Synthesis and Oxidation of Lignin-Carbohydrate Model Compounds

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SYNTHESIS AND OXIDATION OF LIGNIN-CARBOHYDRATE MODEL COMPOUNDS

By

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MODEL COMPOUNDS

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To decrease our dependence on foreign oil supply and in an effort to decrease the amount of green house gases into the atmosphere, researchers are focusing on ways of producing fuels and chemicals from renewable resources. A renewable source such as wood has great potential for the production of biofuels and biochemicals. An integrated biorefinery process that maximizes the usage of wood and complements existing pulp and paper technology was proposed to extract underutilize hemicelluloses and covert them into bioethanol.

In this process, not only hemicelluloses are extracted; lignin, since it is covalently bonded to hemicelluloses is extracted as well. It is reported that lignin is an inhibitor for enzymes and yeast during the conversion of hemicelluloses into ethanol, which decreases the yield of ethanol and increases the cost of production. Therefore, lignin needs to be removed prior to the production of bioethanol from hemicelluloses.

Based on previous work in our lab, my project focuses on investigating oxidative methods to cleave lignin from hemicelluloses. Two glycosidic lignin-carbohydrate
compounds were synthesized for this study. Knowledge of the mechanisms will allow us to determine the potential for such processes in the production of biochemicals and biofuels.

We want to apply the oxygen delignification process to study this mechanism. Due to the presence of many oxygen species during this process, the mechanism is complicated. These oxygen species can be generated using \( \text{H}_2\text{O}_2 \) and UV radiation at 254 nm. Oxidative mechanisms of lignin-carbohydrate compounds were studied with superoxide anion (pH 12) and hydroxyl radical (pH 9.5).

We are able to cleave the glycosidic bond between carbohydrate and lignin. Further oxidation of aromatic and carbohydrate was occurred. Less than 50% of glucose is obtained after the majority of lignin-carbohydrate model compound is reacted and none of the lignin portion is obtained any point of the reaction. Hence, this technique is useful for the cleavage of the glycosidic linkages between lignin and carbohydrate. In order for this process to be a useful technique to obtain sugar from hemicelluloses, a process that is able to stop the further oxidation of carbohydrate must be developed.
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CHAPTER 1-INTRODUCTION

With the concern over the decreasing supply of fossil fuel and global warming due to the increasing amount of greenhouse gases, many researchers are looking to find an alternative source of energy that is renewable but also environmentally friendly. Biomass is a source that addresses both problems. Biomass is abundant on earth. It is anything that is bio-organic in nature and not predicated on fossil fuel. It consists of wood, fast growing trees, agricultural crops, and also residues from forests, mills, and livestock (Lucia et al., 2006).

Currently, corn is the main material that is being converted into bioethanol. The majority of corn and ethanol production is located in the Midwestern United States. The high demand for corn to make into ethanol has driven the price of corn up significantly, which in turn has increased the price of food. Therefore, researchers are interested in finding alternative sources of biomass for the production of ethanol. In our work, we are investigating wood as a source of biomass for biochemicals and biofuels.

TYPES OF WOOD

Wood is divided into softwoods and hardwoods. Softwoods are gymnosperms and are also called conifers. Hardwoods are angiosperms and also are called deciduous trees. A distinct difference between softwoods and hardwoods is that softwoods have needles instead of leaves.

COMPONENTS IN WOOD

Wood is composed of three major polymeric components including cellulose, hemicelluloses, and lignin, plus a large number of smaller molecules that are collectively
called extractives. Cellulose and the hemicelluloses are polysaccharide polymers while lignin is a complex phenolic polymer.

Wood cell wall

The wood cell wall consists of several layers. The primary wall is about 0.1-0.2 \( \mu \)m thick and consists of cellulose, hemicelluloses, pectin, and protein. The secondary wall consists of three layers: inner and outer which are thinner compared to the thick middle layer. The majority of the wood cell wall mass is in the secondary wall.

![Figure 1. Wood cell wall (Lawoko, 2005).](image)

Cellulose

Cellulose is the major component in both softwoods and hardwoods. The amount of cellulose ranges from 41-45 % for both types of wood. Cellulose is a homogeneous linear polymer that consists of about 10,000 \( \beta \)-D-glucose units joined together by 1,4-\( \beta \)-glycosidic linkages (Figure 2) (Sjostrom, 1993). Also, because of the 1-4 linkage and the way each chain aligns in the wood cell wall, these cellulose chains are hydrogen bonded through the hydroxyl groups by intra- and inter- molecular bonding. Therefore,
cellulose is a strong and rigid homopolymer. It contains both a crystalline (70%) and non-crystalline (30%) structure.

![Cellulose chain](image)

**Figure 2.** Cellulose chain.

**Hemicelluloses**

The next major components after cellulose are the hemicelluloses which comprise 25-35% of the wood composition. Hemicelluloses are amorphous heterogeneous polymers with degrees of polymerization between 100 and 200. They are made mainly from five sugars: D-glucose, D-mannose, D-galactose, D-xylose, and L-arabinose. Small amounts of L-rhamnose, 4-O-methyl-D-glucuronic acid, and D-galacturonic acid can be found in some of the hemicelluloses. Some hemicelluloses are acetylated also. The sugars are linearly linked with extensive branches to form the hemicelluloses. The number of branches of hemicelluloses depends on the species and type of wood.

**Softwood hemicelluloses**

Softwoods consist of 20-25% galactoglucomannans. Figure 3 is representative of a softwood galactoglucomannan. The backbone of galactoglucomannan is made up of β-D-1,4-linked glucose and mannose, whereas the α-D-galactose units are branches off the backbone attached by 1, 6 linkages. On average, one out of every three to four of these hexose units is acetylated at the C-2 or C-3 positions. The galactoglucomannans are
further divided into two categories depending on the abundance of galactose. With a ratio of 0.1:1:4 of galactose: glucose: mannose, glucomannan makes up of 15-18% of the softwood galactoglucomannans. The other portion of galactoglucomannan has a 1:1:3 ratio of galactose: glucose: mannose. Hydrolysis of galactoglucomannan into individual hexose units can be done easily by acid, whereas removal of acetyl groups is easier with alkali than with acid (Sjostrom, 1993).

![Diagram of galactoglucomannan](image)

**Figure 3.** Softwood galactoglucomannan

Softwood xylan, also referred to as arabinoglucuronoxylan, is a minor component of softwood hemicelluloses. Figure 4 is a representative model of a softwood xylan which comprises 10-15% of softwood hemicelluloses (Sjostrom, 1993). The backbone of softwood xylan is made up of β-1,4-xylose units. Branches occur at the C-2 and C-3 position of softwood xylan. For about every ten units of xylose, there are two 4-O-methyl-α-D-glucuronic acid groups substituted at the C-2 position. For every 10 xylose units, one α-L-arabinose unit substitutes at the C-3 position. L-arabinose and 4-O-methyl-α-D-glucuronic acid groups help maintain the xylose backbone, otherwise
degraded, during base-catalyzed reaction (Sjostrom, 1993). The 1-3 linkage of the arabinose to the xylan backbone is relatively easily hydrolyzed by acid (Sjostrom, 1993).

![Chemical Structure of Xylose and 4-O-methyl-α-D-glucuronic Acid](image)

**Figure 4.** Softwood arabinoglucuronoxylan (xylan).

**Hardwood hemicelluloses**

Glucuronoxylans (xylans) are the major hardwood hemicelluloses making up about 20-30% of the wood material (Sjostrom, 1993). The backbone of the hardwood xylan is made up of 1,4-β-D-xylose units. For every ten xylose units, one 4-O-methyl-α-D-glucuronic acid is branched off the xylose chain with a 1, 2 linkage between a xylose and the uronic acid. Substitution of hydroxyl groups of xylose by O-acetyl groups occurs at the C-2 and the C-3 positions. For every ten units of xylose, there are about seven O-acetyl groups. Figure 4 is representative of a hardwood xylan. Hydrolysis of the xylose backbone is easily done by acid, whereas the glucuronic acid side chain is very resistant to hydrolysis. The acetyl groups can be removed by alkali (Sjostrom, 1993). Studies also have shown that a D-galacturonic acid group is linked to the reducing end of the xylan chain. At the C-2 position, D-galacturonic acid is linked with an L-rhamnose, whereas
the L-rhamnose is connected to the xylose chain at its C-3 position (Johansson and Samuelson, 1977).

![1,4-β-D-xylopyranose backbone](image)

4-O-methyl-α-D-glucuronic acid

**Figure 5.** Hardwood glucuronoxylan (xylan).

About 2-5% of the hemicelluloses in hardwood are glucomannans (Sjostrom, 1993). The hardwood glucomannan consists of β-D-glucose and β-D-mannose connected by β-1,4 linkages. Depending on the type of hardwood, the ratio of glucose and mannose is between 1:2 and 1:1. Under acidic conditions, hardwood glucomannan can be hydrolyzed into glucose and mannose units.

![β-D-glucose and β-D-mannose](image)

**Figure 6.** Hardwood glucomannan.
Lignin

Lignin is the third major component in wood. It makes up 25-30% of wood, depending on the type of wood. Lignin is a heteropolymer, consisting of many substituted phenylpropane units. The exact structure is still unknown; therefore a simple modified phenylpropane unit represents a monomer of lignin (Figure 7). Depending on the functional groups, there are three types of monomer units: coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol, as shown in Figure 8. Coniferyl alcohol is the main monomer unit that makes up the lignin in softwood. Hardwood lignin is made mainly of sinapyl alcohol and coniferyl alcohol. Both softwood and hardwood lignin also contain a small amount of p-coumaryl alcohol.

