Wood Based Lignin Reactions Important to the Biorefinery and Pulp and Paper Industries

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The cleavage of lignin bonds in a wood matrix is an important step in the processes employed in both the biorefinery and pulp and paper industries. β-O-4 ether linkages are susceptible to both acidic and alkaline hydrolysis. The cleavage of α-ether linkages rapidly occurs under mildly acidic reaction conditions, resulting in lower molecular weight lignin fragments. Acidic reactions are typically employed in the biorefinery industries, while alkaline reactions are more typically employed in the pulp and paper industries, especially in the kraft pulping process. By better understanding lignin reactions and reaction conditions, it may be possible to improve silvicultural and breeding programs to enhance the formation of easily removable lignin, as opposed to more chemically resistant lignin structures. In hardwood species, the S/G ratio has been successfully correlated to the amount of β-O-4 ether linkages present in the lignin and the ease of pulping reactions.

Keywords: Biorefinery; Lignin reactions; Kraft pulping; Cooking; Hardwood; Softwood; Enzymatic hydrolysis; S/G; S/V

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INTRODUCTION

Wood is a naturally occurring mixture of various organic polymers. Cellulose is a partially crystalline polymer that is reasonably chemical-resistant and has the ability to form hydrogen bonds. Cellulose is the major component of fibers and comprises about 50% of the fiber weight. These fibers are locked into wood in a matrix composed of a combination of heterogeneous, three-dimensional, cross-linked, aromatic, hydrophobic polymers. Such polymers are known as lignin, hemicellulose (mainly xylans and mannans), and various lignin-carbohydrate bonds. Lignin consists of three monolignol precursors *p*-coumaryl, coniferyl, and sinapyl alcohols (Fig. 1) reacted via an enzyme-initiated dehydrogenative polymerization and is found in every vascular plant on earth. Lignin can be divided into three classes according to its structural elements. Guaiacyl lignin, which occurs in almost all softwoods, is largely a polymerization product of coniferyl alcohol. Guaiacyl-syringyl lignin, typically found in hardwoods, is a copolymer of coniferyl and sinapyl alcohols. While small amounts of *p*-hydroxyphenyl propane units derived from the incorporation of *p*-coumaryl alcohol are found in both softwood and hardwood lignins, substantially more are found in monocot lignin, which is a copolymer of all three lignin precursors (Sarkanen and Hergert 1971). H-type lignin structures are typically found in grasses and non-wood species. The current work focuses upon lignin reactions occurring in wood species, not grasses such as bagasse, straw, or switchgrass.

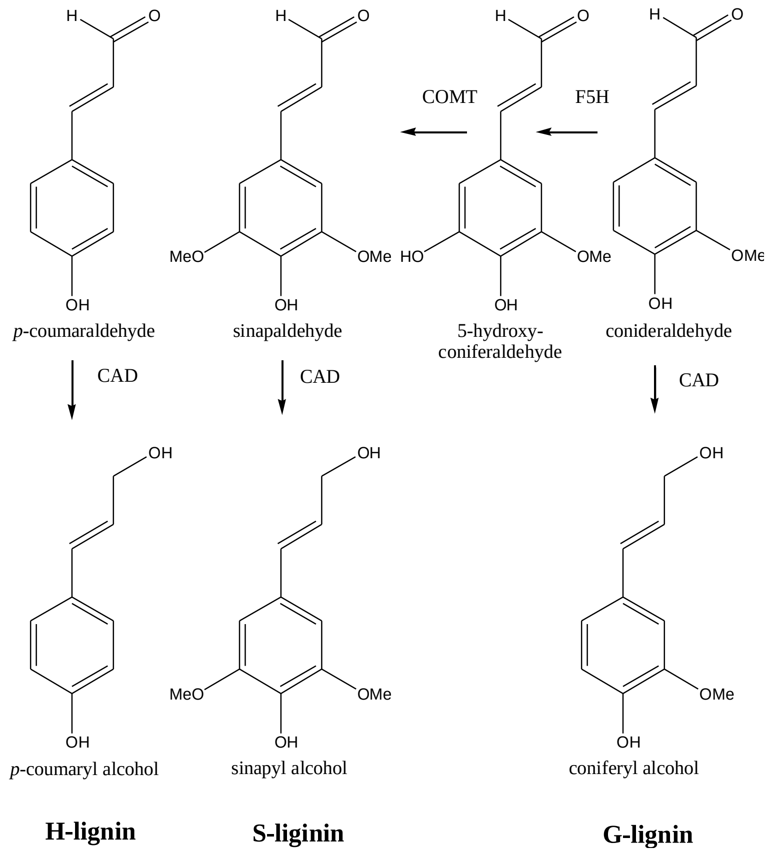


Fig. 1. Biosynthetic route toward the monolignols p-coumaryl, coniferyl, and sinapyl alcohol. The *p*-hydroxyphenyl (H), syringyl (S), and guaiacyl (G) structures of lignin. F5H: ferulate 5-hydroxylase; COMT: caffeic acid/5-hydroxyferulic acid *O*-methyltranferase; CAD: cinnamoyl alcohol dehydrogenase

The dominant linkages between the phenylpropane units as well as the abundance and frequency of some functional groups have been determined and are shown in Table 1 (Argyropoulos *et al*. 2002; Froass *et al*. 1996; Kukkola *et al*. 2004). Linkages between the phenylpropane units and the various functional groups on these units give lignin a unique and very complex structure. The lignin macromolecule also contains a variety of functional groups that have an impact on its reactivity. Lignin mostly contains methoxyl groups (92-96 per 100 phenyl propane units [PPUs]), free phenolic hydroxyl groups (15-30 per 100 PPUs), carbonyls (10-15 per 100 PPUs), benzyl alcohols (15-20 per 100 PPUs) and few terminal aldehyde groups.

Table 1. Predominant Lignin Linkages (Patil 2012)

Linkage Type	Dimer Structure	% Linkages in SWD	% Linkages in HWD
β-O-4	Arylglycerol β−aryl ether	45-50	60
5-5	Biphenyl and Dibenzodioxocin	18-25	20-25
β -5	Phenylcoumaran	9-12	6
β -1	1,2-Diaryl propane	7-10	7
α-O-4	Phenylpropane α−aryl ether	6-8	7
4-O-5	Diaryl ether	4-8	7
β – β	β-β-linked structures	3	3

Only a small proportion of the phenolic hydroxyl groups are free since most are occupied in linkages to neighboring phenylpropane linkages. Carbonyl and alcoholic hydroxyl groups are incorporated into the lignin structure during enzymatic dehydrogenation.

Studies on the association of lignin and carbohydrates have demonstrated that not only do covalent bonds exist between lignin and all major polysaccharides (arabino-glucuronoxylan, galactoglucomannan, glucomannan, pectins, and cellulose), but that cross-linkages also exist (Lawoko *et al.* 2005a; Lawoko *et al.* 2005b; Li *et al.* 2010). Lignin Carbohydrate Complex (LCC) content is known to be responsible for low delignification and/or lignin that is difficult to remove during the residual stage of cooking (Balakshin *et al.* 2007). While evaluating LCC linkages, Obst (1982) observed that pine and aspen had different LCC contents. While pine LCC content (ester + ether) was determined to be 4.7 per 100 monomeric lignin units, aspen had only 0.9 LLC linkages per 100 monomeric lignin units. More recently, using more advanced techniques, Balakshin (2007) found variation in LCC content among different wood species. While total LCC content (ether + phenyl glycoside + esters) in pine was determined to be 7.7, birch was determined to have 10.2 LCCs per 100 monomeric lignin units. Phenyl glycosides, benzyl ethers, and benzyl esters have been suggested to be the main types of lignin-carbohydrate bonds in wood (Balakshin *et al.* 2007). Figure 2 shows some typical LCC structures commonly believed to be present within the wood matrix.

