeavours. The UV absorption spectra of lignins may also be used to estimate their free phenolic hydroxyl content [313]. The method is based on the fact that ionized phenolic groups in lignin absorb at 300 nm fully obeying the Beer-Lambert law. The use of this technique allowed the determination of the phenolic hydroxyl content in the secondary wall, which was found to be 50% greater to that of the middle lamella [314]. The characteristic UV absorption spectra of lignin have been used for the quantitative and

qualitative determination of native lignins [315, 316], lignosulfonates [317] and the study of delignification of wood during pulping [318, 319]. Despite its numerous advantages the method can give erroneous results in the presence of contaminants that are strong UV absorbers. Its reproducibility has also been questioned as a consequence of an international round robin study involving many lignin standards [320].

In recent years the application of nuclear magnetic resonance spectroscopy to the characterization, classification and detailed structural elucidation of lignins has seen widespread utility. The fact that the proton nucleus is of 100% natural abundance and of high sensitivity made the ^{1}H NMR experiment rather popular in the early days of the application of this technique to lignin [321–323]. Almost invariably, ^{1}H-NMR is used on acetylated lignins [322–326] since this affords better signal resolution. ^{1}H-NMR signal assignment has been based on model compound [327–329], decoupling [330], and 2D-NMR experiments [331]. Nevertheless, there are some essential limitations to ^{1}H-NMR spectroscopy of lignins. These include the rather limited range of chemical shifts (12 ppm), extensive signal overlapping and proton coupling effects. The technique is only suitable for the qualitative study of the proton distribution in lignins, while carbon containing groups and labile proton functionalities (OH, COOH, SH) remain beyond the capabilities of the technique. Recent developments have claimed that after appropriate derivatization it is possible to quantitatively determine the phenolic hydroxyl groups in lignin [332].

The early work of Ludemann and Nimz [49] has allowed the use of solution ^{13}C-NMR spectroscopy to become an indispensable tool for the structural elucidation of the carbon skeleton of lignin. Compared to proton NMR, the ^{13}C-NMR spectra of lignin offer considerably better resolution, with no coupling effects, over a much wider chemical shift range (200 ppm). Convenience, speed, and a wealth of even quantitative information [333, 334, 337] have contributed to the widespread use of ^{13}C-NMR for the structural analysis of lignins. Furthermore, a variety of newly developed pulse sequences such as DEPT [335], INADEQUATE [336], HMQC [81], as well as acquisitions on ^{13}C-enriched lignin samples [337–339] have improved our understanding of lignin structure and reactivity. Despite all these, some drawbacks remain as far as the efficient use of ^{13}C NMR is concerned. These stem from the fact that the ^{13}C nucleus is not sensitive enough (1/5800 compared to proton) and that it is only 1.1% naturally abundant. Consequently, relatively large sample sizes and lengthy acquisition protocols are essential. When quantitation is sought extreme care is required to allow for complete relaxation of the magnetization which translates to very lengthy acquisition protocols and the need of external methoxyl analyses so that the OCH_3 signal to be used as internal standard [333, 340].

Advances in instrumentation have made the use of solid-state NMR spectroscopy a routine operation [341, 342]. As such, various solid-state ^{13}C-NMR experiments can now be performed routinely on lignin [343, 344] and solid wood or plant samples [345, 346, 95]. It is thus possible to obtain some information about the lignin within a sample without its prior isolation [87]. Dipolar

dephasing when used as part of the acquisition protocol of solid-state spectra of wood and pulps has been documented to yield significant information in relation to the degree of chemical modification occurring in lignin [347].

Efforts to overcome some of the limitations imposed by proton and ^{13}C-NMR spectroscopies have prompted the examination of other NMR-active nuclei which when covalently linked to lignin by appropriate derivatization procedures may provide additional structural information for these heterogeneous biopolymers. Early attempts examined the potential of silylation followed by silicon NMR for the determination of hydroxyl groups in kraft lignin and related model compounds [348, 349]. While the method offers resolved signals for aromatic, phenolic and carboxylic acids, large sample concentrations and long acquisition protocols are essential due to the low natural abundance, low magnetic moments and high relaxation times of the ^{29}Si nuclei. ^{19}F-NMR has also been proposed as a means of detecting different functional groups in lignins [350–353].

Finally, ^{31}P-NMR spectroscopy has provided a new analytical tool capable of detecting a variety of functional groups in isolated soluble lignins and within lignin containing papers in the solid-state [354]. More specifically, solution ^{31}P NMR has been used to examine soluble lignins [355] and carbohydrate [356] samples after phosphitylation with 1,3,2-dioxaphospholanyl chloride and 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane [357, 358]. From a single quantitative ^{31}P-NMR experiment it is possible to determine the three principal forms of phenolic hydroxyls present in lignins i.e. *p*-hydroxyphenyl, guaiacyl, and syringyl structures as well as primary hydroxyls, carboxylic acids, and the two diastereomeric forms of arylglycerol-beta-aryl ether units (β-*O*-4 structures) [359]. Most significantly, however, the technique offers additional rapid (1–2 h acquisition time) information in relation to the condensed structures in lignin that can only be obtained by permanganate oxidation [360–364].

Acknowledgments. The authors would like to acknowledge the following individuals for having critically examined the manuscript prior to dissemination: Dr. R.M. Berry, Professor K.E. Eriksson, Dr. I. Falkehag, Professor R. Francis, Dr. G. Leary, Dr. L. Jurasek, Professor R.H. Marchessault, Mr. Y. Sun and Dr. S. Shevchenko.

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