**Figure 7.** Basic monomer unit of lignin.
There are seven common linkages between the monomer units of lignin when considering the dimer unit of lignin, as shown in Figure 9 (Sjostrom, 1993). The major linkage in softwood and hardwood lignin is a β-O-4, aryl ether. This linkage type comprises approximately 48% of the lignin in softwood and 60% in hardwood. The next three major linkages are α-O-4 (2-8%), β-5 (9-12%), and 5-5(10-11%). Linkages at the 5-position in hardwood are less common compared to softwood, since in hardwood a methoxyl group is usually substituted at the C-5 position (Cole, 2005). Finally, the last three linkages are 4-O-5, β-β, and β-1, and they make up 4-7%, 2-3%, and 7% of the lignin linkages, respectively (Sjostrom, 1993). Traditional organic methods were used to determine those linkages. Spectroscopic methods such as ultraviolet, infrared, proton nuclear magnetic resonance, and $^{13}$C nuclear magnetic resonance are used to determine functional groups in lignin, since those groups are important in the reactions of lignin (Sjostrom, 1993).
LIGNIN-CARBOHYDRATE COMPLEXES (LCCS)

There is significant evidence that lignin is linked covalently to carbohydrates in wood. In 1866, to explain the difficulty of separating lignin and carbohydrates in wood, Erdmann hypothesized that they must be chemically bonded together, and named them "glycolignose" (Merewether, 1957). Not until many years later was work done to support Erdmann's hypothesis. In 1957, Merewether published a review of earlier works on isolation and identification of different LCC types in wood (Merewether, 1957). This publication also supported the work of Traynard and coworkers (1953) who extracted LCCs from poplar with hot water. Traynard was the first researcher to report extraction of LCCs from wood. His work was followed by many researchers. To this day, it is
accepted widely that lignin is bonded covalently to carbohydrates, but the name "lignin carbohydrate complex" is used still when referring to these compounds.

Evidence for covalent linkages between lignin and the carbohydrates is abundant. Four major types of native lignin-carbohydrate bonds have been proposed: benzyl ethers, benzyl esters, phenylglycosides, and recently, acetal linkages (Figure 10).

![Proposed types of lignin carbohydrate linkages.](image)

**Figure 10.** Proposed types of lignin carbohydrate linkages.

**Biosynthesis of ester and ether LCCs**

The biosynthesis of ester and ether LCC linkages has been proposed. The mechanism is related to the biosynthesis of lignin. As it was mentioned in the lignin section, lignin is a heteropolymer that is made from three main monomer units: coniferyl
alcohol, sinapyl alcohol, and p-coumaryl alcohol. The relative abundances of these monomers depends on the type of wood. The biosynthesis of lignin is initiated by plant peroxidases and phenol oxidases by dehydrogenation and polymerization. Peroxidases or laccases in the wood tissue initiate the dehydrogenation step of the lignin biosynthesis which generates the phenoxy radical and several resonance structures. Figure 11 shows the schematic of all the possible radicals generated from coniferyl alcohol initiated by dehydrogenation of the phenolic hydrogen by peroxidase or laccase (Koshijima and Watanabe, 2003).

Figure 11. All possible radicals generated from coniferyl alcohol initiated by laccase or peroxidase (Koshijima and Watanabe, 2003).

Polymerization of lignin from radicals occurs without enzymatic activity and mainly through coupling. From quantum mechanical calculations, the coupling between phenoxy radicals and β-radicals are most favored, therefore the β-O-4 linkage in lignin is the most abundant linkage. The formation of the β-O-4 linkage leads to a formation of a quinone methide-like structure as shown in Figure 12 (Koshijima and Watanabe, 2003). The α-position is now an electrophile, and it can be attacked by water, alcohol, or carboxyl groups which lead to the formation of benzyl alcohols, benzyl ethers, and
benzyl esters. The biosynthesis of ester and ether LCC linkages has been proposed, but the biosynthesis of glycosidic and acetal LCCs is still uncertain. However, many studies have been done to prove the existence of native LCC linkages.

Figure 12. Biosynthesis of benzyl ether and benzyl ester LCCs (Koshijima and Watanabe, 2003).
Analyses of native LCC linkages

Many methods have been used to study native LCC linkages. Such methods include alkaline degradation, acidic degradation, oxidation, methylation, and a variety of other chemical and biological methods.

Yaku and coworkers (1976), Erikson and coworkers (1980), Obst (1982), Lundquist and coworkers (1983), and Takahashi and Koshijima (1988) used alkaline degradation to study LCC linkages. This method was used to study the ester linkages between carbohydrates and lignin. Carboxyl groups resulting from saponification of ester linkages were analyzed. Also, Lundquist and coworkers (1983) suggested that lignin and xylan linkages could be cleaved under mild alkaline conditions, since they were able to observe xylan after treatment of milled wood lignin (MWL) with a mild alkali.

Acid degradation studies of LCC linkages were done by Eriksson and Lindgren (1977) and Eriksson and coworkers (1980). Along with other analyses, studies of ether linkages between carbohydrate and lignin were done by selectively cleaving the ether linkage under acidic conditions.

Smith oxidation was used by Eriksson and coworkers (1980) and Yaku and coworkers (1981) along with a sodium borohydride reduction of ester LCC linkages to study the ester LCC linkages. A reduction study of LCC linkages using sodium borohydride reduction also was done by Kosikova and coworkers (1979) to study the benzyl ether LCC linkages.

Koshijima and coworkers (1976), Mukoyoshi and coworkers (1981), Takahashi and coworkers (1982), and Azuma and Koshijima (1988) used methylation analysis for their studies of LCC linkages. Methylation has been used to study carbohydrate linkages
for decades. Other methods such as borohydride reduction and acid hydrolysis of glycosidic bonds also have been used in conjunction with methylation prior to chromatographic analysis of LCC linkages. By enzymatic synthesis of glycosidic LCC and with selective hydrolysis and methylation methods, Joseleau and Kesraoui (1986) showed that lignin preferred to link to a furanose ring rather than a pyranose ring.

Other chromatography and spectrometry techniques such as hydrophobic chromatography (Azuma and Koshijima, 1988; Mukoyoshi et al., 1981; Takahashi et al., 1982, and Azuma and Koshijima, 1988), IR (Das et al., 1984), NMR (Merewether et al., 1972), and SEM (Kosikova et al., 1978) also have been used to study LCC linkages. These methods were nonspecific and only could determine that hemicelluloses and cellulose are linked to lignin in the wood cell wall by ether or ester linkages, but could not determine the type of lignin (Koshijima and Watanabe, 2003); therefore, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation was used to determine these LCCs by selectively cleaving the α-ether and α-ester LC linkages to carbonyl groups and analyzing these carbonyl groups by NMR.

Newer biochemical methods for analyzing LCCs also have been developed. Xie and coworkers (2000) used $^{13}$C tracer experiments, analyzed by NMR, to study LCCs. They administered coniferin, $^{13}$C-rich at the α, β, and γ positions, into ginko shoots to produce $^{13}$C-rich wood. From this wood, milled wood lignin enriched with $^{13}$C was obtained. Using NMR analysis, Xie and coworkers were able to determine that the ginko LCC contained ester and ether LC linkages, and at the α-C there was a ketal linkage.
Based on these studies, it is accepted widely that carbohydrates and lignin are linked by covalent bonds. In order to convert the carbohydrates or lignin into valuable chemicals, these bonds must be broken to separate the carbohydrates from the lignin.

VALUE-ADDED CHEMICALS

Bioethanol

To decrease our dependence on a foreign oil supply and because of concern over global warming, many researchers have proposed to produce and use bioethanol as a source of fuel. Bioethanol can be made from sources containing large amounts of saccharides. Today in the United States, the majority of bioethanol is made from starch in corn kernels. Another source for bioethanol is lignocellulosic materials. In this case, cellulose and hemicelluloses are the sources for bioethanol. Hemicelluloses are especially interesting because they are the second most abundant polymer on earth next to cellulose. Also, hemicelluloses generally are underutilized and often simply burned. Therefore, there is much interest in using hemicelluloses to produce valuable products and biofuels.

Biochemicals

In addition to ethanol, other chemicals can be made from hemicelluloses. There are twelve chemicals that have been proposed by researchers at the Pacific Northwest National Laboratory (PNNL) and the National Renewable Energy Laboratory (NREL) as the top value added chemicals from biomass. They are succinic acid, itaconic acid, levulinic acid, glucaric acid, aspartic acid, 3-hydroxypropionic acid, 3-hydroxybutyrolactone, 2,5-furandicarboxylic acid, glutaric acid, sorbitol, xylitol, and
These twelve chemicals were chosen as the top value-added chemicals over a long list of other chemicals through an elimination process based on the idea that they must be derived from saccharides (cellulose, hemicelluloses, and starch). Each chemical must have at least two functional groups and the same number of carbons as the simple sugars. The chemical must have good sales in today’s market and have potential to become a super commodity in the future (Figure 13).

**Figure 13.** Top twelve chemicals.
The top twelve chemicals are divided into two groups. Group one included chemicals that can be obtained by chemical conversion after hydrolysis of the saccharides into simple sugars. For example, group one contains sorbitol made from glucose by catalytic hydrogenation. Xylitol, also produced by this method, uses xylose, whereas treatment of hexose and pentose sugars with acid produces levulinic acid. Group two contains chemicals that can be produced biologically, mainly by fermentation from sugars. Group two contains succinic acid, itaconic acid, 3-hydroxypropionic acid, glycerol, and glutamic acid. These chemicals are widely used in pharmaceutical products and food industries. They can be used to make polymers as well. Not only are these chemicals useful, but now because they can be produced from a biomass source, they are renewable (Table 1).
Table 1. Top twelve value added chemicals and their derivatives.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic Acid</td>
<td>1,4-Butanediol (BDO), g-Butyrolactone, Tetrahydrofurane (THF)</td>
</tr>
<tr>
<td></td>
<td>2-Pyrolidone, N-Methylpyrrolidone (NMP)</td>
</tr>
<tr>
<td>2,5-Furandicarboxylic Acid</td>
<td>2,5-bis(aminomethyl)-THF, 2,5-dihydroxymethyl-THF, 2,5-Dihydroxymethyl-furn</td>
</tr>
<tr>
<td>3-Hydroxypropionic Acid</td>
<td>1,3-Propanediol, Acrylic Acid, Methyl Acrylate, Acrylamide</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>Aspartic Anhydride, Amino-γ-butyrolactone, 3-Amino THF, Aspartic Acid, 2-Amino-1,4-BDO</td>
</tr>
<tr>
<td>Glucaric Acid</td>
<td>Glucaro-γ-lactone, Glucarodilactone, Polyhydroxypolyamides</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Glutaminol, Norvoline, Glutaric Acid, 1,5-Pentadiol, 5-Amino-1-butanol</td>
</tr>
<tr>
<td>Itaconic Acid</td>
<td>3-&amp; 4-Methyl-γ-Butyrolactone, 3-Methyl THF, 2-Methyl-1,4-BDO, 3-&amp; 4-Methyl NMP, 3-Methylpyrrolidine</td>
</tr>
<tr>
<td>Levulinic Acid</td>
<td>2-Methyl THF, g-Valerolactone, Angelilactones, 1,4-Pentanediol, b-Acetylacrylic Acid, Diphenolic Acid</td>
</tr>
<tr>
<td>3-Hydroxybutyrolactone</td>
<td>3-Hydroxy THF, 3-Amino THF, 2-Amino-3-hydroxy THF, Acrylate lactone</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Glyceric Acid, 1,3-Propanediol, Propylene glycol, Branched polyesters and nylons</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>Isosorbide, 1,4-Sorbitan, 2,5-Anhydrosugars, Propylene glycol, Ethylene glycol, Glycerol</td>
</tr>
<tr>
<td>Xylitol</td>
<td>Xylaric Acid, Propylene glycol, Ethylene glycol, Glycerol</td>
</tr>
</tbody>
</table>