Both the pulp and paper and the biofuels industries use established chemical processes to break down wood by initially cleaving lignin structures. The main objective and challenge is to remove lignin in a selective manner, where cellulose and hemicelluloses are preserved to the greatest possible extent. While the pulp and paper industry focuses on fiber liberation (lignin removal with minimal cellulose damage), the biofuels industry works to structurally open the fiber for enzyme accessibility and further breakdown of the cellulose and hemicelluloses into monomeric sugars.

Kraft pulping is currently the dominant chemical process used to produce pulp. As stated, the process was developed with the objective of removing lignin while preserving carbohydrates. During the process, white liquor (a blend of sodium hydroxide and sodium sulfide) promotes lignin dissolution and consequent fiber liberation. It is well documented that kraft pulping occurs in three distinct kinetic phases: the initial phase, bulk phase, and residual phase. During the bulk reaction phase, 60% to 68% of the total delignification of the wood occurs (Gierer 1980; Chiang *et al.* 1987). The use of sodium hydroxide and sodium sulfide offers a number of advantages when compared to other processes, especially in terms of fiber strength. However, there are also disadvantages; the major one being the loss in pulp yield caused by carbohydrate instability and degradation during the alkaline reaction. Typically, for hardwood pulps, delignification reactions are about 13 times faster than carbohydrate degradation reactions (Santos *et al.* 2012a).

Easier lignin breakdown during pulping could potentially result in less drastic process condition requirements, leading to carbohydrate preservation (Santos *et al.* 2011). In order to achieve the structural breakdown and solubilization of lignin, wood chips need to first be impregnated with alkali. The impregnation process consists of transportation of cooking liquor through the surface of the chip, followed by diffusion into the interior. As the chip heats up, lignin chemical reactions start to occur. When chips are being pulped at elevated temperatures, the rate of liquor diffusion into the wood is the rate-determining step. Delignification reactions tend to be faster than the diffusion rate (Stone and Green 1959). This event is followed by diffusion of degradation products to the exterior of the chip and transportation of those products to the cooking liquor.

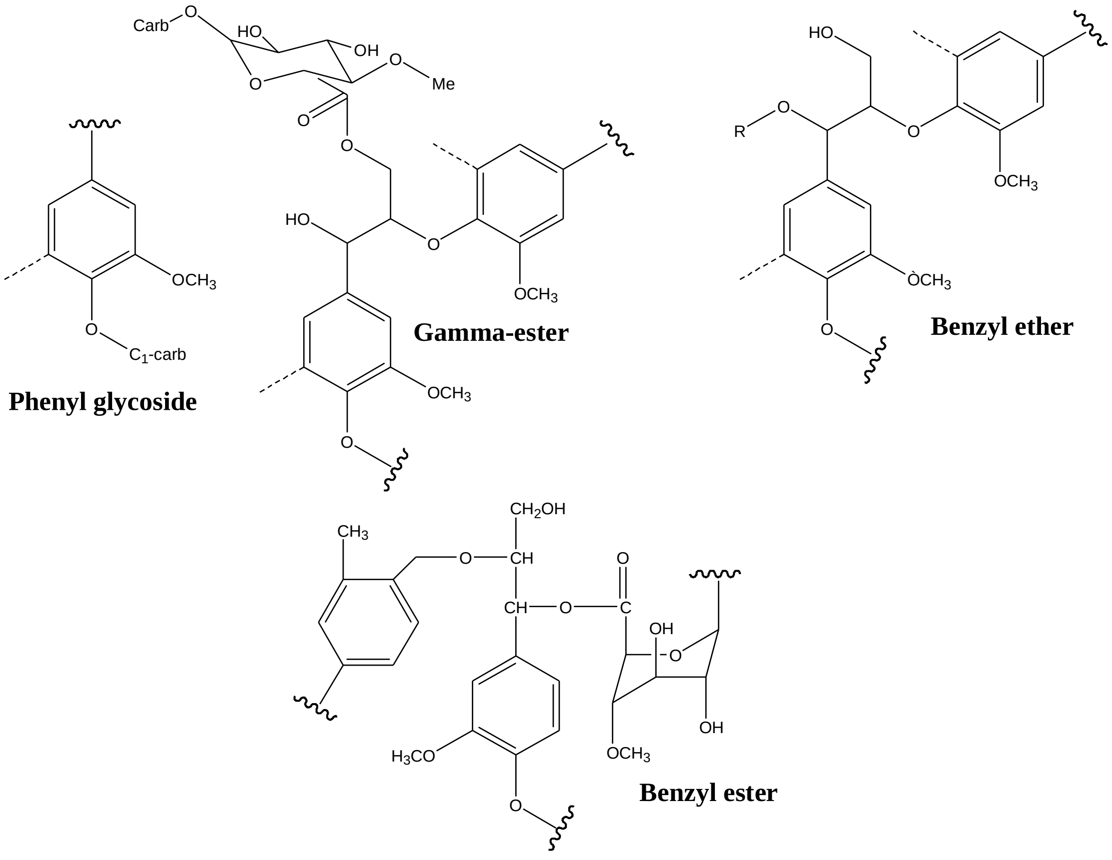


Fig. 2. Typical LCC structures commonly believed to be present within the wood matrix

While penetration is the flow of white liquor to the chip interior (driven by hydrostatic pressure), diffusion is the flow of white liquor ions through the water (present in the chips), which occurs via concentration gradients. Alkali-based reactions that occur when cooking liquor reaches lignin will be examined later in this work.

One of the main goals of the biofuels and biochemicals industries (biorefineries) is to break wood into its component monomeric and oligomeric sugar constituents. Frequently, enzyme-based reactions are employed in at least one step to accomplish this goal. Because it is not as old and well established as the pulp industry, second generation biofuel production has seen the employment of multiple pretreatment methods to make the substrate more amenable to enzymatic hydrolysis. One of the most important considerations in addressing efficient enzymatic hydrolysis is “opening up” the ultrastructure of the lignocellulosic biomass matrix, thus increasing its accessibility to enzymatic penetration and activity. The dominant hurdle for cellulolytic enzymes to overcome is accessing the cellulose chains that are tightly packed in the form of insoluble microfibrils encased in hemicelluloses and lignin (Mansfield *et al.* 1999). This cellulose is buried within a matrix of lignin and part of the highly ordered, tightly packed fibrillar architecture of the cellulose microfibrils, making cellulolytic attacks very difficult (Arantes and Saddler 2010).

Lignin is considered to be a major hurdle to efficient enzymatic hydrolysis. A number of studies have demonstrated a negative correlation between lignin content and carbohydrate conversion efficiency during enzymatic hydrolysis. A comprehensive modeling study of 147 lignocellulosic substrates demonstrated that lignin content has a very clear correlation with the efficiency of enzymatic hydrolysis (Chang and Holtzapple 2000). The negative impact of the presence of lignin is very likely due to the physical barriers it imposes, thus restricting enzyme access to the cellulosic material (Santos *et al.* 2012b; Yang *et al.* 2011).

