CHAPTER 1

A Brief Introduction to Lignin Structure

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1.1 Introduction

Lignocellulosic biomass is a vast resource for the sustainable production of renewable fuels, chemicals, and materials for mankind.[1,2] Biomass, especially wood, has been used for millennia as a building and construction material for myriad applications and a source for heat as a fuel. The majority of mass in plants is in the cell walls, which are primarily composed of the polysaccharides cellulose, hemicellulose, and pectin along with the alkyl-aromatic heteropolymer lignin. The three basic building blocks of lignin, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, are synthesized *via* the phenylpropanoid pathway in plants and differ in their extent of methoxylation (0, 1, and 2, respectively).[3] Lignin is synthesized *via* enzymatic dehydrogenation of these monomers, which form both C–O and C–C bonds, leading to a heterogeneous structure and a three-dimensional structure. As discussed briefly below, additional components of lignin such as hydroxycinnamic acids and flavonoids further complicate the structure and decorate the aromatic heteropolymer with additional linkages and chemical functionality.

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Although under development for at least the last century, the conversion of biomass polysaccharides into fuels and chemicals has especially gained substantial momentum in the past several decades, primarily motivated by the potential to offset petroleum usage with a renewable, sustainable feedstock and to reduce associated global greenhouse gas emissions. For fuels production, the primary biorefinery models examined to date have adopted a strategy to utilize thermochemical pretreatment and enzymatic hydrolysis to produce pentose and hexose sugars for subsequent fermentation to ethanol using natural or engineered yeast or bacterial strains.[1,4] Enormous technical diversity exists around this biomass deconstruction paradigm with many different thermochemical deconstruction/pretreatment strategies being pursued, including (but not limited to) the use of acid, base, hot water, steam, organic solvents, and ionic liquids.[4,5] Industrial enzyme systems to date have primarily focused on the use of carbohydrate-active enzymes from cellulolytic fungi[6] and anaerobic rumen bacteria,[7] but the rise of the (meta)genomics-enabled science has rapidly accelerated the discovery of new polysaccharide deconstruction paradigms and individual enzymes.[8] Both thermochemical and enzymatic polysaccharide deconstruction approaches remain highly pursued areas of research.

Lignin, conversely, is typically relegated for heat and power due to its inherent heterogeneity and recalcitrance.[9] However, techno-economic analysis of lignocellulosic biorefineries is revealing that lignin utilization is a crucial component of integrated biorefineries,[10] and thus new strategies for lignin must be developed. As such, many new discoveries and developments are emerging in the past decade regarding lignin utilization, especially given significant government and industrial funding in large consortia and centers throughout the world. This book brings together world-leading experts in lignin utilization to present and review the most recent discoveries in lignin valorization to highlight opportunities going forward to utilize lignin more efficiently and sustainably. Emphasis is placed on very recent, emerging topics in chemical and biological catalysis for lignin valorization. This introductory chapter primarily focuses on the chemical aspects of lignin structure, as a preface to the subsequent chapters.

1.2 Lignin Structure

Lignin is a polyphenolic material and one of the main components in the plant cell wall. Its biosynthesis occurs through enzymatic dehydrogenation of three phenylpropanoid monomers, *p*-coumaryl alcohol (**2**), coniferyl alcohol (**3**), and sinapyl alcohol (**4**) (Figure 1.1).[11–15] Phenoxyl radials generated from these three monolignols are randomly polymerized to produce a biopolymer with a three-dimensional network. The weight average molecular weight (M_w) of isolated lignin (milled wood lignin) is 6700, 14 900, and 23 500 Da from *Eucalyptus globulus*, Southern pine, and Norway spruce, respectively,[16] with the molecular weight of lignin varying widely with isolation method. Lignin contents, as measured by the Klason method, are