CONVERTING HEMICELLULOSES INTO VALUABLE CHEMICALS

Extracting hemicelluloses

In order to utilize the hemicelluloses for bioethanol and other valuable chemicals, they need to be extracted from wood or biomass. Hot alkaline has been used to extract the hemicelluloses from hardwood (van Heiningen, 2006). This method may be used in an integrated process with the well known Kraft pulping process to maximize the usage of wood. In Kraft pulping, most of the hemicelluloses are extracted by an alkaline sulfide solution forming black liquor which is burned along with lignin to produce energy to
operate the pulp mill. But the energy value of the hemicelluloses is only half that of lignin. Therefore, van Heiningen and others proposed to extract the hemicelluloses prior to pulping so that they can be converted into other valuable chemicals (van Heiningen, 2006; Lopez et al., 1996).

**Lignin-hemicellulose Bonds**

During the synthesis of wood, some lignin is covalently bonded to hemicelluloses. Therefore, lignin gets extracted along with the hemicelluloses. The lignin portion presents a problem in converting the hemicelluloses into valuable chemicals, since it can inhibit enzymes that are used for the hydrolysis of the hemicelluloses into individual sugars. If lignin is still in the mixture during the fermentation process, it can inhibit the yeast as well, which leads to a lower yield of the desired products. Therefore, lignin should be removed prior to the hydrolysis and the fermentation processes to increase the yield of the valuable chemicals (Palmqvist et al., 1997; Jonsson et al., 1998; Palmqvist and Hahn-Hagerdal, 2000; Larsson et al., 2001).

**OXYGEN DELIGNIFICATION**

The chemistry of oxygen (O₂) reacting with organic compounds has been studied for many years, but not until the 1970s was O₂ introduced to remove the lignin in wood in the bleaching process and to replace chlorine (Cl₂) and chloride dioxide (ClO₂) as bleaching reagents (Argyropoulos, 2000). Dioxin and polychlorinated compounds are byproducts that come from Cl₂ and ClO₂ bleaching which are toxic to the environment. O₂ delignification is more environmentally friendly compared to Cl₂ and ClO₂, since the byproducts produced by this method are environmentally benign. Unfortunately, O₂ is
not selective toward lignin due to the fact that there are many oxygen species present during the oxygen based process which lead to degradation of carbohydrates as well. Therefore, there has been great interest to study the reaction of oxygen species with carbohydrate-lignin compounds during the oxygen delignification process.

**Oxygen species**

Oxygen can undergo a series of reductions to water that involve one electron and one proton which produce hydroperoxy radical (HO$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (HO') as intermediate products (Figure 14, (Gratzl, 1992)). With a pK$_a$ of 4.8, the hydroperoxy radical (HO$_2^-$) is in equilibrium with superoxide anion (O$_2^-$). The hydrogen peroxide (H$_2$O$_2$) is in equilibrium with the hydrogen peroxide anion (HO$_2^-$) at pK$_a$ of 11.8. Finally, at pK$_a$ of 11.9, the hydroxyl radical (HO') is in equilibrium with its anion (O$^-$) (Sjostrom, 1993). Therefore, with the high pH of the oxygen delignification process, more than one oxygen species is present.

\[
\begin{align*}
\text{O}_2 & \rightarrow \text{HOO}^- & \rightarrow \text{HOOH} & \rightarrow \text{HOH} + \cdot\text{OH} & \rightarrow 2\text{HOH} \\
pK_a & \ 4.8 & \ 11.8 & \ 11.9 \\
\end{align*}
\]

\[
\begin{align*}
\text{H}^+ + \text{O}_2^- & \quad \text{H}^+ + \cdot\text{OOH} & \quad \text{H}^+ + \cdot\text{O}^- \\
\end{align*}
\]

*Figure 14. Oxygen species present during oxygen delignification. (Gratzl, 1992).*
Molecular Oxygen

Oxygen exists as a diatomic molecule. The molecular orbital diagram of molecular oxygen consists of two atomic orbital diagrams of oxygen. Because of the degeneracy of the \( \pi \) molecular orbitals (Figure 15), molecular oxygen is paramagnetic with two antibonding electrons spinning parallel to each other in the ground state which is the highest occupied molecular orbital (HOMO) (Rayner-Canham, 2000).

\[
\begin{array}{c}
\sigma_{2p}^* \\
\uparrow \downarrow \\
\downarrow \uparrow \\
\pi_{2p}^* \\
\uparrow \downarrow \\
\downarrow \uparrow \\
\sigma_{2p} \\
\end{array}
\]

Energy

Figure 15. Molecular orbital diagram of molecular oxygen.

Molecular oxygen is the most abundant oxygen species during oxygen delignification. Most interactions of molecular oxygen are with substrates that contain hydrogen atoms, unpaired electrons, and single electrons. Molecular oxygen also can abstract an electron from metals and electron rich compounds (Fee et al., 1977).

Hydroxyl Radicals

Of all the oxygen species present during the oxygen delignification, the hydroxyl radicals are the most reactive species (Sawyer, 1991). Due to their high electronegativity, hydroxyl radicals are able to perform many reactions: hydrogen abstraction, electrophilic addition, radical coupling, and electron-transfer reactions (Legrini et al., 1993).
Abstraction of hydrogen from aliphatic C-H bonds can be done by the hydroxyl radical and is controlled by the energy difference between the C-H bond and new O-H bond. Aromatic C-H bond energies are too high for hydrogen abstraction; hence electrophilic attack on the benzene ring occurs. Relocalization of the radical after electrophillic addition of the hydroxyl radical to the ring increases the facility of the reaction (Sawyer et al., 1981). With a high concentration of the hydroxyl radical, there is a high chance that radical coupling can occur which leads to the formation of a hydrogen peroxide molecule. Hydroxyl radicals can couple with other radicals as well. Finally, formation of hydroxide can occur when hydroxyl radicals abstract an electron from other molecules (Legrini et al., 1993).

During oxygen delignification, the hydroxyl radical not only reacts with lignin but also can attack and degrade carbohydrates. It is the hydroxyl radical that is believed to be responsible for the most degradation of carbohydrates during oxygen delignification. The mechanisms of carbohydrate degradation by hydroxyl radicals have been proposed by several researchers (Gierer, 1997; Guay, 1999). The hydroxyl radical can be generated by radiating 254 nm UV light on hydrogen peroxide at pH of about 9.5 (Legrini et al., 1993), which has been demonstrated in prior research in our group (Guay, 1999; Hausman, 1999). Further information regarding reaction of the hydroxyl radicals with lignin and carbohydrates is discussed in subsequent sections in this thesis.

*Superoxide Anions*

The hydroperoxyl radical is the first radical formed during the reduction of molecular oxygen to water. Superoxide anion is the hydroperoxyl radical conjugate base that has a pKₐ of 4.8. With a low pKₐ value, under oxygen delignification conditions,
more of the superoxide anion exists compared to the hydroperoxyl radical. The superoxide anion can act as a radical and as a base. With its moderate basicity, the superoxide anion can deprotonate slightly acidic hydrogens. The superoxide anion is also a radical and thus can facilitate electron transfer, abstract labile hydrogen, and can add to a π-system.

It was proposed by Gierer (1997) that the ring opening of the lignin which leads to degradation of lignin occurs by superoxide anion coupling with the phenoxy radical. Hausman (1999) demonstrated that the superoxide anion is more likely to degrade lignin than carbohydrates. The study was done with model lignin compounds and superoxide anions generated from potassium superoxide and also from hydrogen peroxide in a pH 12 buffer with 254 nm UV radiation.

Reaction of Carbohydrates and Lignin with Oxygen Species

Mechanistic studies of the reactions of oxygen species with carbohydrates and lignin have been done. Reaction mechanisms have been proposed for carbohydrates and lignin with hydroxyl radical and superoxide anion with lignin.

Reaction of Carbohydrates

Degradation of carbohydrates during oxygen delignification is due to the strong oxidant property of hydroxyl radicals. According to Gierer and coworkers (1997), hydrogen abstraction of carbohydrates by hydroxyl radicals leads to degradation of the cellulose chain as shown in Figure 16. There is no experimental evidence to support this mechanism, however.
More recent studies by Guay (1999) suggest that the degradation of the cellulose chain by the hydroxyl radical involves attack at the anomeric position of glucose rather than the C-2 position. Figure 17 describes the degradation of cellulose via anomeric attack by the hydroxyl radical.
Once the cellulose is cleaved into glucose units, glucose can be further oxidized into smaller molecules. In the presence of a mild oxidant, glucose is oxidized to the aldonic acid, in this case gluconic acid. Figure 18 shows the mechanism for gluconic acid formation proposed by Guay in 1999. Once the gluconic acid is formed, formation of smaller aldoses can be generated by the Ruff degradation. These aldoses can oxidize to acids. The final product of glucose oxidation is 2-hydroxyacetic acid. This mechanism was supported by product identification and theoretical calculations (Guay, 1999).
Formation of uronic acid from glucose initiated by hydroxyl radicals was also proposed by Guay (1999). This pathway is minor compared to the formation of gluconic acid. In this case, the formation of a carboxylic acid by oxidation of the alcohol is at the C-6 position, as shown in Figure 19. Once the carboxylic acid is formed, decarboxylation can also occur (Figure 20 (Guay, 1999)).
Figure 19. Formation of uronic acid (Guay, 1999).