In addition, lignin provides a surface onto which irreversible and nonproductive adsorption of enzymes occurs, thus severely hampering the reactivity of enzymatic hydrolysis. It has been conjectured that the greater number of pores created by lignin removal allow cellulose and hemicellulose to be more accessible and thus more open to swelling and contact with enzymes (Taherzadeh and Karimi 2007), providing a favorable environment for hydrolysis.

The adsorption of cellulases and hemicellulases onto lignin is believed to be due to hydrophobic interaction or ionic-type lignin-enzyme interactions (Berlin *et al.* 2006; Eriksson *et al.* 2002; Sewalt *et al.* 1997). Nakagame and coworkers prepared isolated lignins from corn stover, poplar, and lodgepole pine. The isolated lignins were mixed with crystalline cellulose (Avicel) to assess the effect of lignin on enzymatic hydrolysis. It was found that the lignin isolated from lodgepole pine and steam-pretreated poplar decreased the hydrolysis yields of Avicel significantly, which provided evidence that supported the supposition of non-productive adsorption of enzymes onto lignins (Nakagame *et al.* 2010).

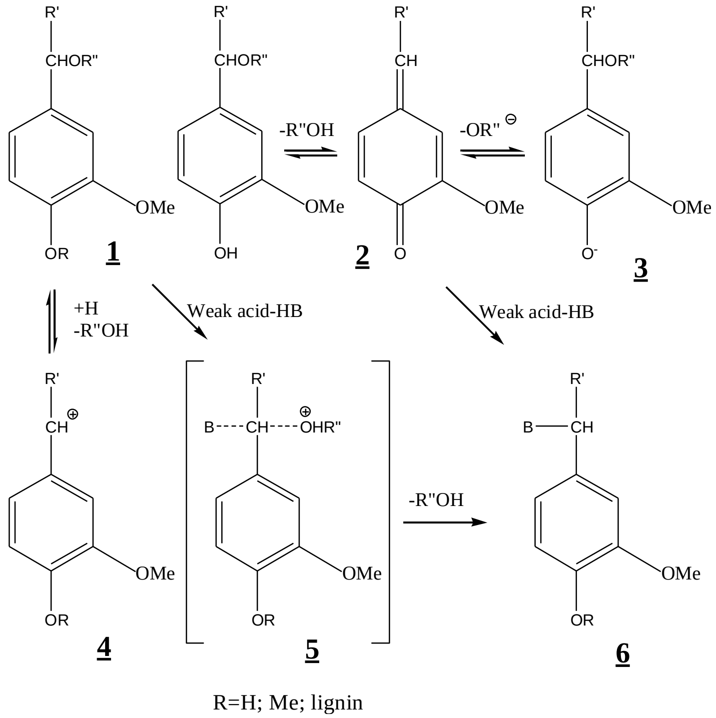


Fig. 3. Benzyl alcohol and benzyl ether group reactions

Overall, lignin presents a unique challenge to the pulping and biofuel processing industries in that it is fairly recalcitrant towards degradation reactions. Unfortunately, lignin needs to be removed to liberate cellulose from the wood. A significant amount of research has focused on methods for enhancing lignin digestion processes and the identification and utilization of more suitable feedstocks.

Lignin removal typically employs either acidic or alkaline degradation techniques. A great amount of effort has been expended in recent years to enhance the biodegradation of lignin through improved understanding of cleavage and displacement reactions that occur when lignin is exposed to alkaline and/or acidic reaction conditions. Benzyl-alcohol and ether groups have been reported to have a major impact on lignin degradation in both acidic and alkaline solutions. Typical benzyl alcohol and benzyl ether reactions are shown in Fig. 3.

LIGNIN REACTIONS UNDER ACIDIC CONDITIONS

β-O-4 Bond Cleavage

Acidic conditions can be created by the simple reaction of water and wood at an elevated temperature. Mildly acidic conditions, also known as acidolysis reactions of wood chips, result in an autocatalytic cleavage of various lignin and hemicellulosic bonds. The most important reaction during acidolysis of lignin is the cleavage of the β-O-4 bonds. This cleavage reaction has been described by several authors (Adler *et al.* 1957; Lundquist and Lundgren 1972; Lundquist 1973; Ito *et al.* 1981; Yasuda *et al.* 1981a, b, 1982, 1985; Yasuda and Terashima 1982; Hoo *et al.* 1983; Karlsson *et al.* 1988). The mechanism for this cleavage reaction (as reported by Lundquist and Lundgren) is shown in Fig. 4. Lignin units are frequently connected to each other via a β-O-4 bond in the β position. During cleavage reactions, the β-O-4 bond at the β-position **I** primarily converts into a benzyl cation type intermediate **II**, and an enol ether type of substructure **III** is formed (route A). The β-O-4 bond of **III** is then hydrolyzed to yield a new phenolic lignin unit **IV** and Hibbert's ketone type substructure **V**.

Another competing route (route B), which also leads to β-O-4 bond cleavage, has been previously described in the literature (Lundquist and Ericsson 1970). Formaldehyde is released from the γ-position of **II** and another enol ether type substructure **VI** forms. The β-O-4 bond of **VI** is similarly hydrolyzed to yield the new phenolic unit **IV** and aldehyde **VII**.

Lundquist and Lundgren (1972) suggested that the rate-determining step of the β-O-4 bond cleavage in route A is the conversion of **II** into **III** and the removal of the β-proton from **II**. This proposal was based on two observations: (1) a model compound analogous to **III** is quite labile under acidolysis conditions, and (2) an acidolysis of this compound yields reaction products identical to those obtained by the same treatment of a model compound analogous to **I**. However, an enol ether compound of type **III** was not detected in acidolysis of the model compound of **I**, so it has not yet been proven whether or not the rate-determining step is definitely the removal of the β-proton from **II**.

Under mild acidic conditions homolytic cleavage of phenolic arylglycerol β-aryl ether bonds is an important reaction particularly for hardwoods (presence of syringyl lignin) (Miksche 1973). This reaction is known to take place via a homolysis of an intermediate quinine methide. The reaction can also occur under elevated temperatures

(Bardet *et al.* 1985), which makes it of significance in processes such as steam hydrolysis, steam explosion, high yield pulping, *etc*.