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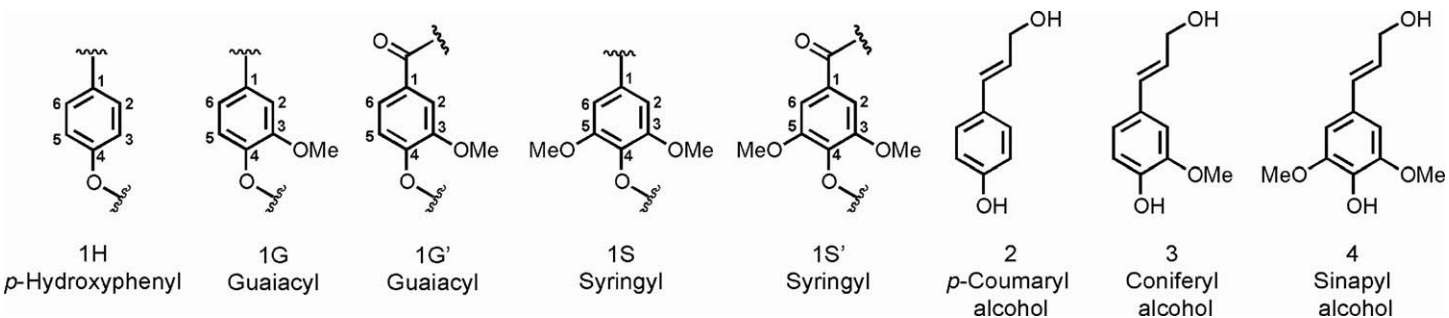


Figure 1.1 Repeating units in lignin.

25–35% in softwood, 20–25% in hardwood, and 15–25% in herbaceous plants.[17] In the cell wall of a hardwood, lignin deposition starts from the middle lamella, then in a primary wall and S1 layer in the secondary wall. Subsequently, lignin is located in the S2 and S3 layers.[18,19]

An understanding of the chemical structure of lignin is critical for developing robust and effective lignin valorization processes. In past decades, many lignin chemists have studied and developed methods for the determination, isolation from biomass, destructive/non-destructive analytical methods, and the degradation of lignin, which is reviewed in more detail in Chapter 15. The main quantitative lignin determination methods are the original Klason acid hydrolysis method, the modified Klason method,[17,20] and the acetyl bromide method.[21] Various lignin isolation methods have also been developed with less structural changes.[22] Among these are milled wood lignin (MWL),[23,24] cellulolytic enzymatic lignin (CEL),[24] kraft lignin, soda lignin, and organosolv lignin,[23] with MWL and CEL considered to retain the native lignin structure. Isolated lignin has been characterized using numerous methods such as solution- and solid-state NMR,[25–27] SEC,[28] GC-MS^n, LC-MS^n, UV-Vis,[29] FTIR,[30,31] Raman,[32] SEM/TEM, and EPR. NMR, especially solution-state NMR, which is a non-destructive method, has provided analytical breakthroughs for insights into the interunit linkages of whole lignin. In addition, several methods have been developed for understanding the chemical structure of lignin based on its degradation products. These include acidolysis,[26,33,34] thioacidolysis,[35] DFRC,[36–39] nitrobenzene oxidation,[40] hydrogenolysis,[41–43] ozonation,[44,45] permanganate oxidation,[46] and nucleus exchange reaction.[47–49] While a large number of such studies have focused on lignin chemical structure, to date the whole lignin chemical structure has still not been determined. It is obvious, however, that the large amount of information based on these instrumental and degradation methods has provided considerable insights into the structure of the polymer. In the next section, insights about the type and frequency of interunit linkages, their side chain composition, and functional groups are described.

The three phenylpropane building blocks of lignin correspond to *p*-hydroxyphenyl (1H), guaiacyl (1G), and syringyl (1S) structures in lignin, respectively (Figure 1.1). Softwood lignin is composed of mainly G-units with a small amount of H-unit lignin, while hardwood lignin consists of both G- and S-units. Herbaceous plant lignin contains all three monolignol units of G, S, and H units, and *p*-coumarate and ferulate, which are incorporated with normal G- and S-units. G-units and S-units with a C_α=O (1G′, 1S′) also exist.