Figure 20. Decarboxylation of uronic acid (Guay, 1999).
Reaction of Lignin

Hydroxyl radicals can not degrade lignin, but they can react with both phenolic and non-phenolic types of lignin. Due to the complexity of lignin, studies were done using lignin model compounds (Gierer et al., 1992; Hausman, 1999). According to Gierer (1992), there are four general oxidation reactions between the phenolic compound and non-phenolic compound with the hydroxyl radical after the formation of a phenoxy radical intermediate: coupling, demethylation, sidechain oxidation, and hydroxylation. An insignificant number of ring opening products were found also for the reaction between the hydroxyl radical and the phenolic and non-phenolic compounds. Therefore, the hydroxyl radical does not lead to the ring opening reaction. The study was done in acidic, neutral, and alkaline media. With the phenolic compound, coupling was the major reaction in the acidic and the alkaline media, whereas in the neutral medium, demethylation, sidechain oxidation, and hydroxylation products were found. For the non-phenolic compound, demethylation, hydroxylation, and sidechain oxidation products were found in all media, and only a small amount of coupling product was found.

Studies by Hausman (1999) found similar results to the study by Gierer and coworkers (1992). Hausman’s study also was done with lignin model compounds. With HOOH/UV reactions at pH 9.5, a majority of products were formed by hydrogen abstraction, hydroxylation, demethylation, and side chain oxidation. According to Hausman, the formation of the phenoxy radical does not depend on the hydroxyl radical, since it can be formed with any oxygen species that is present in the reaction mixture.

After the formation of the phenoxy radical, it is the superoxide anion that degrades the aromatic ring to smaller compounds. For the study by Hausman (1999) with
guaiacol, veratrole, vanillin, and veratraldehyde, low molecular weight acids, ranging from two to four carbons, that are saturated, unsaturated, and hydroxylated were the products of the degradation of these phenolic compounds. The phenoxy radical is the intermediate product of the degradation of the phenolic compound by demethylation, hydroxylation, and decarboxylation by the hydroxyl radical and other oxygen species. The ring opening of the phenoxy radical is caused by the superoxide anion forming a muconic acid intermediate. Figure 21 describes the proposed degradation of the phenolic compound by oxygen delignification by Hausman (1999).

Figure 21. Ring opening mechanism of hydroxylated veratrole (Hausman, 1999).

Under alkaline conditions, studies done by Gierer and coworkers (1994) found similar results with two phenolic compounds, 4-t-butylsyringol and 4-t-butylguaiacol. Ring opening products were found along with products that are formed by radical coupling after the formation of the phenoxy radical.
Based on the results from previous studies on the reactions of oxygen species with carbohydrates and lignin, this thesis focuses on the reactions of oxygen species (hydroxyl radical and superoxide anion) with lignin-carbohydrate compounds. We are interested in the oxidation mechanism of lignin-carbohydrate compounds to see if we can selectively cleave off the lignin portion without degrading the carbohydrate.

**Thesis objective**

In order to study the oxidation mechanisms of lignin-carbohydrate compounds the specific objectives of this project included:

1. Synthesize two glycosidic lignin-carbohydrate model compounds: 4-methoxyphenyl-β-D-glucopyranoside and coniferin.

2. Oxidize model compounds with generated oxygen species: hydroxyl radical and superoxide anion by irradiation of H₂O₂ with 254 UV at pH 9.5 and 12, respectively.

3. Analyze products by gas chromatogram-mass spectrometry (GC-MS).

4. Propose oxidation mechanisms.
CHAPTER 2-EXPERIMENTAL

This part of the thesis describes the synthesis of lignin-carbohydrate model compounds. It also describes the experimental methods used for the oxidation of the lignin-carbohydrate model compounds by oxygen species. Details of reaction conditions and analytical methods are included.

MATERIALS

\(\beta\)-D-glucose pentaacetate, tetra-O-acetyl-\(\alpha\)-D-glucopyranosylbromine, and phenyl-\(\beta\)-D-galactopyranoside were purchased from Alfa Aesar. Quinoline was purchased from Acros Organic. Glucovanillin was purchased from ChromaDex. Glacial acetic acid, methylene chloride, methanol, 95% ethanol, and concentrated hydrochloric acid were purchased from Fisher Scientific. Xylose, vanillin, 4-methoxyphenol, boron trifluoride etherate, methanolic base, Amberlite IR-120 resin, sodium hydroxide, sodium sulfate, sodium bicarbonate, silver oxide, piperidine, pyridine, toluene, sodium phosphate, monoethyl malonate, diisobutylaluminum in toluene (1M), 30% hydrogen peroxide, xylitol, 1,4-dimethoxybenzene, Sylon TP, and Sylon BTZ were purchased from Sigma-Aldrich. Nanopure water (18.3 M\(\Omega\)) was used from our Barnstead NANOpure Analytical Deionization System D4744 water purifier.

SYNTHESIS OF CONIFERN

The synthesis of coniferin, a model lignin-carbohydrate compound, was carried out using the method of Terashima and coworkers (1995) with some modifications. IR and \(^1\)H-NMR spectra were taken for the products of each step and compared to the reference NMR spectra that were available in the literature (Terashima et al., 1995) to
check and confirm the success of the synthesis. IR and $^1$H-NMR spectra are included in Appendix A and B. GC-MS analysis was also done for coniferin, and the chromatogram and mass spectrum are included in Appendix C.

**Synthesis of 4-O-[tetra-O-acetyl-β-D-gluco]-vanillin**

Vanillin (4.56 g, 30.0 mmol), and tetra-O-acetyl-α-D-glucopyranosylbromide, (12.34 g, 30.0 mmol), were dissolved in quinoline (30.0 mL). While stirring the mixture, silver oxide, (3.95 g, 18.0 mmol), was added in small portions. After the first exothermic reaction ended, the mixture was stirred for an additional 20 minutes. Acetic acid (20.0 mL) was added to the reaction mixture and the combined mixture was then poured into 1.0 L of water. The fine precipitate that formed was collected by vacuum filtration through 2 inches in diameter and 1/10 inch thick mat of pulp that was prepared from disintegrated filter papers. The fine precipitate was washed with water and then extracted with hot ethanol (200. mL, 3X). The solvent was removed by rotary evaporation and the crude product was recrystallized with 95 % ethanol. The percent yield was 38.7. The melting point was 144-146 °C, and the literature value was 141-142 °C (Terashima, 1996). IR: 1753 and 1737 cm$^{-1}$ (C=O, ester), 1694 cm$^{-1}$ (C=O, aldehyde), 838 and 781 cm$^{-1}$ (1,2,4-trisubstituted benzene). $^1$H-NMR (300 MHz, CDCl$_3$): 1.8-2.3 ppm (m, 12H), 3.85 ppm (m, 1H), 3.9 ppm (s, 3H), 4.25 ppm (dd, 2H), 5.1 ppm (d, 1H), 5.2 ppm (m, 1H), 5.25-5.35 ppm (m, 2H), 7.25 ppm (d, 1H), 7.43 ppm (m, 2H), 9.9 ppm (s, 1H).

**Synthesis of 4-O-[tetra-O-acetyl-β-D-gluco]-ferulic acid ethyl ester**

A mixture of 4-O-[tetra-O-acetyl-β-D-gluco]-vanillin, (4.80 g, 10.0 mmol), monoethyl malonate (1.45 g, 11.0 mmol), pyridine (4.00 mL) and piperidine (0.150 mL)
was heated at 100 °C for 1.5 hours. The mixture was poured into water (200. mL) while stirring. The mixture was then filtered to collect the precipitate and recrystallized with 95% ethanol. The percent yield was 64.4. The melting point was 134-136 °C, and the literature value was 134-137 °C (Terashima, 1996). IR: 1759, 1728, and 1712 cm⁻¹ (C=O, ester), 1636 cm⁻¹ (C=O, ester conjugated), 843 and 802 cm⁻¹ (1,2,4-trisubstituted benzene). ¹H-NMR (300 MHz, CDCl₃): 1.35 ppm (t, 3H), 1.8-2.3 ppm (m, 12 H), 3.8 ppm (m, 1 H), 3.87 ppm (s, 3H), 4.15-4.25 ppm (m, 2H), 4.28 ppm (q, 2H), 5.0 ppm (m, 1H), 5.15 ppm (m, 1H), 5.3 ppm (m, 2H), 6.35 ppm (d, 1H), 7.08 ppm (m, 3H), 7.6 ppm (d, 2H).

Synthesis of coniferin

4-O-[tetra-O-acetyl-β-D-gluco]-ferulic acid ethyl ester (3.30 g, 6.00 mmol) was dissolved in dry toluene (200. mL) and diisobutylaluminum hydride (DIBAL-H, 1.0 M in toluene, 75.0 mL, 75.0 mmol) was added while stirring under nitrogen at 0-5 °C over 1 hour. The mixture was stirred for another hour after the addition of DIBAL-H. The reaction mixture was quenched by a slow addition of dry ethanol (20.0 mL) while cooling. The solvent was removed by a rotary evaporator at 50 °C. Water (100. mL) was added to form two layers. The top layer which was toluene was further removed with the rotary evaporator. The aqueous mixture was warmed in a boiling water bath, filtered, and extracted with boiling water (100. mL, 2X). The water solutions were combined and concentrated with the rotary evaporator at 60 °C. The crude product was recrystallized with hot water. The percent yield was 41.4. The melting point was 186-188 °C, and the literature value was 183-185 °C (Terashima, 1996). IR: 3285 cm⁻¹ (-OH, stretch), 1515 cm⁻¹ (C=C, stretch), 859 and 804 cm⁻¹ (1,2,4 trisubstituted benzene). ¹H-NMR (300 MHz,
DMSO$_{d6}$: 3.67 ppm (m, 1H), 3.78 ppm (s, 3H), 4.09 ppm (t, 2H), 4.52 ppm (t, 1H), 4.82 ppm (t, 1H), 4.89 ppm (d, 1H), 5.0 ppm (d, 1H), 5.08 ppm (d, 1H), 5.22 ppm (d, 1H), 6.28 ppm (dt, 1H), 6.48 ppm (d, 1H), 6.89 ppm (dd, 1H), 7.01 ppm (d, 2H).

\[ \text{tetra-}O\text{-acetyl-}\alpha\text{-D-glucopyranosyl bromide} \]
\[ \text{Vanillin} \]
\[ \text{4-O-[tetra-}O\text{-acetyl-}\beta\text{-gluco]-vanillin} \]
\[ \text{4-O-[tetra-}O\text{-acetyl-}\beta\text{-gluco]-ferulic acid ethyl ester} \]
\[ \text{Coniferin} \]

Figure 22. Synthesis of coniferin

SYNTHESIS OF 4-METHOXYPHENYL-\(\beta\)-D-GLUCOPYRANOSIDE

The synthesis of 4-methoxyphenyl-\(\beta\)-D-glucopyranoside was done using a method described by Ohlson and Magnusson (2000) with minor modifications. Melting point, IR, $^1$H-NMR, and GC-MS of the silylated product were taken for product
characterization. The IR spectrum $^1$H-NMR spectrum, gas chromatogram and mass spectrum are included in Appendix A, B, and C.