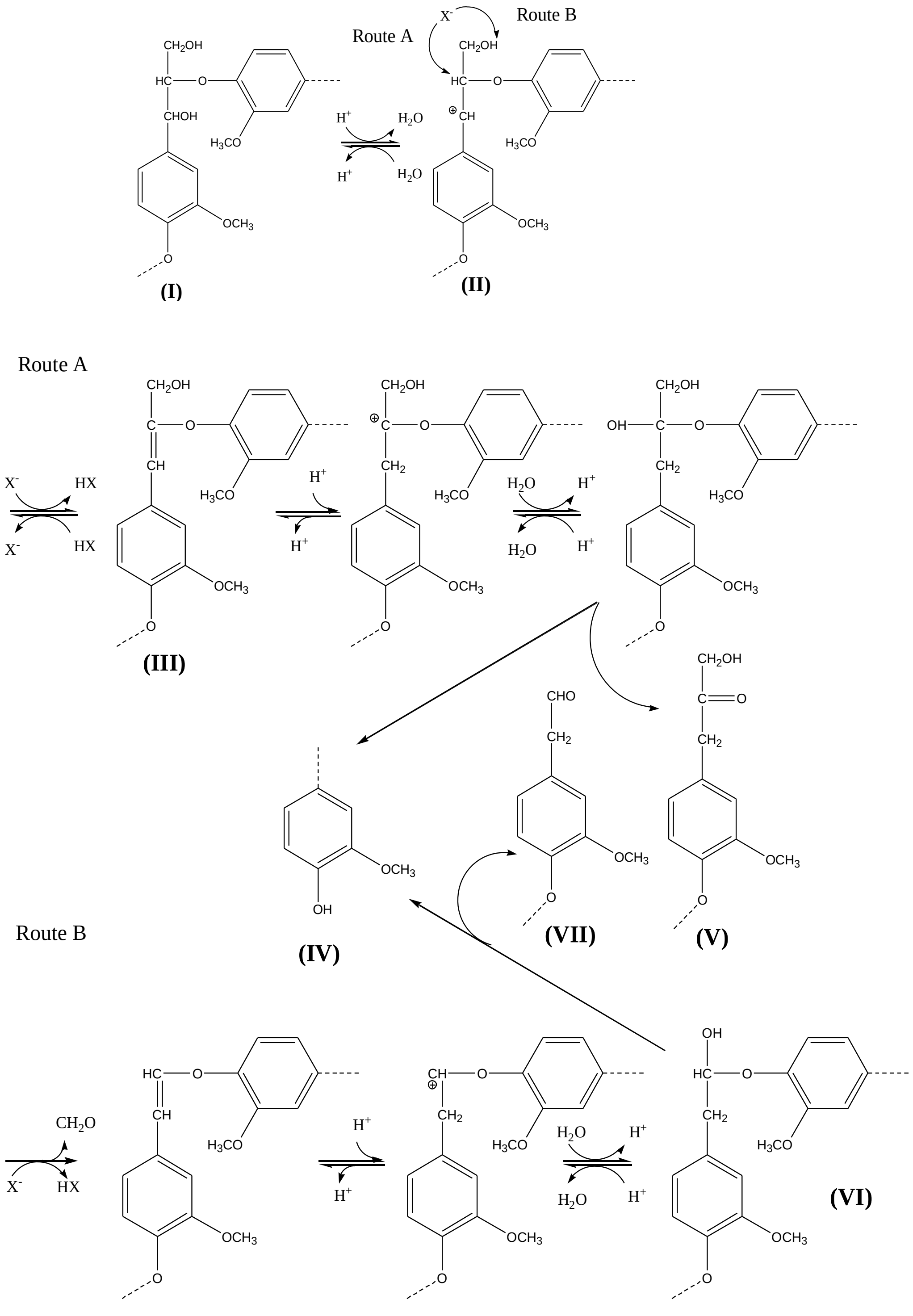


Fig. 4. Mechanism of the β-O-4 bond cleavage, based on Lundquist and Lundgren's research (1972)

Impact of Acid Type on β-O-4 Cleavage Reaction Pathways

Under mildly acidic reaction conditions, such as those occurring during autocatalytic hydrolysis reactions between wood and water, both α- and β- ether linkage cleavage occurs. Figure 5 shows examples of both α- and β- ether linkage structures found in lignin. In fact, the α-ether linkage has been reported to have a higher rate of reaction under mildly acidic conditions than the β-ether linkage and will be reviewed later.

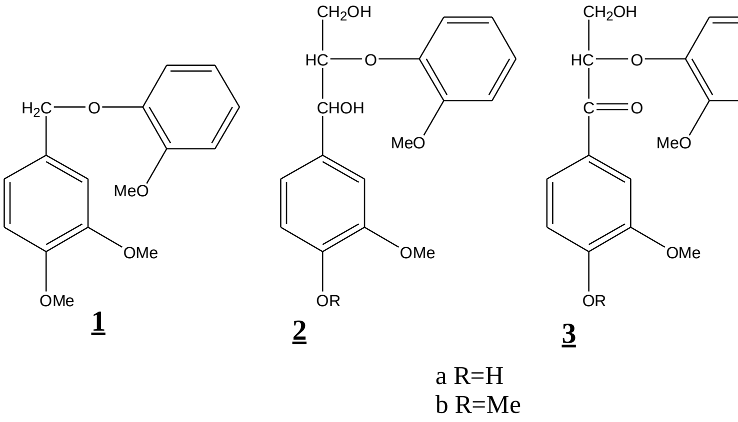


Fig. 5. α- (1) and β- (2, 3) ether linkage substructures found in lignin

Speculation about the impact of different acids and the ability of their conjugate bases to abstract a β-proton from **II** has been discussed in the literature (Ito *et al.* 1981; Yasuda *et al.* 1985; Karlsson *et al.* 1988). It has been reported that the rate of β-O-4 cleavage is dependent upon the type of conjugate base employed and that the rate of cleavage reactions follows the order HBr > HCl > H_2SO_4. More recently, the Matsumoto group from the University of Tokyo published a series of papers re-examining the β-O-4 cleavage reaction (model compound based) in the presence of different types of acids (Yokoyama and Matsumoto 2008, 2010; Ito *et al.* 2011; Imai *et al.* 2011). The work by these authors is in good agreement with the existing theories about β-O-4 cleavage (Yasuda *et al.* 1985; Karlsson and Lundquist 1992). However, these authors determined that the reaction pathway for β-O-4 cleavage was dependent upon the type of acid employed. The authors determined that the predominant reaction routes when using HCl are **I** and **III**, shown in Fig. 6, while route **II** (also shown in Fig. 6) is predominant when H_2SO_4 is employed. When employing HBr, reaction routes **I** and **III** were found to predominate only after the first 2 hours of reaction time.

Acidic Alcohol Reactions

The use of alcohol in conjunction with acidic liquor has been explored as a possible biomass pretreatment to either remove lignin for downstream value-added processing or as a pretreatment for enzymatic hydrolysis. The predominant alcohol of choice to date has been ethanol. It has been found that normal primary alcohols are better delignification reaction agents than secondary or tertiary alcohols. The mixture of n-butyl-alcohol-water appears to be the most efficient at removing lignin from wood (Yawalata 2001). Reactions occurring in this medium introduce alkoxy groups into the lignin structure. Additionally, lignin has been found to be soluble and can be dissolved by ethoxylation reactions occurring in alcohol-water reaction media (West *et al.* 1943;

Schuerch 1950). During the course of ethanolysis, the phenolic hydroxyl content of the lignin increases, which indicates that phenyl ether linkages are broken, forming γ-methyl groups and β-carbonyl groups (MacGregor *et al.* 1944). Evidence of condensation reactions of Cα, C5, and C6 with the formation of carbonyl groups has been found and reported in the literature (Sarkanen and Schuerch 1957).