1.2.1 Side Chain Structure in the End-group

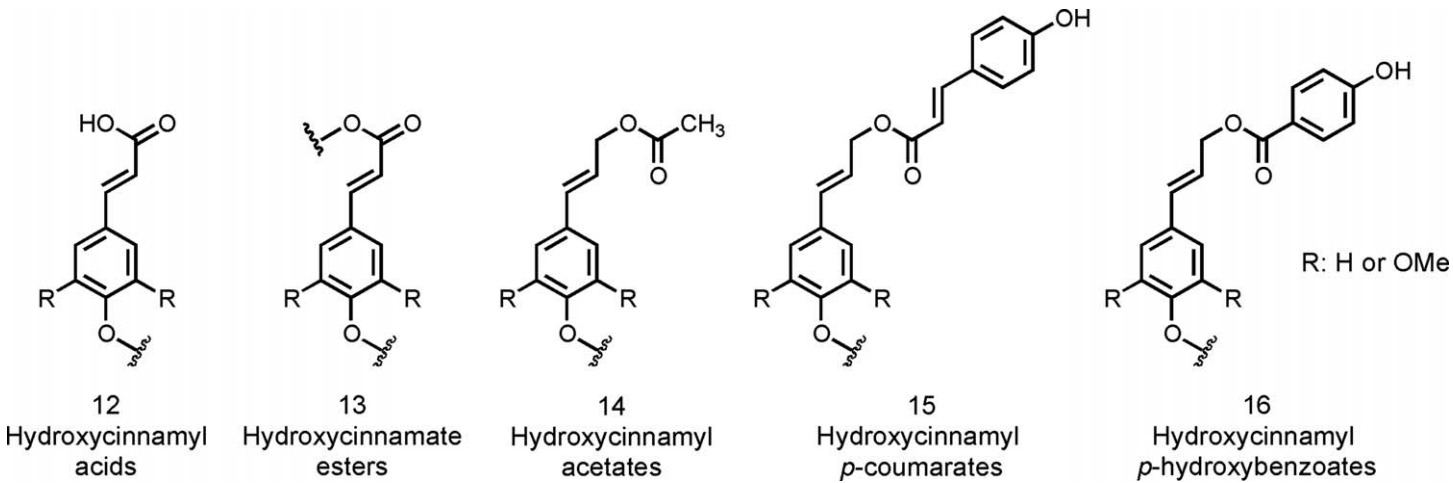
Based on analyses of degradation products, *p*-coumarate (**5**), ferulate (**6**), hydroxycinnamyl alcohol (**7**), hydroxycinnamaldehyde (**8**), and arylglycerol end-units (**11**) have been identified in lignin (Figure 1.2).[11–13,33,41]

Figure 1.2 Side chain structure in end-groups in lignin.

Using a color reaction method the abundance of hydroxycinnamaldehyde and hydroxycinnamyl alcohol in spruce MWL were determined to be 0.02–0.04/OAr, and 0.02, respectively.[20] The cinnamyl alcohol (including 5-hydroxyconiferyl alcohol) units are more abundant in hardwoods than softwoods and herbaceous plants as detected by solution-state HSQC NMR, while *p*-coumarate and ferulate end-units are found in mainly in herbaceous species.[50] Dihydrocinnamyl alcohol end-units (**9**) are incorporated primarily into softwood lignin.[51] This unit is homo- and cross-coupled with coniferyl alcohol to generate the dibenzodioxocin unit, as described below.[52] Guaiacylpropane-1,3-diol end-units (**10**) are derived from dihydrocinnamyl alcohol end-units (**9**), and found in low concentrations in pine and in high concentrations in CAD-deficient pine mutants.[53] The arylglycerol end-unit (**11**) was detected in the hydrolysates from dioxane–H_2O and hot water,[54] but specific methods for its determination have not yet been established.

1.2.2 Acylated End-groups

Various products with acyl groups from incomplete monolignol biosynthesis are generated, such as hydroxycinnamic acids (**12**; *p*-coumaric acid, ferulic acid, sinapic acid, and *p*-hydroxybenzoic acid) and hydroxycinnamaldehydes (**8**). These molecules incorporate into the lignin polymer through ester linkages to create various acylating end-groups at the γ-OH position in many lignins, such as acetate (**14**), *p*-coumarate (**15**), and *p*-hydroxybenzoate (**16**) (Figure 1.3). For instance, over 50% of kenaf bast fiber lignin contains γ-acetylate (**14**),[55] and γ-*p*-coumarate (**15**) makes up about 10% of grass lignins.[56] The γ-*p*-hydroxybenzoate substituents (**16**) with an aliphatic hydroxyl group is present up to a level of 10% in aspen native lignin.[57] Additionally, γ-*p*-hydroxybenzoate was also detected in poplar lignin, palm, and willow.[58–62] Sinapyl *p*-hydroxybenzoate has also been isolated in lignifing xylem tissue in poplar, and its synthetic mechanism was proposed to be a coupling reaction of sinapyl alcohol (**4**) and sinapyl *p*-hydroxybenzoate (**16**).[63] Recently, it was shown that the *p*-hydroxybenzoate selectively acylates the γ-hydroxyl group in the S unit.[64] More importantly, it was found that these acylating groups are not introduced from acylation of the lignin polymer, but that acylated lignin monomers are generated first and then these monomers are polymerized through radical coupling to incorporate into acylated lignin.[64,65] This indicates that these acylated compounds are pre-acylated lignin precursors in parallel to the classical three monolignols.[64] With regard to the enzymes that promote the acylated monolignol in the lignin biosynthesis pathway, *p*-coumaroyl-CoA:monolignol transferases in grasses and feruloyl-CoA:monolignol transferases in engineered poplar promote *p*-coumaroylation and feruloylation of monolignols, respectively.[66,67] These results imply the existence of *p*-hydroxybenzoyl-CoA:monolignol transferase.[64]



12 Hydroxycinnamyl acids | 13 Hydroxycinnamate esters | 14 Hydroxycinnamyl acetates | 15 Hydroxycinnamyl *p*-coumarates | 16 Hydroxycinnamyl *p*-hydroxybenzoates

Figure 1.3 Acylated end-groups in lignin.