1, 2, 3, 4, 6-penta-O-acetyl-$\beta$-D-glucopyranose (5.00 g, 13.3 mmol) was dissolved in 25.0 mL of CH$_2$Cl$_2$ and 4-methoxyphenol (2.08 g, 16.8 mmol) was added to the mixture. The reaction mixture was cooled to 0 °C in an ice bath. While stirring, 2.00 mL (19.3 mmol) of BF$_3$Et$_2$O was added dropwise into the reaction mixture. After continual stirring for two hours, the reaction mixture was allowed to reach room temperature. Next, the reaction mixture was diluted with 25.0 mL of CH$_2$Cl$_2$ and then washed with 2.00 M NaOH (2X, 25.0 mL) and with H$_2$O (2X, 25.0 mL). The washed CH$_2$Cl$_2$ solution was dried over NaSO$_4$, filtered, and rotary evaporated to remove the solvent. The residue was dissolved in 30.0 mL of CH$_3$OH, and 0.25mL of 0.5 M solution (Sigma-Aldrich) of CH$_3$ONa was added. The reaction mixture was allowed to react overnight while stirring at room temperature. The solution was neutralized with Amberlite IR-120 resin, filtered, and rotary evaporated to remove the CH$_3$OH. The crude product was recrystallized with 95% ethanol. The percent yield was 40.0. The melting point was 177-180 °C, and the literature value was 175-177 °C (Shafizadeh, 1972). IR: 3306 cm$^{-1}$ (-OH, stretch), 1508 cm$^{-1}$ (C=C, stretch), 822 cm$^{-1}$ (1,4 disubstituted benzene). $^1$H-NMR (300 MHz, DMSO$_{d6}$): 3.1-3.6 ppm (m, 6H), 3.7 ppm (s, 3H), 4.6 ppm (t, 1H), 4.7 ppm (d, 1H), 5.1 ppm (dd, 2H), 5.3 ppm (d, 1H), 6.9 ppm (d, 2H), 7.0 ppm (d, 2H).
CHARACTERIZATION BY IR AND $^1$H-NMR

A PerkinElmer Spectrum One infrared spectrometer was used to obtain all IR spectra of products. All $^1$H-NMR spectra were taken with a Varian Gemini 300 spectrometer. Samples were dissolved in DMSO$_{d16}$. Samples were pulsed for one second, and four acquisitions were obtained.

OXIDATION OF MODEL LIGNIN-CARBOHYDRATE COMPOUNDS

Preparation of pH 9.5 sodium bicarbonate buffer solution

To make a sodium bicarbonate buffer with a pH of 9.5, 125. mL of 1.00 M sodium bicarbonate and 26.75mL of 2.00 M sodium hydroxide were added into a 250.00 mL volumetric flask and diluted to the mark with deionized water (Lide, 1993).
Preparation of pH 12 sodium phosphate buffer solution

To make a sodium phosphate buffer with a pH of 12, 125 mL of 0.20 M sodium hydrogenphosphate solution and 67.3 mL of 0.4 M NaOH solution were added to a 250.00 mL volumetric flask and diluted to the mark with deionized water (Lide, 1993).

Control reactions

For both model compounds, control reactions were done to check for the effect of H$_2$O$_2$ and 254 nm radiation on the model compounds in each buffer system. Samples were dissolved in pH 9.5 and pH 12 buffers. H$_2$O$_2$ was added to the sample and followed by work-up procedure. Samples were subjected to 15 minutes of 254 nm UV radiation without H$_2$O$_2$ added and the work-up procedure was followed.

UV-Vis of coniferin

UV-Vis spectra of coniferin were taken in each buffer to check for UV absorption of coniferin. Spectra are included in Appendix D.

Oxidation of model lignin-carbohydrate model compounds at pH 9.5 and 12

Lignin-carbohydrate model compound 30.0 mg, (coniferin or 4-methoxyphenyl-β-D-glucopyranoside), was dissolved in 30.0 mL of the appropriate buffer. H$_2$O$_2$ (30% solution), 108 μL for 4-methoxyphenyl-β-D-glucopyranoside and 90 μL for coniferin, was added to the mixture so that the molar ratio of the model compound and the H$_2$O$_2$ was 1:10. The reaction mixture was divided equally into three quartz test tubes, and each test tube was capped with a septum. Each test tube was purged with N$_2$ for 15 minutes, and the outlet syringe was left in each test tube to let out any gas that evolved during the UV reaction. The test tubes were placed into a Rayonet photochemical reactor equipped
with 16 bulbs radiating 254 nm and with a cooling fan. Reaction times were 5, 10, 15, 20, and 30 minutes.

Work-up procedures

After being taken out of the photochemical reactor, the solutions in the three test tubes were combined and neutralized with 6.00 M HCl. After neutralization, the solution was then extracted with CH₂Cl₂ (3X, 10.0 mL). The CH₂Cl₂ layers were combined and removed with the rotary evaporator. The residue was dissolved in 1 mL (measured with a 1000 µL micro pipet) of ethyl acetate for GC-MS analysis. After freeze drying, residues were extracted with CH₃OH (3X, 10.0 mL). The CH₃OH layers were centrifuged and concentrated with the rotary evaporator with a 60 °C water bath. The residue was then silylated with a 1 mL ampule of Sylon TP (Supelco) overnight for GC-MS analysis. Coniferin samples and some 4MPG samples were silylated with Sylon BTZ (1 mL of Sylon BTZ and 2 mL of pyridine) overnight before GC-MS analysis to check for carboxylic acids since Sylon TP does not silylate carboxylic acid groups.

Quantification

Internal standards that were used for the quantification of products were: xylitol, 4-methoxyphenyl-β-D-mannopyranoside, phenyl-B-D-galactopyranoside, 1,4-dimethoxy benzene. These were chosen as internal standards because they have similar properties to the compounds that were quantified.

Each time the experiment was run in triplicate to calculate for experimental error. Each of the samples was injected three times into the GC-MS to calculate for instrumental error.
Gas Chromatograph-Mass Spectrometry (GC-MS) Analysis

Samples were dissolved in ethyl acetate or silylated with Sylon TP or Sylon BTZ. Two similar temperature programs were used for samples dissolved in ethyl acetate and samples silylated with Sylon TP. Samples were analyzed on an Agilent Technologies 6975 Network GC System 5975B VL MSD equipped with an HP-5MS 5% Phenyl Methyl Siloxane capillary column.

The GC conditions were as follows for samples dissolved in ethyl acetate: injector temperature was 250 °C, and the oven temperature was 70 °C for 6 minutes, and increased 10 °C per minute until it reached 300 °C. The solvent delay was 4 minutes. The amount of the sample injected was 1 μL with splitless injection. Full scan with mass range from 50-500 m/z was set for the MS.

For samples silylated with Sylon TP or Sylon BTZ, the GC conditions were as follows: injector temperature was 250 °C, the oven temperature was 70 °C for 6 minutes, increased 10 °C per minute until it reached 300 °C, and held at 300 °C for 2 minutes. The solvent delay was 15.5 minutes. The amount of sample injected was 1 μL with a split of 1:20 injection. Full scan with mass range from 50-500 m/z was set for the MS.
CHAPTER 3-RESULTS AND DISCUSSION

For this study, we focus on the oxidation mechanism of lignin-carbohydrate compounds that exist in native wood. Following the results from the previous research in our group with carbohydrates and lignin model compounds, we apply similar oxidation conditions for this study of lignin-carbohydrate compounds. We want to know if we can selectively cleave lignin without degrading the carbohydrate. In order to study the oxidation mechanism of the lignin-carbohydrate compounds, two aryl glycoside model compounds were synthesized by literature methods, because it is not possible to extract and isolate individual lignin-carbohydrate compounds from wood.

SYNTHESIS OF MODEL COMPOUNDS

For this study, we decided to work with glycoside linkages, one of the principal lignin-carbohydrate bonds. The two glycosidic lignin carbohydrate model compounds that were synthesized are 4-methoxyphenyl-β-D-glucopyranoside (4MPG) (Ohlson and Magnusson, 2000) and coniferin (Terashima et al., 1995). Even though the yield from our syntheses was not comparable to the references, with repetitive syntheses we were able to obtain enough of the model compounds to use for all oxidation experiments.

GENERATION OF HYDROXYL RADICALS AND SUPEROXIDE ANIONS

Photolysis of hydrogen peroxide was used to generate hydroxyl radicals and superoxide anions. At a pH of about 9.5, with 254 nm UV light radiation, hydroxyl radicals are generated from hydrogen peroxide (Legrini et al., 1993). At pH of 12, also
with 254 nm UV radiation, the majority of the oxygen species are superoxide anions.

The lignin-carbohydrate model compounds were subjected to both reaction conditions.

\[
\begin{align*}
\text{HOOH} & \quad \xrightarrow{254 \text{ nm}} \quad 2 \text{HO}^\bullet \\
\text{pH 9.5} & \\
\text{HO}^\bullet & \quad + \quad \xrightarrow{254 \text{ nm}} \quad \text{H}_2\text{O} \quad + \quad \text{O}_2^\bullet \\
\text{pH 12} & \\
\text{HOO}^- & \\
\end{align*}
\]

**Figure 24.** Oxygen species present during photochemical reactions.

**REACTION CONDITIONS**

The standard reaction conditions were 30.0 mg of substrate and 108 µL of 30% H\(_2\)O\(_2\) for 4MPG and 90 µL for coniferin, a 1:10 molar ratio in a buffer. Work-up involved neutralization with 6.0 M HCl, extraction with CH\(_2\)Cl\(_2\) and freeze drying of aqueous layers prior to analysis by GC-MS. Irradiation was conducted for 5, 10, 15, and 30 minutes at the ambient temperature of the reactor, about 35 °C. Control reactions were done for both lignin-carbohydrate compounds. Each compound was subjected to UV radiation without H\(_2\)O\(_2\) present to check for the effect of UV radiation for both pH conditions. Also H\(_2\)O\(_2\) was added to samples that were not subjected to UV radiation to check the effect of H\(_2\)O\(_2\) on model compounds.
RESULTS

Control reaction of 4-methoxyphenyl-\(\beta\)-D-glucopyranoside

For 4-methoxyphenyl \(\beta\)-D-glucopyranoside, minor cleavage at the anomeric position occurred in both pH systems in the absence of \(\text{H}_2\text{O}_2\), but the amount was not significant and was not quantified. Figure 25 is the gas chromatograph of the aqueous layer of the control with no \(\text{H}_2\text{O}_2\) and 15 minutes UV radiation at pH 9.5. Figure 26 is the analogous control at pH 12.

