LIGNIN EXTRACTION, BIODEGRADATION AND USAGE

by

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Abstract

Lignin is a complex biopolymer abundantly found in all vascular plants. It plays a key role in building connective tissues and giving them strength, rigidity, and resistance to environmental factors such as pathogens. Extracted lignin finds diverse applications in the commercial sector with immense potential in novel value-added applications. Therefore, it is important to develop optimum and sustainable processes for lignin extraction. To this end, one of the aims of the present research was to examine different lignin extraction methods on common wood species present in Newfoundland, Canada – balsam fir, pine, spruce (softwood), birch, maple, and oak (hardwood). Two different lignin extraction methods were studied: (1) the Formacell method, which uses acetic acid/formic acid/water; and (2) the BioEB method, which uses only formic acid/water.

Various parameters were tested, including solvent concentration, temperature, cooking time, to determine the most optimal lignin extraction conditions. The results of this study can be applied to inform and improve industrial lignin extraction processes to obtain better yields in the most optimal manner.

This thesis also discusses the latest developments in value-added uses of extracted lignin for the preparation of novel bio-based materials. Lastly, it provides a review of the mechanisms of microbial biodegradation of lignin. These microbial ligninolytic mechanisms provide a host of possibilities to overcome the challenges of using harmful chemicals to degrade lignin biowaste in many industries.

Acknowledgments

First and foremost, I want to express my gratitude to my supervisors Dr Joseph Banoub and Dr Christopher Parrish for all their help, advice, and encouragement during my programme.

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List of Symbols and Abbreviations

Abbreviation	Description
Acetyl CoA	Acetyl Coenzyme A
APPI-MS	Atmospheric Pressure Photoionization Mass Spectrometry
BDO	Butanediol
BSTFA	N, O-bis(trimethylsilyl) trifluoroacetamide
CID-MS/MS	Collision-induced Dissociation Tandem Mass Spectrometry
CIMV	la Companie Industrielle Masse Vegetal
CW	Cell Wall
DEPT	Distortionless Enhancement by Polarization Transfer

DFRC	Derivatization Followed by Reductive Cleavage
ECH	Epichlorohydrin
EI-MS	Electron Impact Mass Spectrometry
EtOH	Ethanol
FT-NMR	Fourier Transform Nuclear Magnetic Resonance
GC	Gas Chromatography
GPC	Gel Permeation Chromatography
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Correlation
IL	Ionic Liquid

IPCC	Intergovernmental Panel on Climate Change
IR	Infrared
LCA	Life Cycle Assessment
LUC	Land Use Change
MALDI	Matrix Assisted Laser Desorption/Ionization
MDL	Method Detection Limit
MeOH	Methanol
Mn	Number average molecular weight
MS	Mass Spectrometry
Mw	Weight average molecular weight
MWL	Milled Wood Lignin

PBS	poly (1,4-butylene succinate)
PEG	Polyethylene glycol
PEGDGE	Polyethylene glycol diglycidyl ether
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHB4B	Poly (3-hydroxybutyrate-co-4hydroxybutyrate)
PLA	Polylactic acid
PTT	poly(trimethyleneterephthalate)
Py-GC-MS	Pyrolysis Gas Chromatography Mass Spectrometry
Qq-TOF	Quadrupole-quadrupole time of flight

SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction
TMAH	Tetramethylammonium hydroxide
TMCS	Trimethylchlorosilane
UV	Ultraviolet
VPO	Vapor Pressure Osmometry
VRL	Virgin Released Lignins

List of Appendices

Co-authorship Statement

Two of the Chapters of the present thesis have been submitted thus far for publication in peer-reviewed journals. Co-authorship information about these manuscripts is presented below:

Chapter 6: LIGNIN DEGRADATION BY MICROORGANISMS: A REVIEW

Submitted to: Biotechnology Progress

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APPENDIX A: ENVIRONMENTAL IMPACT OF BIOPLASTIC USE: A REVIEW

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Chapter 1: INTRODUCTION

1.1. Introduction and Motivation

In the last decade in Canada, the pulp and paper industry has faced a descending period because of a reduction in the demand for traditional paper products as well as an increase in competition from international companies. To increase the revenue and sustainability of this industry, various studies have attempted to design and manufacture new non-paper products from different components of wood.

It has also been proposed that existing paper and pulp mills be converted to integrated forest biorefinery plants as this would allow for the transformation of lignocellulosic biomass into novel value-added commodities while also producing the conventional paper products. [1,2]

All vascular plant cell walls and woods are composed of three main components: cellulose, hemicelluloses, and lignins.