1.2.3 Lignin Interunit Linkages

To identify interunit linkages, lignin is generally degraded to low molecular weight products of monomers, dimers, and oligomers, and analyzed using GC-MS, LC-MS, and NMR. Based on these techniques and computational modeling, estimates of the type and relative abundance of interunit linkages have been determined. The common interunit linkages found are summarized in Figure 1.4. Among these, the β-O-4, β-β, β-5, and 5-5′ units are the main structures, with dibenzodioxocin, spirodienone, and benzodioxane representing recently discovered units. Other linkages such as β-1, 4-O-5 diphenyl, and α-O-4 are relatively uncommon. The relative abundance of each linkage depends on the species of biomass, lignin type, and isolation method.[68–70]

1.2.3.1 Aryl Ether Unit (β-O-4)

The most frequent interunit linkage in lignin is the arylglycerol-β-aryl ether (β-O-4) bond, which is considered to occur at levels of 40–60% in softwood and hardwood.[15,71] Adler *et al.* reported that the arylglycerol-β-aryl ether structure was degraded to Hibbert ketones through acidolysis, and estimated the β-O-4 content at 0.25–0.3/OAr in spruce lignin.[72] Lapierre *et al.* found that thioacidolysis could specifically cleave the β-O-4 structure in lignin and reported both the levels of β-O-4 units and H/G/S ratio from various lignin samples and biomass species including herbaceous plants.[35] More recently, Ralph *et al.* developed the derivatization followed by reductive cleavage (DFRC) method, which can also degrade β-O-4 units using AcBr and is followed by reductive cleavage with zinc.[36–39] Using spectroscopic analyses, the β-O-4 linkage can be estimated by ^{1}H NMR of acetylated lignin samples and by quantitative ^{13}C NMR of non-derivatized lignin samples. More recently, it has been demonstrated that the advanced two-dimensional NMR technique HSQC could measure relative amounts of β-O-4 units in the whole cell wall.[50,73] Based on these results from chemical degradation and spectroscopic methods, it has been confirmed that the β-O-4 unit is the predominant interunit linkage in lignin and is critical for depolymerization.

1.2.3.2 Resinol Unit (β-β)

The pinoresinol structure was first detected in the enzymatic dehydrogenation polymer (DHP) of coniferyl alcohol,[74] while other resinol units such as syringaresinol, episyringaresinol, lariciresinol, and dimethoxylariciresinol have also been isolated in percolation hydrolysate and hydrogenation products from hardwood.[75–77] The amount of resinol has been estimated to be 0.02 and 0.03–0.05/OAr in spruce and birch lignin, respectively. Based on the quantitative data of these resinol units, it was found that hardwood lignin contains more resinol units than softwood, but maize lignin has no resinol structures.[50]