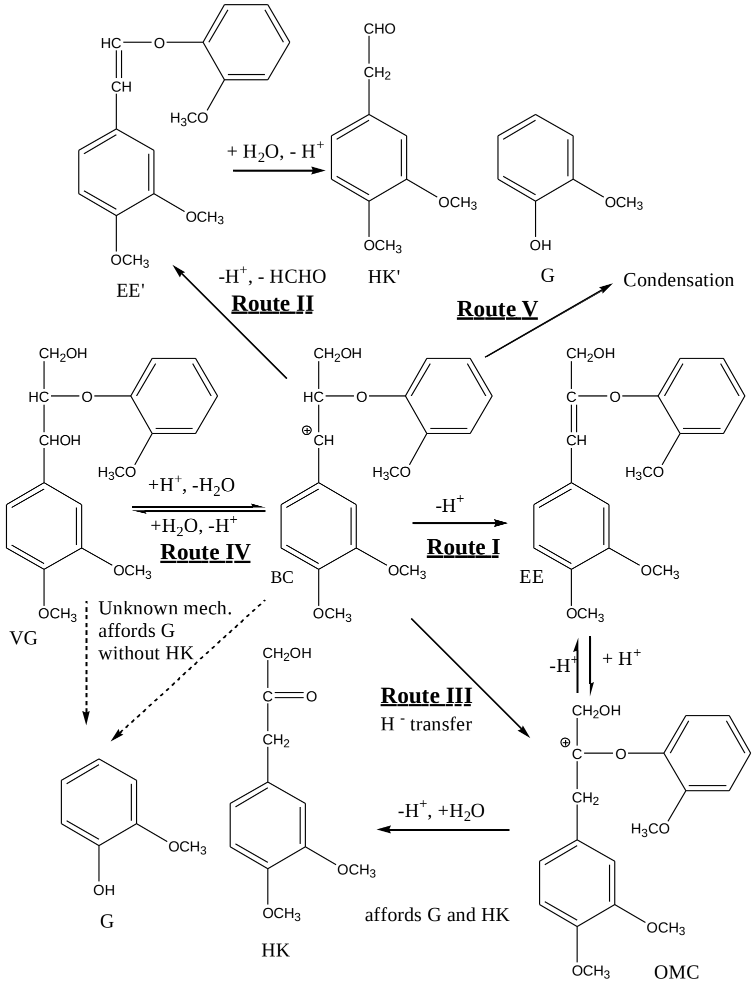


Fig. 6. Cleavage of lignin (model compounds) using different types of acids. Reaction routes I and III are predominant using HCl, while route II is predominant when using H_2SO_4. VG: 2-(2-methoxyphenoxy)-1-(3,4-dimethoxyphenyl)propane-1,3-diol; G: 2-methoxyphenol; HK: 1-hydroxy-3-(3,4-dimethoxyphenyl)propan-2-one; OMC: β-oxymethyne cation; BC: benzyl cation-type intermediate; EE: 2-(2-methoxyphenoxy)-3-(3,4-dimethoxyphenyl)prop-2-en-1-ol; EE': 1-(2-methoxyphenoxy)-2-(3,4-dimethoxyphenyl)ethane; HK': 3,4-dimethoxyphenylacetaldehyde.

Another acid-catalyzed reaction known to occur is the elimination of the γ-hydroxy function from β-5 and β-1 dilignols to form phenylcoumarone (formation of water), stilbenes (elimination of formaldehyde), 1,1-diguaiacyl-2-propanone, and 1,2-diguaiacyl-1-propanone. These structures are shown in Figs. 7 and 8. They are: phenylcoumarone (Fig. 7b), stilbenes (Fig. 7a and Fig. 8e), 1,1-diguaiacyl-2-propanone (Fig. 8g), and 1,2-diguaiacyl-1-propanone (Fig. 8h) (Adler *et al.* 1966; Lundquist 1964).

It has been shown that propyl side chains containing β-aryl linkages and a γ-methylol group react under mildly acidic conditions via the loss of water to form stilbene and a new γ-methyl group. Reactive methylol groups may also be lost through the formation of formaldehyde. Formaldehyde production may result from reversed aldol condensation reaction, which takes place via a benzyl carbonium ion intermediate.

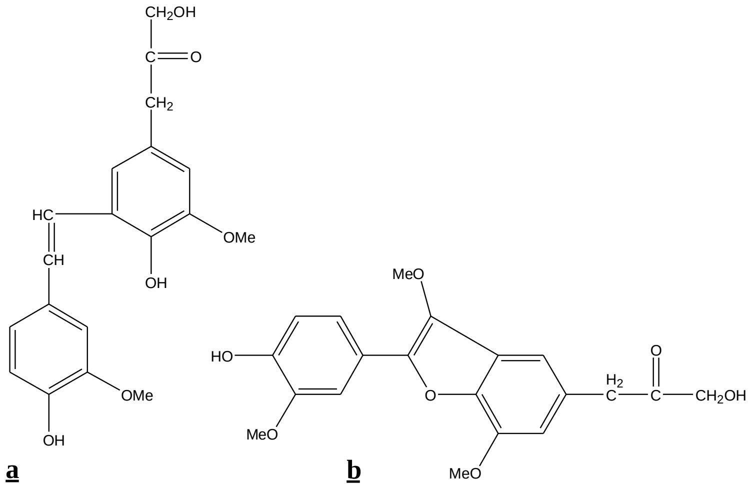


Fig. 7. Stilbenes (a) and phenylcoumarone (b)

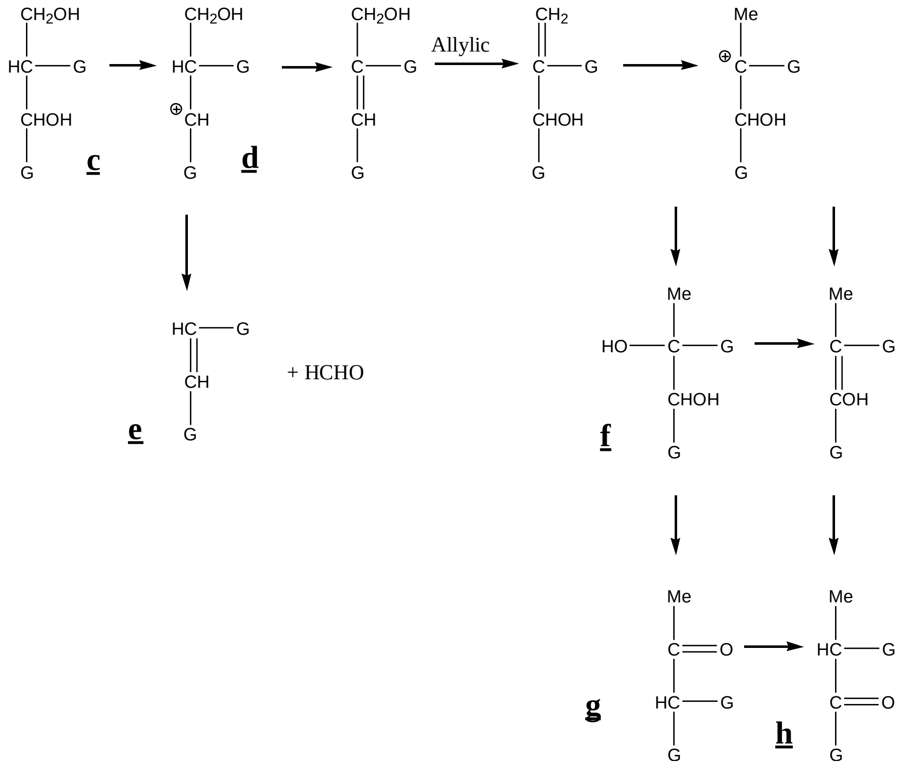


Fig. 8. 1,2-diguaiacyl-1,3-propandiol (c), benzyl carbonium ion (d), Stilbenes (e), diol intermediate (f), 1,1-diguaiacyl-2-propanone (g) and 1,2-diguaiacyl-1-propanone (h)

α-Ether Linkage Cleavage Reactions

Another lignin cleavage reaction that occurs under mildly acidic conditions is the breaking of α-ether bonds. This reaction requires the presence of a quinonemethide intermediate. Similar to β-ether linkages, α-aryl ethers are promptly hydrolyzed (Kratzl and Silbernagel 1952) and are largely responsible for the formation of low molecular weight lignin components. Cleavage of α- and β- structures that are responsible for the formation of vanillin and vanillic acid can occur under acidic conditions. To date, the mechanism for this reaction has not been clarified, but it is believed to be the result of oxidative reactions (Nimz 1967).