Plants utilise solar energy to produce these three components in large quantities. The plant cell wall (CW) structure can be divided into two parts: primary and secondary.

Cellulose, which is the primary component of all skeletal plant structures, builds the framework of both the CWs, while hemicelluloses are responsible for cross-linking cellulosic and non-cellulosic polymers.[1-4] Cellulose fibers contain around 8×10^3 D-glucopyranose residues which are covalently linked via β-D-(1→4)-glycosidic bonds.[5]

Around 40% of these glycan chains are clamped together by hydrogen bonds, forming cellulose microfibrils that are arranged randomly in the primary CW. These cellulose microfibrils are linked to hemicellulosic polysaccharides, such as mannans, xylans, galectins, or combinations of these, via hydrogen and covalent bonding (Figure 1.1). [5,6,7]

The secondary plant CW is formed by hemicelluloses (10-40%) that are embedded in lignin (25%). Lignin is the second most plentiful biopolymer in nature after cellulose. It is located in all vascular plants, mostly between the cells and within the cells in the CWs. [3,5]

It is accepted that lignin biomolecules are intimately mixed with the carbohydrate components of the CWs of vascular plants and form the complex glycolignin network. Needless to say, cellulose and hemicelluloses are structurally more organized in the secondary CW than in the primary CW. Lignin plays a key role in building constructive tissues, giving them strength, rigidity, and resistance to environmental factors.[1] In recent years, it has been found that the lignin biomolecule structures depend on the botanical source and chemical composition of the vegetal fibers.[11] However, because of the diverse and complex nature of lignin and hemicellulosic moieties in the ligno-hemicellulosic complexes, it is very difficult to predict the structure of the secondary CW (Figure 1.1).[3,5]