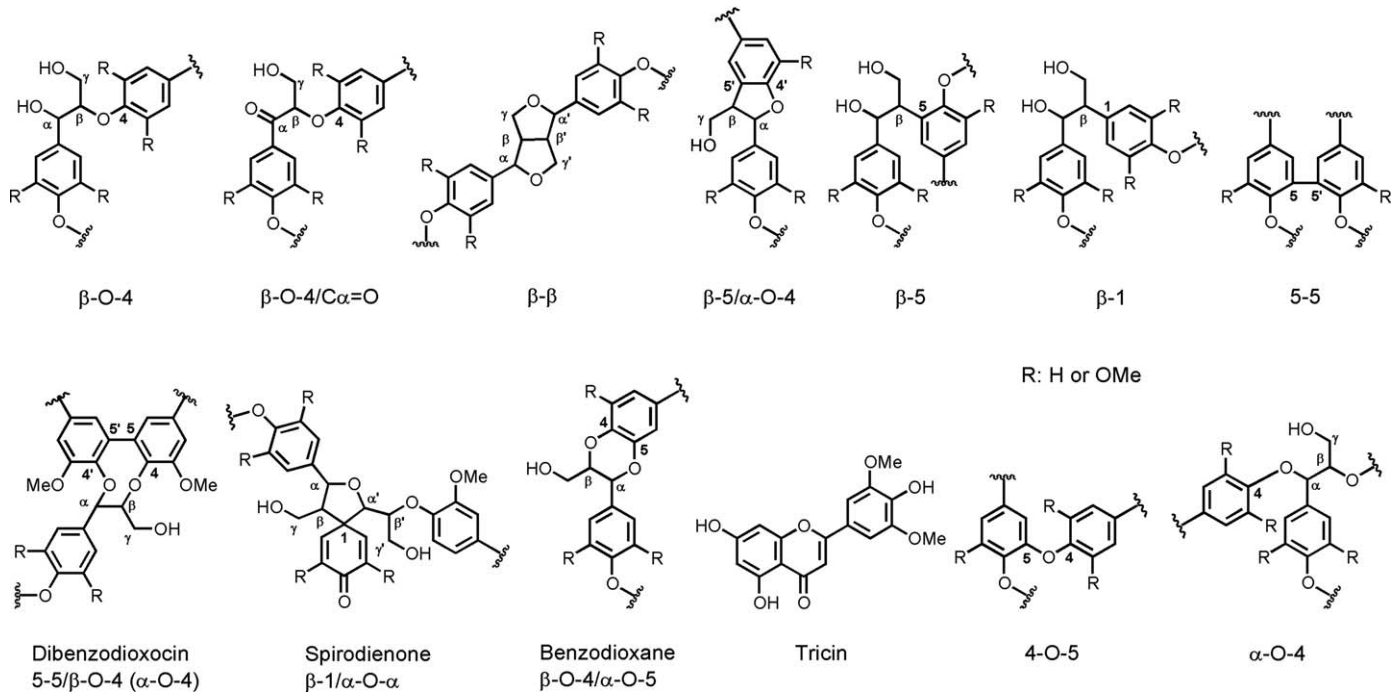


Figure 1.4 Main interunit linkages in lignin.

1.2.3.3 Phenylcoumaran Unit (β-5)

The β-5 structure was found in dioxane–H_2O (1 : 1, v/v) hydrolysate.[78,79] The ring-opened β-5 dimer/trimers were isolated after hydrogenolysis.[80] The β-5 unit was determined to be 0.11/OAr by quantification of phenylcoumarone structure by UV-Vis, which was converted from the phenylcoumaran structure after acidolysis.[81] Based on quantitative analysis of isohemipic acid after potassium permanganate oxidation, the amount of β-5 units was also estimated to be 0.05 and 0.09–0.12/OAr from birch and spruce lignin, respectively.[82,83] The solution-state HSQC NMR spectra of whole cell wall samples revealed that poplar had fewer β-5 units than pine and that maize lignin had no β-5 structures.[50]

1.2.3.4 Biphenyl Unit (5-5′)

The biphenyl structure was confirmed by the isolation of dehydrodivanillin from nitrobenzene oxidation products.[84] Other biphenyl dimers have been also detected in the hydrogenolysis products. The amount of biphenyl was estimated to be 0.045, 0.09–0.11, and 0.023/OAr in birch, spruce, and beech, respectively, calculated from the amount of the corresponding oxidative degradation products.[82,83,85]

1.2.3.5 Dibenzodioxocin (5-5′/β-O-4) and Spirodienone (β-1/α-O-α) Units

The dibenzodioxocin structure was discovered in softwood lignin using several two-dimensional NMR techniques (HMQC and HOHAHA) by Karhunen *et.al*.[86] This structure has also been found in MWL of hardwood in low amounts.[86,87] Later, using confocal laser-scanning fluorescence microscopy (CLSM), Kukkola found that dibenzodioxocine was localized in the S3 layer in Norway spruce and silver birch xylem.[88] Spirodienones have also been observed with 0.03/OAr abundance in spruce and with 0.018/OAr abundance in aspen lignin.[89] The unit contains the β-1 structure and therefore has been considered as a precursor of β-1 linkage. According to studies using 1D and 2D NMR techniques (quantitative ^{13}C, HSQC, HSQC-TOCSY, and HMBC), it has been found that the spirodienone structure is the predominant form of the β-1 structures present in both softwood and hardwood native lignin, and that a spirodienone structure of the guaiacyl type exist in spruce lignin and the syringyl type is predominant in kenaf and birch lignin at levels of 0.03–0.04/OAr.[89,90]