Figure 25. GC of 4MPG control reaction at pH 9.5 with 15 minutes UV radiation and no \(\text{H}_2\text{O}_2\).
Figure 26. GC of 4MPG control reaction at pH 12 with 15 minutes UV radiation and no H₂O₂.

The results for these control reactions with UV radiation and no H₂O₂ are similar to the control reaction with H₂O₂ and no UV radiation. Therefore, the amount of glucose produced under this reaction condition is due to the cleavage of the glycosidic bond by the hydroxyl radical or the superoxide anion, and the cleavage is not caused by UV radiation or H₂O₂.

Internal standards

Only glucose and 4-methoxyphenyl β-D-glucopyranoside were quantified. Xylitol was used as an internal standard for glucose, and 4-methoxyphenyl β-D-mannopyranoside was used as the 4-methoxyphenyl β-D-glucopyranoside internal standard. The internal standard for 4-methoxyphenol was 1,4-dimethoxy benzene. These internal standards were added to the reaction mixture after the UV reaction and before neutralization to eliminate reaction of the internal standards with oxygen species during the photochemical reaction. We were trying to keep these internal standards as close as
possible to the true internal standard, where the internal standards would be added in the beginning of the oxidation process.

**Oxidation of 4MPG at pH 9.5**

Quantification of glucose and 4MPG was done for reaction times of 5 to 30 minutes with a 10:1 ratio of H$_2$O$_2$ to 4MPG. Table 2 contains the results. At each reaction time, three set of samples were done simultaneously to measure the experimental error. For the percent yield of glucose, the error ranges from about one to three percent. For the percent yield of 4MPG, the percent error ranges from less than one percent to about nine percent.

**Table 2.** Percent recovery of glucose and 4MPG after 5, 10, 15, and 30 minutes of 254 nm UV radiation at pH 9.5 with a 1:10 ratio of 4MPG: H$_2$O$_2$.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Yield of Glucose pH 9.5</td>
<td>17.6 ± 1.3</td>
<td>25.8 ± 3.9</td>
<td>28.1 ± 3.1</td>
<td>29.1 ± 1.3</td>
</tr>
<tr>
<td>% Yield of 4MPG pH 9.5</td>
<td>27.0 ± 8.7</td>
<td>21.4 ± 4.3</td>
<td>17.3 ± 1.7</td>
<td>1.54 ± 0.29</td>
</tr>
</tbody>
</table>

Figure 27 shows a graph of the results described Table 2. Based on these results, the majority (73%) of 4MPG had reacted during the first 5 minutes and the last 27% had reacted over the next 25 minutes. While glucose was released during the first 5 minutes, less than 18% of the glucose was recovered after the 5 minute reaction. More glucose was recovered during the next 25 minutes. The implication is that in the early stages, while the H$_2$O$_2$ concentration is relatively high, glucose is further oxidized as it is
formed. When the concentration of \( \text{H}_2\text{O}_2 \) becomes low in the latter half of the reaction, glucose tends to survive. We tried to quantify the amount of \( \text{H}_2\text{O}_2 \) after the oxidation process using \( \text{KMnO}_4 \) but were unsuccessful because the buffer solution interfered with the analysis. Overall, about 30% of glucose was recovered for the oxidation of 4MPG at pH 9.5.

![Oxidation of 4MPG at pH 9.5](image)

**Figure 27.** Percent recovery of glucose and 4MPG at pH 9.5 (lines are guides connecting data points).

**Oxidation of 4MPG at pH 12**

The quantification of the oxidation of 4MPG at pH 12 was done exactly like the oxidation of 4MPG at pH 9.5. The results for this reaction are summarized in Table 3.
Table 3. Percent recovery of glucose and 4MPG after 5, 10, 15, and 30 minutes of 254 nm UV radiation at pH 12 with a 1:10 ratio of 4MPG: H₂O₂.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>% Yield of Glucose pH 12</th>
<th>% Yield of 4MPG pH 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>26.4 ± 1.0</td>
<td>13.9 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>21.9 ± 1.2</td>
<td>7.32 ± 0.52</td>
</tr>
<tr>
<td>15</td>
<td>22.0 ± 1.4</td>
<td>4.40 ± 0.57</td>
</tr>
<tr>
<td>30</td>
<td>21.9 ± 1.2</td>
<td>3.04 ± 0.39</td>
</tr>
</tbody>
</table>

At pH 12, about 86% of the 4MPG reacted during the first 5 minutes and about 27% of glucose was recovered. Over the next 25 minutes, the majority of the remaining 4MPG reacted and the amount of glucose recovered dropped to about 22%. Results from Table 2 are also shown graphically in Figure 28. It appears that superoxide steadily consumes glucose throughout the reaction. As for the 4MPG, it consumes more H₂O₂ during the first 5 minutes of the reaction and therefore less H₂O₂ is present afterward. This causes the cleavage of the 4MPG rate to slow down because there is less H₂O₂, since some of the H₂O₂ is consumed by glucose and 4-methoxyphenol throughout the 30 minute reaction. Here again, the quantification process of H₂O₂ was not possible because of the buffer system.
Figure 28. Percent recovery of glucose and 4MPG at pH 12 (lines are guides connecting data points).

Additional experiments and summary for the oxidation of 4MPG

Cleavage of 4MPG at the glycosidic bond occurred under both pH conditions, as indicated by observation of varying amounts of glucose. However, 4-methoxyphenol, the lignin portion of the LCC, could not be detected under either set of conditions.

The amount of H$_2$O$_2$ was reduced so that the ratio between 4MPG and H$_2$O$_2$ was about 1:1 to see if 4-methoxyphenol could be recovered, and also to see if the reaction trend is rapid oxidation of 4MPG when there is an excess amount of H$_2$O$_2$. The results of these reactions are shown in Table 4. Only at pH 12 was 4-methoxyphenol recovered after a 30 minute reaction. The results in this table represent simple date points so standard deviations are not available.
Table 4. Oxidation of 4MPG at pH 9.5 and 12 with 1:1 4MPG:H$_2$O$_2$.

<table>
<thead>
<tr>
<th></th>
<th>pH 9.5 (5min)</th>
<th>pH 9.5 (30 min)</th>
<th>pH 12 (30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-methoxyphenol</td>
<td>X</td>
<td>X</td>
<td>8.89%</td>
</tr>
<tr>
<td>Glucose</td>
<td>12.2%</td>
<td>13%</td>
<td>11.7%</td>
</tr>
<tr>
<td>4MPG</td>
<td>25.5%</td>
<td>9.8%</td>
<td>24.9%</td>
</tr>
</tbody>
</table>

Dimers of 4-methoxyphenol were recovered based on their mass spectrum but they were not quantified for both pHs. From observation of the chromatogram the amount of dimers is not very significant (Figure 29). This implies 4-methoxyphenol degraded faster at pH 9.5 than at pH 12. These results suggest that there is less reaction occurring at pH 12 than at pH 9.5. For example, at 30 minutes, more 4MPG reacted at pH 9.5 than at pH 12. Also, more 4-methoxyphenol could be recovered at pH 12. Therefore, the reaction of 4MPG and 4-methoxyphenol at pH 12 is more dependent on the concentration of H$_2$O$_2$ than at pH 9.5 because more 4-methoxyphenol was recovered at pH 12 than at pH 9.5 after 30 minutes.

Figure 29. Oxidation of 4MPG at pH 12 for 30 minutes (organic layer).
Glucose also was recovered but only about 12% at both pHs, but a little more glucose was obtained at pH 9.5 than at pH 12 after 30 minutes. So, glucose consumes more of H$_2$O$_2$ at pH 12 compared to pH 9.5. This is the similar to the pattern of the 4MPG reaction when there is an excess amount of H$_2$O$_2$.

Figure 30 shows the trend of the reaction rate of 4MPG at pH 9.5 with a lower amount of H$_2$O$_2$ and with an excess amount of H$_2$O$_2$. This trend is similar at pH 12 as well. Based on these results, most of the H$_2$O$_2$ is consumed after 5 minutes. At pH 12, 4MPG uses up more H$_2$O$_2$ during the first 5 minutes. Therefore, glycosidic cleavage of 4MPG after 5 minutes at pH 12 is less compared to pH 9.5.

![Graph showing the trend of 4MPG recovered based on the amount of H$_2$O$_2$.](image)

**Figure 30.** 4MPG recovered based on the amount of H$_2$O$_2$ (lines are guides connecting data points).

That 4-methoxyphenol or any possible partial degradation products are not observed suggests that it rapidly is oxidized to CO$_2$. Headspace analysis was done for the oxidation of 4MPG where the molar ratio between 4MPG and H$_2$O$_2$ was 1:10. This
experiment was done only for the pH 12 buffer system which is a phosphate buffer. Since sodium bicarbonate was used to make the pH 9.5 buffer, CO₂ analysis would not be meaningful since the CO₂ could be coming from the buffer or from the oxidation of 4-methoxyphenol. At pH 9.5, however, a substantial amount of CO₂ was detected but not quantified. GC-MS spectra for the CO₂ analysis are included in Appendix C.

In the reaction described above, the amount of glucose recovered was not very high, less than 50 %, even when most of the 4-methoxyphenyl-β-D-glucopyranoside was reacted. As demonstrated by Guay (1999), glucose can be degraded to lower sugars such as arabinose and gluconic acid. In this case, arabinose and gluconic acid were detected by the GC-MS. These compounds were not quantified since there were only trace amounts of these compounds.