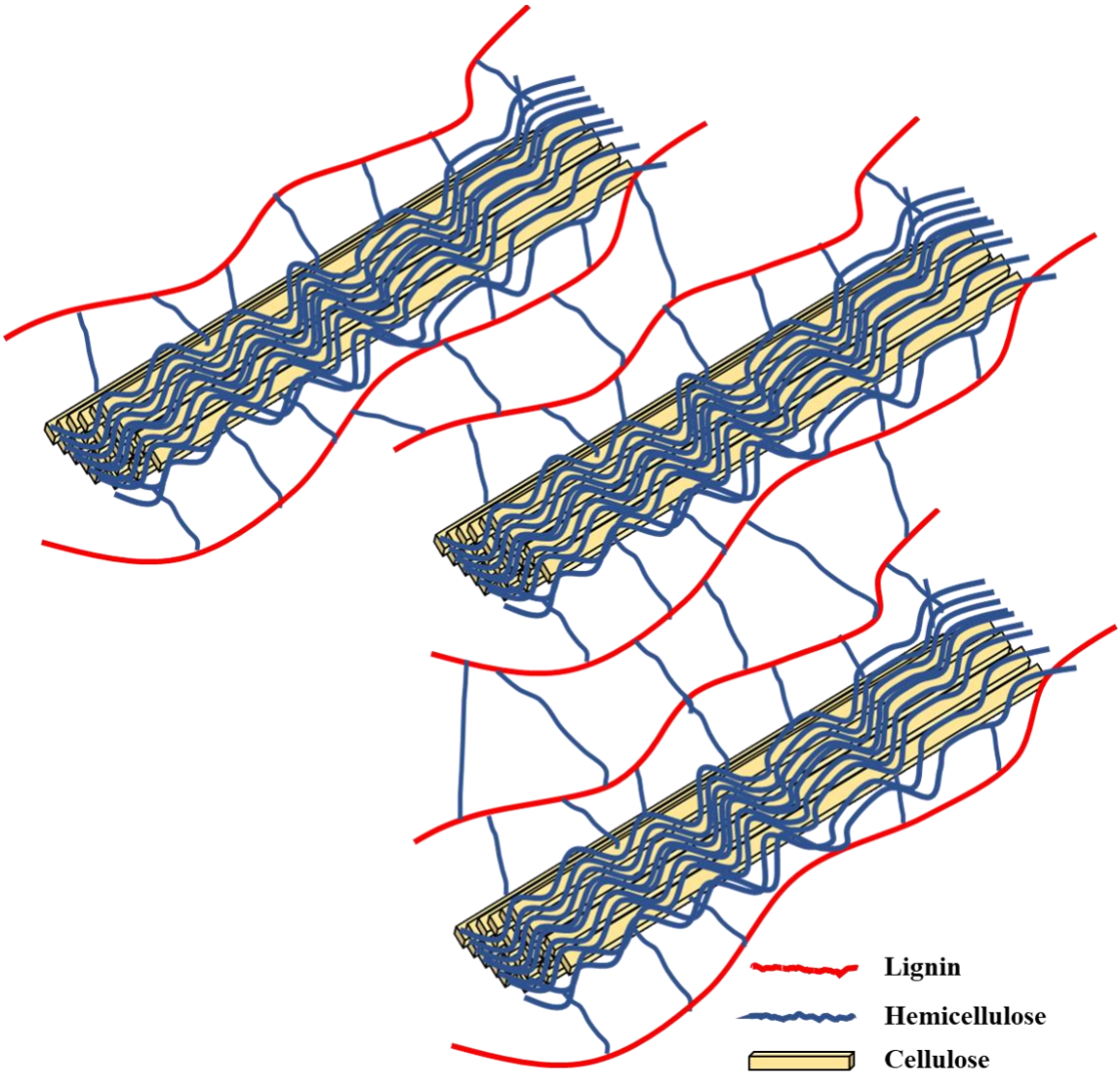


Figure 1.1. General structure of plant matter.

The word lignin comes from the Latin name for wood, “lignum”. It is described as the encrusting material in wood. While it has been known that lignin contains higher carbon content than carbohydrates, little was known about its chemical nature for a long time. In fact, lignin structure in the last century was defined simply as a complex polymer of

irregular branched units.[8] As research progressed through the years, woody lignin biomass was found to contain a polyphenolic structure whose role is to give resistance to biological and chemical degradation (the hydrophobic nature and insolubility of lignin in aqueous systems prevent access to chemicals and organisms' degrading action.)[9] Today, lignin has become a valuable commodity created from a widely available organic source. The annual global production of lignin as a by-product of the woodworking industry exceeds 100 million tons.[10]

During the last century, an incredible effort was made to understand the exact structure of the lignin biomolecules. The accepted concept proposed for the formation of lignin, which is found as a crosslinked amorphous macromolecular substance, consists of phenylpropanoid monomers known as lignols.[12] It is also known that lignin biomolecules are composed of several oligomeric subunits that possess the same phenylpropanoid skeleton. However, their phenyl ring can have a different degree of oxygen substitution. Therefore, lignin biomolecules are composed of the monolignol H-structure, which consisted of a 4-hydroxyphenyl ring, the monolignol guaiacyl G-structure containing one hydroxyl and one methoxyl group, and the monolignol syringyl S consisting of one hydroxyl group and two groups of methoxyl (Figure 1.2).[13]

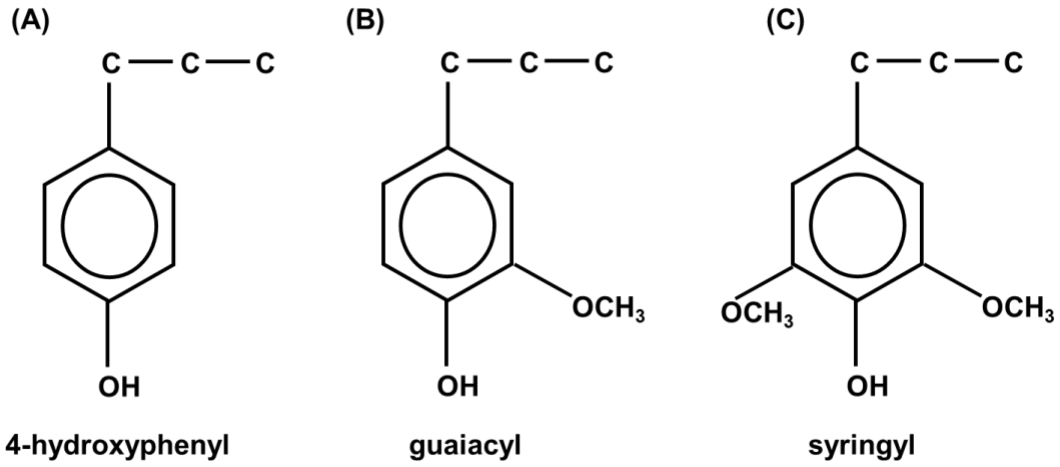


Figure 1.2. Monomeric structures of lignin.