1.2.3.6 Benzodioxane Unit (C-Lignin)

Most recently, a catechyl lignin polymer was found in the seed coats of both monocot and dicot plants.[91,92] This lignin is derived solely from caffeyl alcohol, which was usually not considered as a monolignol, linked in a linear

benzodioxane chain. It has been dubbed "C-lignin" and is observed in the seed coat of the vanilla orchid with high concentration.[91,92] 5-Hydroxyconiferyl alcohol can also be produced by genetic modification of hardwoods that interrupts the formation of sinapyl alcohol. These units have been incorporated into angiosperm lignin. Currently, the 5-hydroxyconiferyl alcohol is also considered to form benzodioxane units.[93]

1.2.3.7 Tricin Unit

Tricin is a flavonoid that exists in wheat, oat bran, sugarcane, and maize. It is recognized as a valuable compound due to its antioxidant, antiaging, and anticancer properties.[94] Recently, the flavonoid tricin has been implicated as a monomer in monocot lignin by assignment of unknown spectra on HSQC of whole cell wall and MWL of wheat straw[95] and other monocots.[96,97] However, the mechanism of incorporation of tricin with lignin and the role of tricin remained unclear. Most recently, by comparison of DHP from tricin-monolignols with maize stover lignin, substantive findings showed that tricin is incorporated into monocot lignins *via* a free radial coupling mechanism, that there is a covalent bond between tricin and the lignin polymer through the β-O-4 linkage, and that tricin functions as a nucleation site for the growth of the lignin polymer chain in monocots.[98] Based on these findings, it was found that tricin is the only non-cinnamyl alcohol derived from outside of the canonical cinnamyl alcohol biosynthesis pathways. Due to the structural analogy between tricin-lignin oligomer/polymer and MWL, they were renamed flavonolignin units.[98] The abundance of lignin-integrated tricin has also been estimated using thioacidolysis at levels of 0.0331 and 0.0327/OAr of lignin in oat straw and wheat straw, respectively.[99] Using thioacidolysis, it was also revealed that the amount of tricin integrated into lignin is much higher than the extractable tricin, indicating that lignin in cell wall and solubilized waste lignin from biorefinery processing have potential to provide the valuable compound tricin.[99]

1.2.4 Lignin Functional Groups

Lignin has various functional groups such as phenolic hydroxyl, aliphatic hydroxyl, benzyl alcohol, noncyclic benzyl ether, carbonyl groups, and methoxyl groups. The abundance of these functional groups directly affects reactivity of the lignin in various chemical reactions. This section introduces the determination methods of main functional groups and their roles.

1.2.4.1 Phenolic and Aliphatic Hydroxyl Groups

Lignin has phenolic and aliphatic hydroxyl groups, with the former playing an important role for both lignin biosynthesis[100] and degradation reactions.[101,102] For instance, phenolic hydroxyl groups are directly related to growth of lignin. In the cell wall, phenoxy radicals are generated by

peroxidase from phenolic hydroxyl groups in the three monolignols, and the generated radical resonance structures couple with each other randomly to create various C–C and C–O–C bonds in lignin.[11] In the ''endwise'' polymerization process, a cinnamyl phenoxy radical abstracts a hydrogen from the end of the polymer chain. The newly formed radical then couples with another cinnamyl phenoxy radical to increase the chain length. The phenolic hydroxyl group also increases by cleavage of the β-O-4 bond in the oxidative and base-catalyzed depolymerization of lignin.[101,102] In addition, the amount of phenolic hydroxyl groups in crude pulp contributes to the degree of brightness and stability. Therefore, the phenolic hydroxyl group is an important functional group in lignin. On the other hand, aliphatic hydroxyl groups on C_α and C_γ positions effect catalytic and chemical depolymerization reactions, especially acidolysis.[26]

As reviewed in more detail in Chapter 15, there are several quantitative analytical methods for the determination of phenolic hydroxyl groups in lignin[103] such as UV-vis,[24,104] aminolysis,[105,106] acetylation/titration,[107,108] acetylation/quantitative ^{13}C NMR,[109–111] and periodate oxidation[24,106] methods. Based on these methods, the abundance of free phenolic hydroxyl groups was estimated to be 0.1–0.13/OAr and 0.09–0.1/OAr in spruce and aspen wood meal, respectively,[106] and to be 0.2–0.33/OAr in spruce MWL.[24,103,105] Quite recently, quantitative ^{31}P NMR methods have been developed to estimate individual phenolic hydroxyl groups in P, G, and S units in isolated lignins.[112–114] These methods can provide information on phenolic hydroxyl groups as well as aliphatic hydroxyl group and carboxylic acids.