Proposed mechanisms

From the results of the oxidation of 4MPG at both pHs, possible reaction mechanisms are suggested. These mechanisms were also based on the proposed mechanisms from previous studies by Guay (1999) and Hausman (1999). The reactions begin with cleavage of the glycosidic bond between glucose and 4-methoxyphenol by ipso attack on the aromatic ring and not at the anomeric carbon of the glucose, due to the ability of the aromatic ring to stabilize the odd electron by resonance. Molecular orbital calculations (B3LYP/6-31+G*) of energies of the transition state for anomeric attack and the intermediate for aromatic attack were carried out. The transition state for anomeric attack was 45.7 kcal/mol higher than the aromatic attack for hydroxyl radical, and 27.5 kcal/mol higher for superoxide anion. Thus, the computations strongly support the initial attack on the aromatic ring. Structures of the modeled species are shown in Table 5.
Table 5. Relative energies of possible reaction of 4MPG with hydroxyl radical (pH 9.5) and superoxide anion (pH 12).

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Energy, h (\text{kJ/mol} )</th>
<th>Relative Energy, kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="OH" /></td>
<td>-1108.145339</td>
<td>0.0</td>
</tr>
<tr>
<td><img src="image2.png" alt="OH" /></td>
<td>-1108.218102</td>
<td>-45.7</td>
</tr>
<tr>
<td><img src="image3.png" alt="OH" /></td>
<td>-1182.762825</td>
<td>0.0</td>
</tr>
<tr>
<td><img src="image4.png" alt="OH" /></td>
<td>-1182.806640</td>
<td>-27.5</td>
</tr>
</tbody>
</table>

Hydroxyl radical

At pH 9.5, after the initial addition of the hydroxyl radical to the aromatic ring, the bond between the oxygen and aromatic ring would break to restore the aromaticity of the aromatic ring, producing the glucoxy radical (Figure 31).
Figure 31. Hydroxyl attack on the aromatic ring of 4MPG leading to ipso cleavage to give 4-methoxyphenol and glucoxy radical.

The glucoxy radical abstracts hydrogen from H₂O₂ to form glucose, which is detected, or it may oxidize further to compounds such as gluconic acid and arabinose (Figure 32). Arabinose and gluconic acid were detected with gas chromatography and were confirmed with standards, but these compounds were not quantified. The reaction mechanisms for formation of these products were proposed by Guay in 1999.

Figure 32. Formation of glucose.
A pathway from 4-methoxyphenol to CO₂ can be suggested. A hydroxyl radical abstracts a hydrogen atom from the 4-methoxyphenol hydroxyl group to form a phenoxy radical. Delocalization of the phenoxy radical places odd electron density at the carbon meta to the methoxy group. A hydroperoxy radical attacks there, and subsequently forms a dioxetane. The dioxetane ring opening leads to the formation of a muconic acid. Figure 33 describes the proposed mechanism of the oxidation of 4-methoxyphenol to the dioxetane.

4-Methoxyphenol

Dioxetane

**Figure 33.** Formation of dioxetane from 4-methoxyphenol reaction with hydroxyl radical at pH 9.5.

There are two proposed pathways for the opening of the dioxetane ring, as described in Figure 34. One pathway depends on the presence of UV radiation and the
other does not. The one step dioxetane ring opening to muconic acid, a reverse $2\pi + 2\pi$ cycloaddition, was proposed by Gierer in 1997. In 1999, Hausman proposed a new mechanism by modifying the Gierer mechanism. Here we propose that a two step ring opening process without the need of UV is an electrocyclic reaction mechanism that would involve a $6e^-$ disrotatory process followed by an $8e^-$ conrotatory process to form a muconic acid. Degradation mechanisms of muconic acid into smaller acids have been proposed by several researchers (Sun et al., 1998; Hausman, 1999).

![Dioxetane to Muconic Acid](image)

**Figure 34.** Formation of muconic acid from the dioxetane.

**Superoxide anion**

The reaction between 4MPG and superoxide anion (pH 12) is similar to the reaction with the hydroxyl radical (pH 9.5). Superoxide anion attacks the aromatic ring of the 4MPG leading to formation of glucoxyl radical and a phenyl peroxy radical (Figure 35).

![4MPG Reaction with Superoxide Anion](image)

**Figure 35.** Reaction of 4MPG at pH 12 (superoxide anion).
Similarly to the pH 9.5 system, the glucoxyl radical can further react or abstract hydrogen from H$_2$O$_2$ to form glucose. The phenyl peroxy anion abstracts a proton from H$_2$O$_2$ to form a phenyl hydroperoxide. UV radiation causes homolysis of the bond of the peroxide to form a phenoxy radical and a hydroxyl radical. The phenoxy radical couples to the superoxide anion leading to the formation of the dioxetane ring as before. Figure 36 describes the mechanism of the phenyl peroxy anion reaction with oxygen species to form the dioxetane ring. At this stage, the formation of muconic acid follows the same mechanism as pH 9.5 system. The phenoxy anion also may abstract hydrogen from H$_2$O$_2$ to generate 4-methoxyphenol, since some 4-methoxyphenol was observed during the control reaction with a 1:1 ratio between 4MPG and H$_2$O$_2$.

![Reaction Mechanism](image)

**Figure 36.** Formation of dioxetane by oxidation of 4MPG at pH 12.
Overall, cleavage of the glycosidic bond did occur for 4MPG at both pHs. The majority of 4MPG reacted during the first five minutes of the reaction. The reaction of 4MPG was faster at pH 12 compared to pH 9.5 during the first five minutes. But for the next 25 minutes the reaction of 4MPG was faster at pH 9.5 compared to pH 12. Glucose was recovered but not in a substantial amount, less than 40 % for both pHs. No 4-methoxyphenol was recovered at either pH when there was an excess amount of H$_2$O$_2$ present. Dimers of 4-methoxyphenol and 4-methoxyphenol were recovered with a lower concentration of H$_2$O$_2$.

Results for the oxidation of coniferin

*Control reactions*

Coniferin differs from the simpler 4-methoxyphenol analog in that it contains a conjugated double bond in the side chain of the aromatic group. Owing to the conjugated double bond, control reactions for coniferin were more complicated than control reactions of 4-methoxyphenyl $\beta$-D-glucopyranoside. In the control reaction at both pHs with H$_2$O$_2$ but no UV radiation, minor cleavage of the glycosidic bond occurred. Oxidation of coniferin occurred for both pHs under UV irradiation for 15 minutes in the absence of H$_2$O$_2$. Quantification of the amount of coniferin that was lost during UV radiation was carried out. From GC-MS data of control reactions for coniferin, included in Appendix C, the majority of cleavage of the glycosidic bond between glucose and coniferyl alcohol is due to oxidation chemistry by oxygen species.
Figure 37. Reaction of coniferin with UV radiation and oxygen species.

On the other hand, it is likely that the oxidation of the coniferyl alcohol portion was due to the UV irradiation. Studies of the photochemical reactions of coniferyl alcohol in CH$_2$Cl$_2$ after degassing done by Jaeger and coworkers (1993) identified several products that form. Reaction pathways were proposed (Figure 38).
Figure 38. Schematic of photodegradation products of coniferyl alcohol (Jaeger et al., 1993). X signifies products not possible under our conditions.

Using thioacidolysis, they were able to determine two other oligomers from UV irradiation of coniferyl alcohol (Figure 39).
Figure 39. Products generated by photodegradation that were identified after thioanalysis.

For our controlled reaction conditions, we should expect the majority of these compounds, except the chlorinated products, since there was no chlorine in our system. A control photolysis of coniferin was also done in water without the presence of H₂O₂. UV-Vis spectra were taken before and after 15 minutes UV irradiation at 254 nm UV light. Figure 40 shows a significant change in the UV absorption.

Figure 40. UV-vis spectra of coniferin before and after 15 minutes UV irradiation.
Therefore, information from the manufacturer of the photoreactor was obtained to see the range of wavelength and radiation intensity that would be produced by this specific photochemical reactor. Figure 41 is the graph of the wavelength and intensity of the radiation produced depending on the distance from the UV lamp and at the center of the equipment. For our experiments, the sample vessels were located about 2.5 inches away from the lamps. It can be seen that the lamps do produce modest amounts of radiation at wavelengths that are absorbed by coniferin.

![Intensity Reading for Rayonet 254 nm Photochemical Reactor](image)

**Figure 41.** Wavelength and intensity of the Rayonet 254 nm photochemical reactor.

Internal standards

We were able to quantify glucose, coniferin, and glucovanillin from the oxidation of coniferin. These compounds were in the aqueous layer after the oxidation and work-
up process. Xylitol was used as the internal standard for glucose. The internal standard for coniferin and glucovanillin was phenyl-β-D-galactopyranoside.

**Oxidation of coniferin at pH 9.5**

At pH 9.5, we were able to identify and quantify glucose, glucovanillin, and coniferin. Similarly to the oxidation of 4MPG, no aromatic component was found. Table 6 contains the results of the oxidation at pH 9.5.

**Table 6.** Results for the oxidation of coniferin at pH 9.5.

<table>
<thead>
<tr>
<th>Aromatic(s)</th>
<th>10 minutes</th>
<th>15 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Glucose</td>
<td>17.9 ±7.1</td>
<td>27.9 ±4.9</td>
<td>30.4 ±5.1</td>
<td>36.7 ±3.0</td>
</tr>
<tr>
<td>Glucovanillin</td>
<td>6.84 ±0.37</td>
<td>6.86 ±0.92</td>
<td>4.70 ±1.0</td>
<td>2.81 ±0.81</td>
</tr>
<tr>
<td>Coniferin</td>
<td>27.3 ±1.1</td>
<td>20.8 ±6.1</td>
<td>5.59 ±1.4</td>
<td>1.15 ±0.76</td>
</tr>
</tbody>
</table>

A graph of the amount of glucose, glucovanillin, and coniferin recovered at each time reaction was done to show the trend in yield of these compounds (Figure 42). About 70% of the coniferin had reacted in the first 10 minutes. About 18% of the glucose was recovered at 10 minutes and almost 40% was recovered after 30 minutes. The amount of glucovanillin stayed consistently at 7% and slowly dropped down to half at 30 minutes. Based on these results, H₂O₂ was consumed during the first 10 minutes of the reaction. As the reaction progressed, the concentration of H₂O₂ decreased leading to less oxidation of the glucose, and thus more glucose was obtained.
Figure 42. Percent of glucose, glucovanillin, and coniferin recovered (lines are guides connecting data points).