The H, G, and S constituents of lignin oligomers are covalently linked and can form ether, ester, and carbon-carbon bonds. These latter structural features can also repeat in a random manner and provide magnificent complexity.[14] Generally, the site of attachment of the covalent linkages can vary between two lignols. Moreover, each monomer's distribution within the lignin biomolecules diverges significantly depending on whether the source plant was a gymnosperm, a monocotyledonous angiosperm, or a dicotyledonous angiosperm. Gymnosperms are typically hardwood and/or coniferous trees such as pine, oak, or spruce and almost exclusively contain the lignol G unit, whereas angiosperms are found in flowering plants such as cereals, vegetables, and fruits. There are two categories of angiosperms, and these are broadleaf weeds that contain almost exclusively the G and S lignol units and the monocotyledons that contain all three lignol units G, S, and H.[15]

It should be mentioned that the main mechanism by which the biosynthetically formed monolignols migrate to the CW and form lignin is not yet known.[16] The most accepted biosynthetic polymerization route agreed to today is the 'combinatory process'. This proposed route posits that lignol radicals produced by oxidizing enzymes combine to form di-, tri-, and oligo-lignols. This biosynthetic route depends only on the chemical control that dictates the monomeric linking and produces all resulting polymeric structures. [16,17,18] It has been confirmed that this polymerization is usually initiated by an oxidative radical ionization of the phenols, which react to form oligomers. The formation of these covalent bonds occurs by combinatorial free radical coupling.[19]

It is generally recognized that lignin biomolecules are comprised of H, G and S monomers attached by degradation-resistant β–O–4′, β–5′, β–β′, β–1′, 5–5′ and 5–O–4′ links. The designation of these formed covalent bonds depends on the atomic centers in the radicals that are connected together in the last stage of lignin biosynthesis. [20,21,22] The phenylpropane units of lignin are connected by various carbon-carbon, ether, and ester bonds, of which the most frequent 5, 6 in proportion to the structure are reported in Figure 1.3.