Side chain rearrangement can also occur in hot water (100 °C) and acid-catalyzed solvolytic reactions to form β-5 dilignol and dihydroconiferyl alcohol (Gierer and Noren 1962) from β-ether cleavage. Figure 9 details these rearrangement reactions under hot water and acidic conditions (only valid for the model compounds shown below). These reactions also result in allylic rearrangement of γ-hydroxy groups to the α-position.

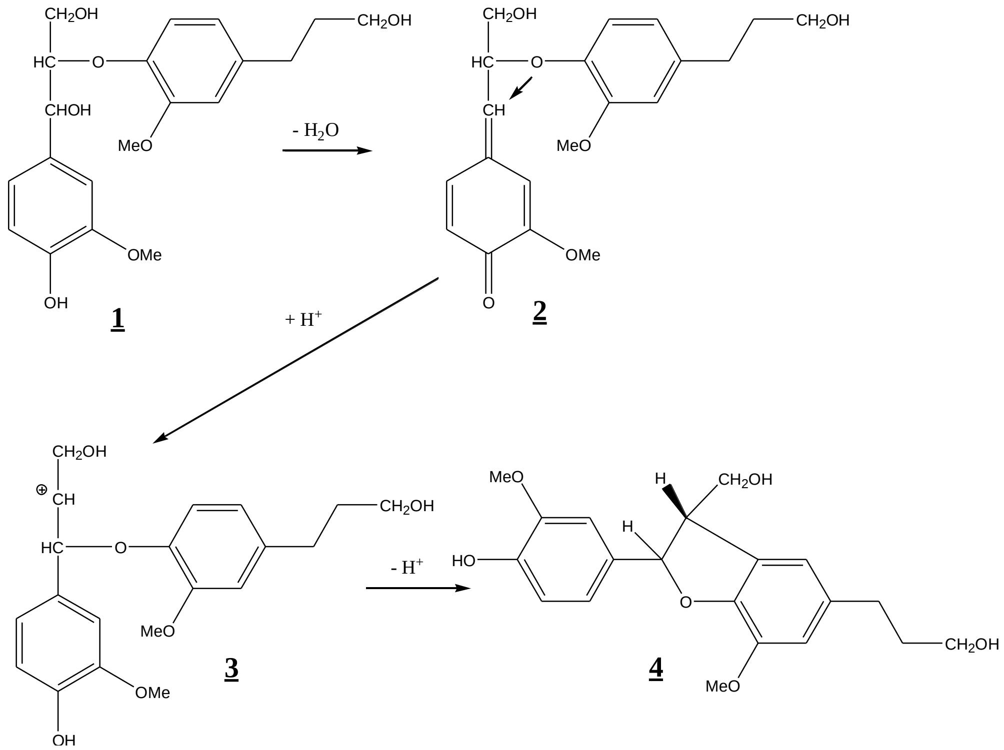


Fig. 9. Side chain rearrangements in hot water

LIGNIN REACTIONS UNDER ALKALINE CONDITIONS

Not many biomass-to-fuel operations use high pH treatments to break down wood fibers, although some studies have been performed with either green liquor or ammonia as a pretreatment to enzymolysis. The predominant use of alkaline lignin reactions is in the kraft pulping process, where lignin undergoes a variety of reactions under high temperatures. Carbon-carbon bonds are relatively stable under these conditions, while carbon-oxygen bonds prove to be quite liable to cleavage (Sarkanen and Hergert 1971).

Demethylation via Nucleophilic Substitution

An important aspect of kraft pulping is the nucleophilic attack of hydrosulfide ion (HS^-), causing demethylation of the kraft lignin. The nucleophilic HS^- reactions greatly increase the rate of lignin fragmentation as compared to the OH^- attack of lignin associated with soda cooking (Walker 2006). Nucleophilic attack of HS^- upon methyl aryl ether bonds results in demethylation. Demethylation reactions form methyl mercaptain and a catechol. The mercaptain may further react by cleaving another methoxy group to form dimethyl sulfide or it may get oxidized to form dimethyl disulfide. These reactions result in the characteristic strong odor associated with kraft pulping.

The fragmentation of lignin during kraft cooking is dependent upon the cleavage of both α- and β- aryl ether linkages which are the dominant linkages in both hardwood and softwood lignins. The α-aryl linkages are hydrolyzed by alkali but only fragments if the β-aryl ether linkage is absent or cleaved in a later reaction step.

α-Ether Cleavage

Cleavage of α-ether bonds has been known to occur when a free phenolic OH group is present in the *para* position to the propyl side chain (Gierer and Noren 1962). Intermediate quinonemethide formation facilitates the ongoing reaction (Larsson and Lindberg 1962).

Non-phenolic α-ethers can also be cleaved, as long as an OH group is located adjacent to the β-position (Gierer and Noren 1962). It is believed that, during this reaction, the neighboring OH group assists the reaction via the formation of an epoxide.

Non-cyclic benzyl ether linkages in lignin (such as α-aryl ethers – Fig. 10) may be cleaved by hydroxide ion to form phenolic hydroxyl groups, causing the content of this functional group to be higher than the actual amount present in the wood or pulp. NMR analysis suggested that residual lignin, compared to the wood lignin, is much less reactive towards pulping chemicals due to the low content of aryl ether linkages and the prevalence of condensed type structures (Froass *et al*. 1996).

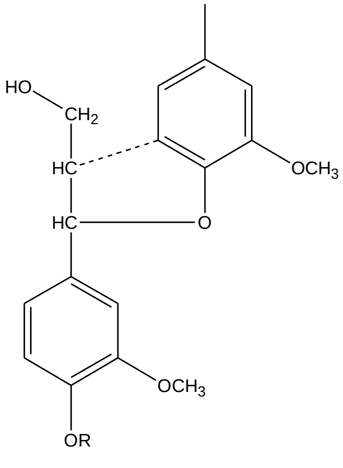


Fig. 10. Non-cyclic benzyl ether linkage in lignin

β-O-4 Cleavage of Non-phenolic and Phenolic Structures

β-ether linkage cleavage of non-phenolic (β-O-4) dilignol occurs if any adjacent OH or carbonyl group is present in either the α- or γ-position (Gierer *et al.* 1964). The reaction is enhanced by the presence of sulfide ions (Sarkanen and Hergert 1971). Figure 11 shows the reaction of the β-ether model compound (1) under alkaline conditions, which forms guaiacol (2) and veratrylglycerol (3).