1.2.4.2 *Benzyl Ether and Benzyl Alcohol Groups*

Benzyl ether and benzyl alcohol groups are critical to chemical reactions in lignin since most degradation reactions, including β-O-4 cleavage, start from the benzyl position. There are three different types of benzyl ethers, the non-cyclic benzyl aryl ether, cyclic benzyl aryl ether such as phenylcoumaran and pinoresinol structures, and benzyl alkyl ether. The determination of these groups is also quite relevant for structural and chemical characterization of lignin. The total content of benzyl ether and benzyl alcohol groups in spruce MWL was determined to be 0.43/OAr by acid hydrolysis in methanol[115] and 0.33/OAr by NMR.[116] The abundance of the benzyl alcohol is also quantified by a combination of several chemical methods to be 0.05/OAr with phenolic units and 0.15/OAr with non-phenolic units.[115–117]

1.2.4.3 *Carbonyl Groups*

There are three different types of carbonyl groups on lignin side chains. These are conjugated carbonyls of the C_α=O and cinnamaldehyde (C_γHO) type and non-conjugated carbonyls of the C_β=O type (Figure 1.1 (G′, S′), Figure 1.2). Other types of carbonyls can be found on the aromatic ring, such

as a quinones.[118] The total carbonyl content has been determined using hydroxylamine hydrochloride at levels of 0.19/OAr in spruce MWL.[119] In this method, one mole of the carbonyl group reacts with hydroxylamine hydrochloride to quantitatively generate one mole of oxime and hydrochloric acid. The generated hydrochloric acid can be determined by titration.[118] Alternatively, a combination of the reduction of carbonyl groups with sodium borohydride and UV-Vis detection is considered to determine all conjugated carbonyl groups.[119] By the reduction difference spectrum in the UV-Vis method ($\Delta\varepsilon_r$ method), total carbonyl groups in the same spruce MWL is estimated to be 0.43/OAr, which is much larger than the value obtained from the hydroxylamine hydrochloride method because the calculation of correct absorptivity for each carbonyl type for UV-Vis is difficult in the $\Delta\varepsilon_r$ method. Based on detailed investigations,[120,121] the hydroxylamine hydrochloride method is currently considered to provide more accurate determination of the carbonyl content in lignin.

1.2.5 Linkages between Lignin and Polysaccharides

Lignin is also present as part of the lignin–carbohydrate complex (LCC). Several different linkages between lignin and polysaccharides have been identified (Figure 1.5A–D): (A) benzyl ether, (B) γ-ester, (C) conjugate γ-ester, and (D) phenyl glycoside bond type.[122,123] To determine the exact linkage positions in both lignin and carbohydrate moieties, various wet chemistry techniques such as DDQ oxidation have been applied to extracted LCC fractions and MWLs.[124–126] It was found that lignin links directly to arabinoglucuronoxylan *via* ester bonds at either benzyl or conjugated ester at γ positions.[127] Recently, spectroscopic methods such as FT-IR, solid-state NMR, and solution-state NMR have also been applied to the analysis of LCC structure. Especially, combinations of quantitative ^{13}C solution-state NMR and 2D NMR techniques are sufficiently advanced to obtain detailed insights into LCC structures.[128–130]

The presence of bonds between polysaccharides and hydroxycinnamic acids such as *p*-coumaric acid, ferulic acid, and sinapic acid in the cell wall has been proposed.[131,132] Based on intensive research into relationships among lignin, cellulose, and hemicellulose in the past several decades, it was found that ferulic acid residues linked with arabinoxylan *via* ester linkages between carboxylic acid in ferulic acid and primary alcohol at C5 position in arabinose group,[133] and that ferulic acid also linked to lignin monomers through oxidative coupling pathways to form ferulate–polysaccharide–lignin complexes *via* ether bonds (Figure 1.5E and G).[134,135] Another cross-linked structure between ferulates and arabinoxylan *via* diferulate esters might occur (Figure 1.5F).[135] These results indicate that ferulates could have important roles as initiation or nucleation sites for lignification,[136,137] a key component of the cross-linked structure between lignin and carbohydrates[135] and therefore a critical functional group for reducing recalcitrance to hydrolysis.[138]