Oxidation of coniferin at pH 12

Table 7 contains the results for the oxidation of coniferin at pH 12. A graph is also used to show the trend of each component (glucose, glucovanillin, and coniferin) (Figure 43). The majority of coniferin (74%) reacted during the first 10 minutes. The rest of the coniferin reacted slowly for the next 25 minutes. Only 24% of glucose was recovered after 10 minutes photochemical reaction, and during the next 25 minutes only 6% more of glucose was recovered. About 5% of the glucovanillin was recovered after 10 minutes. It increased slightly at 15 minutes and slowly dropped to about 1%. Again, this parallels the amount of H$_2$O$_2$. Also similar to the oxidation of 4MPG at pH 12, glucose consumed H$_2$O$_2$ throughout the 30 minute reaction. Therefore, the amount of glucose recovered was less compared to the amount of glucose recovered at pH 9.5.
Table 7. Results for the oxidation of coniferin at pH 12.

<table>
<thead>
<tr>
<th>Aromatic(s)</th>
<th>10 minutes</th>
<th>15 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Glucose</td>
<td>23.8 ±0.38</td>
<td>35.0 ±0.52</td>
<td>24.0 ±6.1</td>
<td>29.7 ±2.7</td>
</tr>
<tr>
<td>Glucovanillin</td>
<td>4.69 ±0.46</td>
<td>6.50 ±0.38</td>
<td>2.53 ±0.35</td>
<td>1.1 ±0.56</td>
</tr>
<tr>
<td>Coniferin</td>
<td>26.2 ±2.0</td>
<td>24.1 ±1.4</td>
<td>17.1 ±4.1</td>
<td>1.08 ±2.7</td>
</tr>
</tbody>
</table>

Figure 43. Percent recovery of each component at each time interval for oxidation of coniferin at pH 12 (lines are guides connecting data points).

Because we can observe the glucose, we can conclude that cleavage at the glycosidic bond did occur. It could happen as direct cleavage from the coniferin or could be from modified coniferin compounds, such as glucovanillin (Figure 44), produced by side-chain oxidation by irradiation or oxygen species. For both pHs, the reaction rate is similar for the first 10 minutes but also for the next 25 minutes. More glucose was recovered at pH 9.5 after 30 minutes (40%) than at pH 12 (30%). Therefore, glucose is oxidized to compounds such as arabinose and gluconic acid more readily at pH 12.
Figure 44. Glucovanillin

No aromatic fragment was recovered at either pH. We suggest that they are also oxidized to CO₂, similar to the oxidation of 4-methoxyphenol, following cleavage at the glycosidic bond.

Proposed mechanism

The cleavage mechanism for both pHs could be similar to the cleavage mechanism for 4MPG as was shown in Figure 31. At pH 9.5, the hydroxyl radical likely attacks the aromatic rather than the anomeric position and breaks the glycosidic bond at the ipso position (Figure 45). This mechanism at pH 12 is likely the same with superoxide anion replacing the hydroxyl radical in the first step. Further oxidation of glucose would be the same as Guay (1999) proposed.
Coniferin/Modified coniferin(s)

React further

H₂O₂

React further

Figure 45. *Isopo* cleavage of the glycosidic bond of coniferin by hydroxyl radical.

Our results suggest that photochemical and peroxide-based oxidation compete with cleavage of the glycosidic bond, with chemistry occurring at both the double bond and phenol.
CHAPTER 4-CONCLUSIONS AND SUGGESTED WORK

CONCLUSIONS

Two model compounds representing lignin-carbohydrate “complexes” were synthesized, 4-methoxyphenyl-β-D-glucopyranoside and coniferin. Cleavage of the glycosidic bond of these model compounds was successful. In the presence of an excess amount of H$_2$O$_2$, the aromatic portion is proposed to further oxidize to smaller acids and eventually to CO$_2$. We are able to detect the CO$_2$ via GC-MS headspace analysis. With a lower amount of H$_2$O$_2$, aromatic compounds were recovered (monomer and dimer units). Glucose was recovered as well in the presence of excess H$_2$O$_2$ but not in substantial amounts. Glucose was further degraded to arabinose and gluconic acid and eventually also to CO$_2$. The degradation rate of glucose was slower than the degradation rate of the aromatic component.

For both model compounds, most of the oxidation takes place during the first five to ten minutes. During the first 5 minutes in the presence of excess H$_2$O$_2$ (10:1, H$_2$O$_2$:4MPG), 4MPG reacted faster at pH 12 compared to pH 9.5. From 5 to 30 minutes, it was the opposite; 4MPG reacted faster at pH 9.5 than at pH 12. When the ratio between 4MPG and H$_2$O$_2$ was changed to 1:1, more of 4MPG reacted at pH 9.5 after 30 minutes than at pH 12. This implies that reaction of 4MPG at pH 12 is dependent on the concentration of H$_2$O$_2$. The quantification of H$_2$O$_2$ was unable to perform due to the buffer systems.

We proposed a plausible mechanism that the hydroxyl radical (at pH 9.5) or the superoxide anion (at pH 12), attacks the aromatic ring which leads to ipso cleavage of the
glycosidic bond for both model compounds. The glycoxyl radical generated by this
process then abstracts a hydrogen from H₂O₂ to form glucose or reacts further. Our
results suggest that, in this case, the glucoxyl radical oxidized faster to form arabinose
and gluconic acid than it was able to abstract a hydrogen to form glucose, since the
amount of glucose that formed was 40% or less for both lignin-carbohydrate compounds
at both pHs. The degradation mechanism of glucose is likely the same as was proposed
by Guay (1999).

Since no aromatic portion of the model compound was detected during GC-MS
analysis, the degradation of the aromatic portion is clearly much faster than the
degradation of glucose. Degradation of 4-methoxyphenol to smaller acids and CO₂ is
proposed.

SUGGESTED WORK

This work concluded that the hydroxyl radical and the superoxide anion can
cleave the glycosidic linkage at the ipso position. This hypothesis is based on a
computational study of the oxidation of 4-methoxyphenyl-β-D-glucopyranoside.
Additional computational study is needed regarding the oxidation of coniferin to confirm
that ipso cleavage also occurred for coniferin. Also, to further confirm this specific
cleavage, H₂O₂ labeling could be done to see if the labeled oxygen of the H₂O₂ ends up
on the aromatic ring. Another labeling experiment that would confirm an ipso
cleavage is if the phenoxy oxygen of the aromatic is labeled prior to the synthesis of the
model compound so that the label would be on the glucose.
More work is needed to elucidate and determine mechanisms for the degradation of the aromatic portion of coniferin. In order to propose these degradation mechanisms, the products generated by the oxidation process must be identified with authentic samples. Since these authentic samples are not available commercially, syntheses of these compounds must be done.

Oxidation studies should also be done with other lignin-carbohydrate model compounds that represent other likely linkages to determine the usefulness of oxidation as a means of cleaving lignin-carbohydrate linkages so that these components can be utilized. This work will require synthesizing these compounds.

Also, different ratios between model compound and hydrogen peroxide should be used for further study to see if more glucose and more lignin can be obtained. Recently, NREL published a list of valuable products that can be generated from lignin (Bozell et al., 2007), so if we can control the oxidation to obtain the lignin fragments without degrading them, more valuable products might be generated. Therefore, quenching the reaction to stop the lignin portion from reacting further to CO₂ must be done. Finally, when the optimal conditions for oxidation of model compounds are found, these conditions should be used to study actual lignin-hemicellulose samples. Other oxidizing agents, such as singlet oxygen and ozone, also should be investigated.
REFERENCES


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Figure A1. $^1$H-NMR spectrum of 4-methoxyphenyl-β-D-glucopyranoside.
Figure A2. $^1$H- NMR spectrum of 4-O-[tetra-O-acetyl-β-gluco] vanillin.
Figure A3. $^1$H- NMR spectrum of 4-O-[tetra-O-acetyl-β-gluc] ferulic acid ethyl ester.
Figure A4. $^1$H- NMR spectrum of coniferin.
Figure B1. IR spectrum of 4-methoxyphenyl-β-D-glucopyranoside.
Figure B2. IR spectrum of 4-O-[tetra-O-acetyl-β-D-gluco] vanillin.
Figure B3. IR spectrum of 4-O-[tetra-O-acetyl-β-D-gluco] ferulic acid ethyl ester.
Figure B4. IR spectrum of coniferin.
Figure C1. GC-MS of 4-methoxyphenyl-β-D-glucopyranoside
Figure C2. GC-MS of coniferin.
Figure C3. GC-MS of 4-methoxyphenyl-β-D-mannopyranoside
Figure C4. GC-MS of glucovanillin.
Figure C5. GC-MS of phenyl-β-D-galactopyranoside (PβGal).
Figure C6. GC-MS of glucopyranose.
Figure C7. GC-MS of arabinose.
Figure C8. GC-MS of xylose.
Figure C9. GC-MS of xylitol
Figure C10. GC-MS of vanillin.
Figure C11. GC-MS of coniferyl alcohol.
Figure C12. GC-MS of 1,4-dimethoxy benzene.
Figure C13. GC-MS of 4-methoxyphenol.
Figure C14. GC-MS of gluconic acid.
Figure C15. GC-MS of headspace analysis of pH 12 oxidation of 4MPG (blank).
Figure C16. GC-MS of headspace analysis of pH 12 oxidation of 4MPG.
Figure C17. GC of 4MPG aqueous layer after oxidation (pH 9.5).
Figure C18. GC of 4MPG aqueous layer after oxidation (pH 12).
Figure C19. GC of coniferin aqueous layer after oxidation (pH 9.5).
Figure C20. GC of coniferin aqueous layer after oxidation (pH 12).
**Figure D1.** UV-vis spectrum of coniferin in pH 9.5 buffer.
Figure D2. UV-vis spectrum of coniferin in pH 12 buffer.
Figure D3. UV-vis spectrum of coniferin in water.
Figure D4. UV-vis spectrum of coniferin in water after 15 minutes UV irradiation.
BIOGRAPHY OF THE AUTHOR

Mai Nguyen was born in Ho Chi Minh City, Vietnam on March 6, 1979. In March of 1991, she and her family moved to New Holland, Pennsylvania. Mai went to Garden Spot High School, New Holland, PA, and graduated in May of 1998. In the fall of 1998 she enrolled at Millersville University, Millersville, PA. She received her Bachelor of Science in Chemistry from Millersville University in May, 2003.

In July of 2003, Mai entered the graduate program at the University of Maine. She is a candidate for Doctor of Philosophy in Chemistry from the University of Maine in August, 2008.