Figure 12 shows a sulfide ion reacting with quinonemethide (4) leading to episulfide (5) and guaiacol formation (Sarkanen and Hergert 1971). Di-aryl ether cleavage products have also been obtained from alkaline hydrolysis of lignin but should be regarded as only minor reactions occurring under these conditions (Freudenberg and Chen 1967).

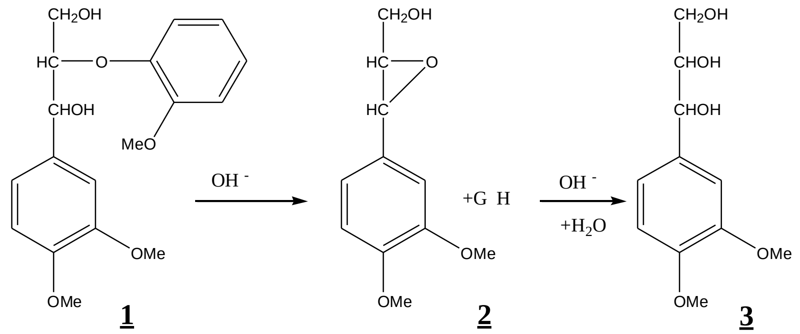


Fig. 11. Reaction of β-ether model compound (1) to form guaiacol (2) and veratrylglycerol (3)

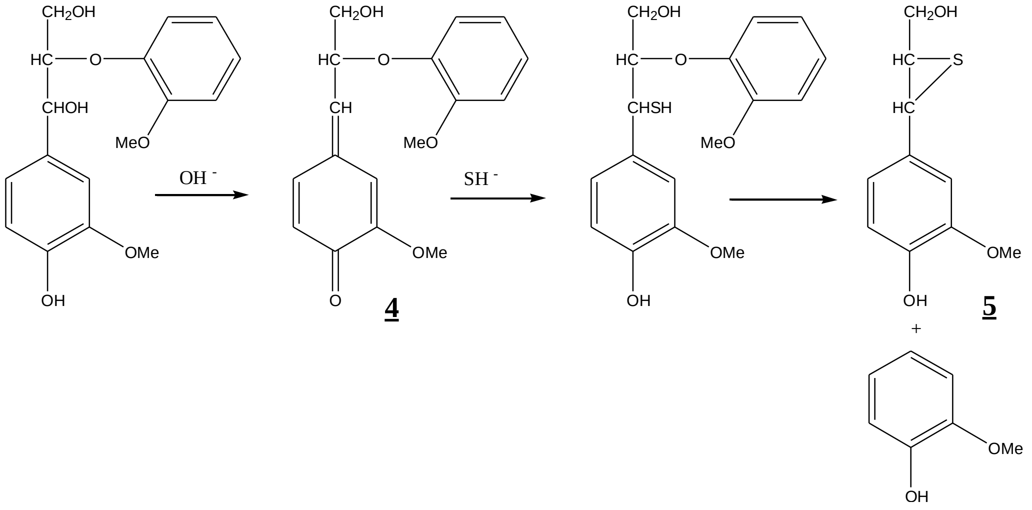


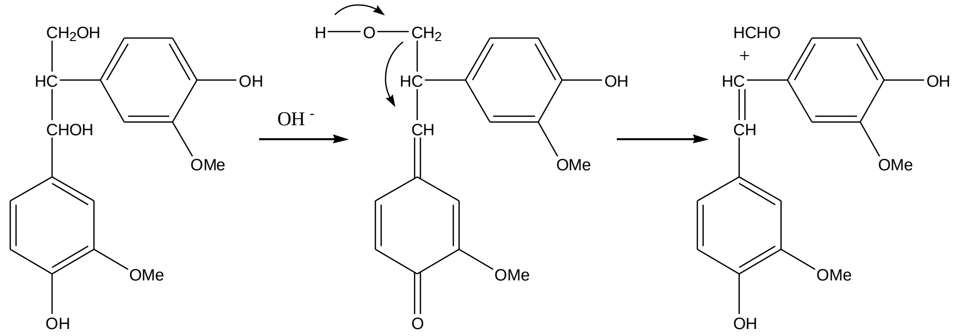
Fig. 12. Impact of hydrosulfide ion reacting with quinonemethide (4), leading to episulfide (5) and guaiacol formation. Phenolic structure.

Methoxyl Cleavage

If the experimental temperature conditions rise to around 200 °C, methoxyl lignin groups may undergo hydrolysis. Sarkanen *et al.* (1967) demonstrated that methoxyl groups from strongly acidic phenol, such as vanillin, are hydrolyzed much more quickly than the methoxyl lignin groups from weakly acidic phenols. There are fast and slow hydrolysis reaction phases that take place as a result of these different types of lignin methoxyl groups. When the reaction temperature is raised above 200 °C under alkaline conditions with NaSH present (kraft reaction conditions), principally homolytic cleavage reactions of lignin occur, resulting in a range of products (Turunen 1963; Enkvist and Turunen 1964). As with pure alkaline conditions, alkali with NaHS present results in primarily ether-type linkages being cleaved with limited cleavage of carbon-carbon linkages occurring.

β-γ Cleavage

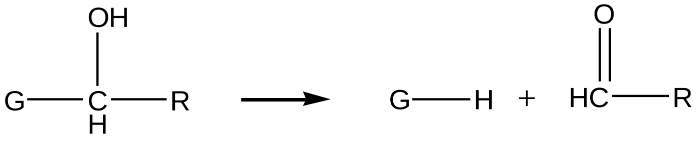
A prominent example of C-C cleavage reaction is the cleavage of γ-methylol during hydrolysis of β-5 and β-O-4-linked dimers, resulting in formaldehyde formation. The formaldehyde breakdown reaction only occurs if the structure contains free OH groups where it is possible to form a quinonemethide intermediate. Additionally, formation and liberation of formaldehyde under alkaline conditions can lead to the formation of a methylol derivative by electrophilic substitution onto an aromatic nucleus. Figure 13 shows β-1 dilignol (1) giving rise to formaldehyde. The reaction proceeds with quinonemethide formation (2) and formation of stilbene (3) via the loss of formaldehyde.



1 **2** **3**

Fig. 13. β-1 dilignol giving rise to formaldehyde with quinonemethide formation (2) and formation of stilbene (3) via the loss of formaldehyde

Because guaiacol can be found when wood undergoes alkaline treatment, it is assumed (Sarkanen and Hergert 1971) to result from the reaction of a guaiacylpropane carrying an α-hydroxyl substituent, resulting in aldehyde formation (see Fig. 14).



$$G-\underset{H}{\overset{OH}{C}}-R \longrightarrow G-H + HC(=O)-R$$

Fig. 14. Aldehyde formation from guaiacylpropane

Impact of Syringyl-to-Guaiacyl Ratio of Lignin on Biomass Conversion Processes

While softwood lignin appears to vary little among species (Sarkanen *et al.* 1967; Akiyama *et al.* 2005; Creighton *et al.* 1944; Leopold and Malmstrom 1952; Bland *et al.* 1950; Tower and Gibbs 1953), there is increasing evidence that the structure of hardwood lignin varies greatly from one species to another. Monolignol-forming lignin structures are of major importance when discussing lignin reactivity under either acidic or alkaline conditions. This is especially true for hardwood species. Early studies on nitrobenzene oxidation of hardwoods clearly showed wide variation in the syringaldehyde-to-vanillin ratio (S/V ratio) among different species (Creighton *et al.* 1944; Leopold and Malmstrom 1952; Bland *et al.* 1950; Tower and Gibbs 1953). While guaiacyl lignin (G) was found in vessels and cell corners, syringyl-guaiacyl lignin (S) was found in the secondary wall of fibers and rays, in which the S/G ratio varied from species to species among hardwoods. More recent studies demonstrated that the S/G ratio varies not only among different species (Akiyama *et al.* 2005; Pinto *et al.* 2005), but also among species of the same genus (Gonzalez-Vila *et al.* 1999; Bose *et al.* 2009), and among clones of the same species (Guerra *et al.* 2009).