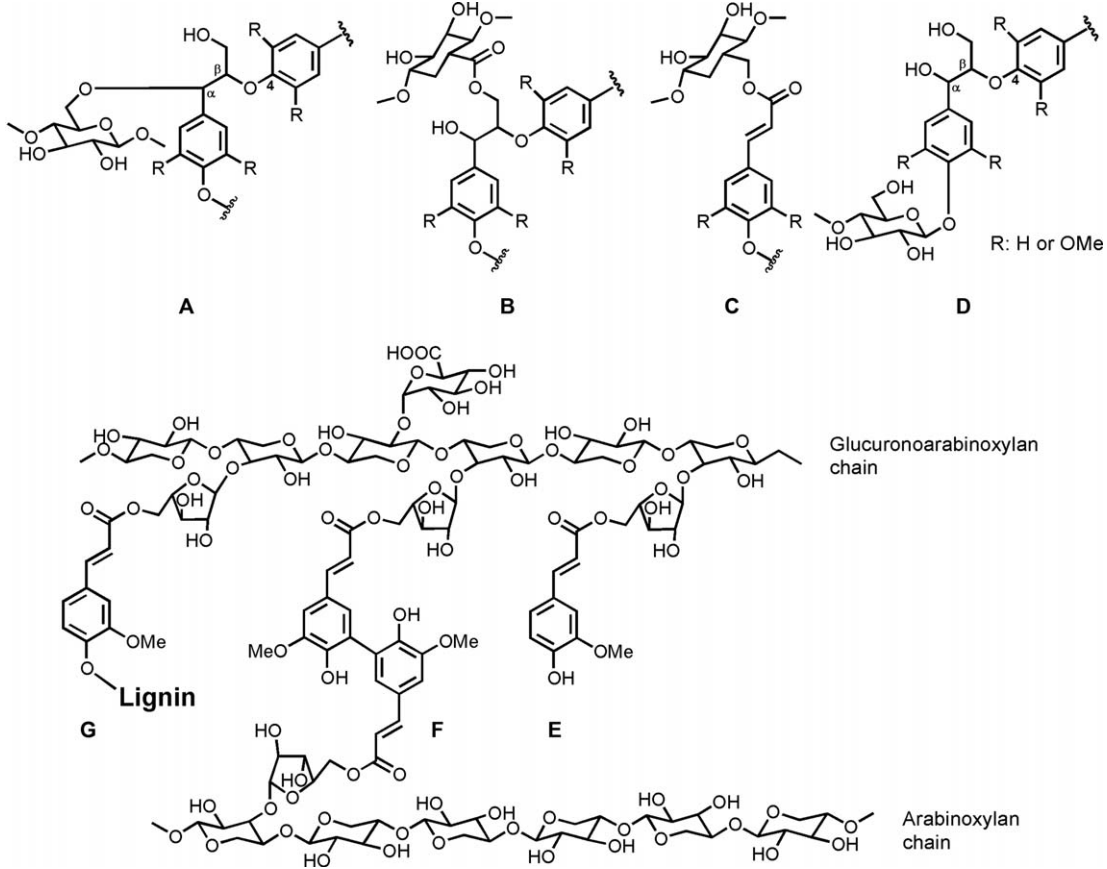


Figure 1.5 Possible lignin–carbohydrate structures (A–D); (A) benzyl ether, (B) γ-ester, (C) conjugate γ-ester, (D) phenyl glycoside type, and feruloylated glucuronoarabinoxylan; (E) ferulic acid residue esterified to an arabinose residue, (F) diferulic acid cross-linking two arabinoxylan chains, and (G) ferulic acid residue etherified to lignin moiety.

1.3 Scope of This Book

This book consists of chapters aimed at reviewing the latest breakthroughs and challenges in upgrading lignin to biofuels and biochemicals. In assembling this book, the specific aims were to bring together experts from biology, catalysis, engineering, and analytical chemistry to present a comprehensive, interdisciplinary picture of how lignocellulosic biorefineries could potentially employ lignin valorization technologies. Specific focus is on (i) methods for isolating lignin in the context of the lignocellulosic biorefinery, (ii) thermal, chemo-catalytic, and biological methods for lignin depolymerization, (iii) chemo-catalytic and biological methods for upgrading lignin, (iv) characterization of lignin, and (v) techno-economic and life-cycle analysis of integrated processes to utilize lignin in an integrated biorefinery. Chapters will specifically focus on the production of fuels and chemicals (not materials) from lignin. We also note that an exciting amount of *in planta* lignin modifications has emerged in the past decade, but these are not reviewed in this book. For materials from lignin and genetic modifications to lignin biosynthesis, readers are encouraged to consult many of the recent, excellent reviews on these topics.[139–143]

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