A correlation has been established between the structural form of the β-O-4 structures found in lignin and the S/G ratio, indicating that the syringyl/guaiacyl composition is the key factor governing the proportion of erythro and threo forms of β-O-4 structure in hardwood lignin (Santos *et al.* 2012c). Studies have shown that syringyl-type lignin impacts cell wall degradability positively, although the mechanism responsible for this phenomenon is still unclear (Xu *et al.* 2010).

It has been proposed that during acidic treatment of wood under high temperature, lignin is softened and relocalized to the outer surface of the cell wall (Kristenen *et al.* 2008; Selig *et al.* 2007). Because guaiacyl-rich lignin has a more branched structure and a higher degree of polymerization than syringyl-rich lignin (Sterwart *et al.* 2009), it has been speculated that syringyl-rich lignin may have a lower softening point and is more easily relocated to the surface than guaiacyl-rich lignin. This phenomenon therefore leads to improved enzymatic digestibility (Li *et al.* 2010).

The observation that hot water washing after liquid hot water (LHW) pretreatment significantly increases the initial saccharification rate of the high syringyl sample suggests that some inhibitory compounds are removed. It has been shown that LHW pretreatment of wet cake (solids left after corn is fermented to ethanol) releases some phenolics, specifically the less substituted ones, (Nishikawa *et al.* 1988) and water-soluble xylo-oligosaccharides that can inhibit cellulases and β-glucosidases (Ximenes *et al.* 2010).

On the other hand, Davison *et al.* (2006) reported that a decrease in S/G ratio resulted in significant improvement in the rate of dilute acid hydrolysis and sugar release during pretreatment. It has been speculated that when guaiacyl type of lignin is present, a thinner cell wall is formed and it is easier to degrade. The same was found in a similar study by Fontaine *et al.* (2003), and they sustained the hypothesis that a thinner cell wall contained more guaiacyl lignin than a thicker cell wall.

By means of the same dilute acid hydrolysis pretreatment, Samuel *et al.* (2010) observed a 36% reduction of β-O-4 units and a slight decrease of β-β and β-5 linkages in switchgrass lignin. It was also detected that a decrease in S/G ratio resulted in an increase in guaiacyl phenolic OH and condensed phenolic OH concentration. Studer *et al.* (2011) also found that β-O-4 cleavage had the biggest impact on sugar release when auto-hydrolysis (mildly acidic) pretreatment was applied.

The fact that β-O-4 is the major lignin structure affected by acid pretreatment is not unexpected, as discussed in a previous section of this review. No matter the pretreatment method employed or the wood composition, lignin is a major constituent that results in enzymes' decreased ability to hydrolyze plant cell walls (Chang and Holtzapple 2000; Chen and Dixon 2007; Dien 2009; Vermerris *et al.* 2007; Wyman 2009).

When it comes to alkaline treatment of wood, considerable variation in kraft pulping performance of different hardwood species has been reported (Collins *et al.* 1990; Pinto *et al.* 2005; González-Vila *et al.* 1999; Bose *et al.* 2009). A study with model compounds indicated that the β-aryl ether of syringyl lignin is cleaved much more easily than that of guaiacyl lignin (Tsutsumi *et al.* 1995). Since β-O-4 is a dominant structure in lignin and appears to be strongly correlated to the S/G ratio, there have been many studies investigating the effect of the S/G ratio on kraft pulping of hardwoods (Collins *et al.* 1990; Pinto *et al.* 2005; González-Vila *et al.* 1999; Bose *et al.* 2009; Chang and Sarkanen 1973; Gomes *et al.* 2008). Even within the same species of *E. globulus* wood, evidence of the influence of S/G ratio on pulp yield and ease of pulping has been reported, leading to the inclusion of lignin S/G ratio as a selection parameter in clonal breeding programs for pulpwood production (González-Vila *et al.* 1999; del Río *et al.* 2005). Similar results were also reported for various clones of *E. grandis* and the hybrid of *E. grandis* × *E. urophylla* (Gomide *et al.* 2005; Guerra *et al.* 2009). The correlation was not as strong, leading to the suggestion that other lignin structural features, in addition to the S/G ratio, may also play a role (Guerra *et al.* 2009).

More recently, the S/G ratio has been reported as a key factor affecting the rate of hardwood kraft pulping (Santos *et al.* 2011). The same authors also reported that the S/G ratio appears to control β-O-4 content, E/T ratio (Erythro/Threo), degree of condensation, and methoxyl content (Santos *et al.* 2012c). A lignin with a high syringyl content has a higher proportion of β-ether linkages than a pure guaiacyl lignin. This is due to the fact that the 5-position is blocked by a methoxyl group in syringyl monolignols. Thereby it is not surprising that plants with a high S/G-ratio are easier to pulp (more β-ethers and less condensation) or that the erythro diastereoisomer is more prevailing since this is preferred for S-type β-ethers. These results have practical implications for tree breeding and energy plantation programs, as S/G ratio in lignin can be manipulated by genetic transformation.

CONCLUSIONS

1. Understanding the underlying reactions of lignin and various lignin constituents is an important part of improving reaction engineering and biological silvicultural efforts designed to enhance both the wood-to-biopolymers and the pulp and paper industries.
2. Lignin cleavage and its selective removal are required for both of these industries. In the biopolymer industry, the isolation of lignin may be desirable as a feedstock for further downstream, value-added processing. For the wood-to-fuels portion of this industry, lignin has been shown to be a major inhibiting constituent of enzymatic hydrolysis, and removing selected portions of the lignin prior to this reaction step helps enhance sugar yields.
3. For the kraft pulp and paper industries, lignin is typically considered to be an undesirable wood constituent that is costly and difficult to remove. Forestry and clonal breeding programs that enhance the percentage of easily removed lignin may allow for more selective and gentler reaction conditions to be employed during the lignin removal steps of the pulping and bleaching process.
4. A significant number of lignin cleavage reactions may occur in both acidic and alkaline reaction conditions. The cleavage of the β-O-4 ether linkage is a critical

reaction under both acidic and alkaline conditions. In general, under alkaline conditions, the carbon-carbon bonds are reasonably stable, while the carbon-oxygen bonds are liable to cleavage. Under acidic conditions, side chain rearrangement and cleavage of α-ether linkages leads to the formation of lower molecular weight lignin fragments.

5. For hardwood species, the relative amounts of syringyl (S) and guaiacyl (G) lignin have been successfully correlated to the number of β-O-4 linkages present in the wood matrix. The S/G ratio has been found to correlate with more easily pulped lignin. It is well known that the S/G ratio may be controlled via selective breeding programs. Thus, the potential to genetically "build" easier to pulp trees may exist.

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