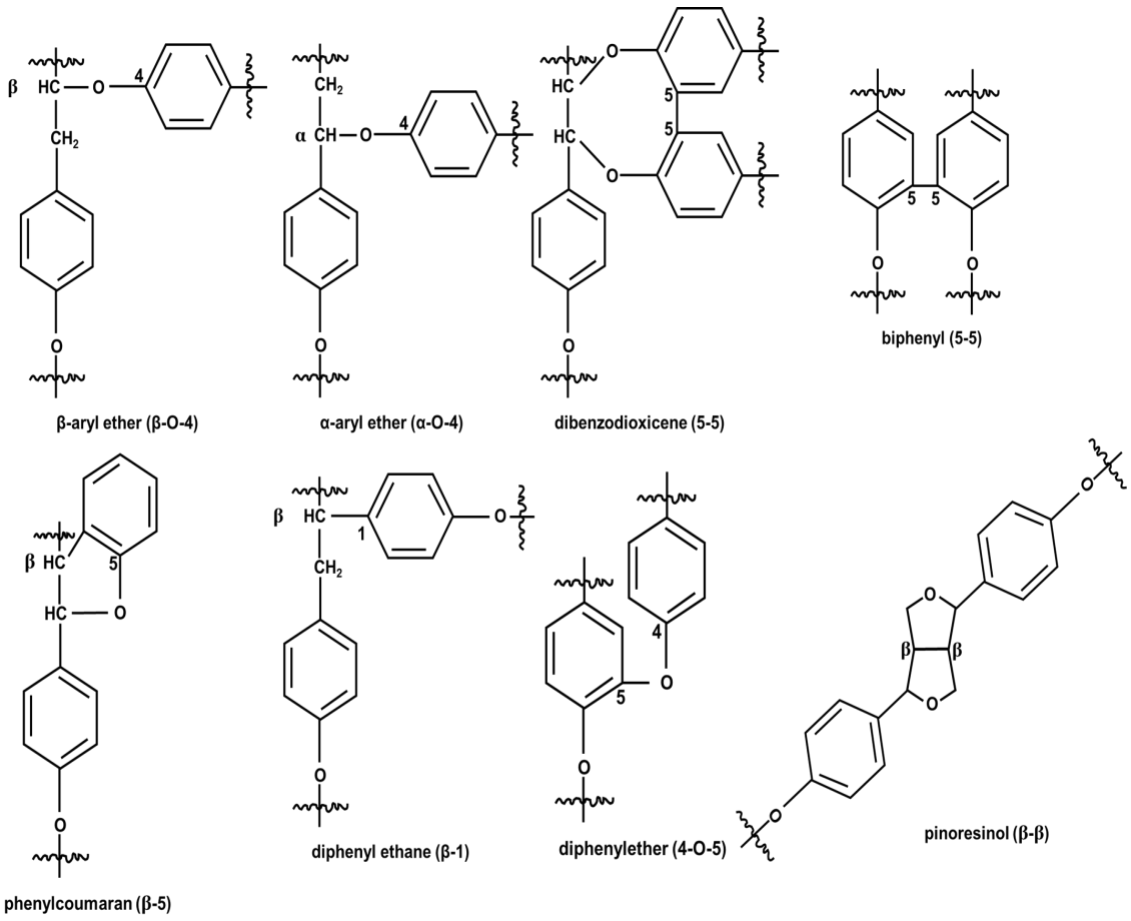


Figure 1.3. Lignin linkages: ether bonds, carbon-carbon bonds, and more complex linkages.

The industrial significance of lignin is ever-increasing, making lignin an important topic of research. However, to the best of our knowledge, comprehensive review studies that compile all the different industrial applications of lignin are absent from the literature. Moreover, given the increasing significance of lignin in a large variety of industrial sectors (which will be discussed in detail in Chapter 4), lignin extraction has also become an important field of scientific research. As such, a variety of lignin extraction methods have been developed, each with a different efficacy, specificity, eco-friendliness, cost-

effectiveness and feasibility. In order to meet the increasing industrial demands of lignin, it is important to develop extraction methods that not only operate at feasible ranges of parameters such as temperature, and yield high lignin concentrations, but are also cost-effective and eco-friendly. However, a comparative study of the efficacy of cost-effective and eco-friendly lignin extraction methods is absent from the literature. Moreover, lignin derivatives, such as lignin-epoxy resins, find application in a wide variety of industries too. Studies elucidating the synthesis and characterization of such epoxy resins in the context of the type of epoxy source used are absent from the literature. Besides lignin extraction, lignin degradation is also fast becoming a commercially viable avenue for its applications in a variety of industries. At the same time, lignin degradation by microbial organisms is an important area of research for its biological value. However, comprehensive review articles that discuss microbial biodegradation in the context of its growing industrial applications are absent from the literature. To address the gaps in research identified above, the present thesis had four primary objectives, as discussed below.

1.2. Research Objective and Contributions

The present dissertation has the following primary objectives:

(1) To investigate two lignin extraction methods (Formacell and BioEB methods) for their optimum yields, reaction times, and parameters in 6 different wood species, and to investigate structural changes in lignin as a function of the extraction method.

(2) To review the current literature to understand the most recent developments in the industrial applications of lignin.

(3) To synthesise and characterise lignin-crosslinked epoxy resins and deacetylated crosslinked lignin- PEGDGE resins.

(4) To review the mechanisms of lignin biodegradation by microorganisms (fungi and bacteria).

1.3. Thesis Structure

To meet the above-mentioned objectives, the rest of this thesis has been divided into 5 chapters. Chapter 2 provides a general overall review of literature related to lignin extraction and structural analysis methods. Chapter 3 discusses and summarizes experimental results from lignin extraction experiments conducted on 6 different wood species (3 hardwood and 3 softwood). It also discusses the results from lignin structural analysis as a function of extraction method and extraction parameters. This chapter further presents the mass spectrometric characterization of oak lignin and its composition. Thereafter, Chapter 4 provides an in-depth review of the literature about the most recent advances in lignin industrial applications. Chapter 5 discusses and summarizes experimental results from the synthesis of lignin-crosslinked epoxy resins and deacetylated crosslinked lignin-PEGDGE resins. Lastly, Chapter 6 subsequently focuses on a literature review about microbial biodegradation of lignin.

Chapter 2: LITERATURE REVIEW

2.1. Extraction of Lignin from Biomass

The pulp and paper industry in 2002 produced approximately 52 million dry tons of lignins.[23] During large-scale industrial preparation of cellulose fibers used to create paper, lignins are released from biomass degradation. The lignin thus produced is then submitted to a series of transformations and purification by different acidic or basic methods. Consequently, they do not bear any resemblance to their native state. [23,24] Importantly, lignins were considered a by-product waste until recently. Therefore, it is critical to realize that cellulose and lignin extraction are two opposing processes. In the following part of my thesis, I will briefly discuss the extraction and removal of native lignins.

All manufacturing paper production processes that use acid or alkaline processes are operated according to traditional procedures and working conditions and can produce the purest cellulose. To eliminate lignin during paper production, the industry uses chemical reagents and conditions that completely change lignin structure to make it completely soluble in aqueous media. [25,26]

Upon studying lignin reactivity, it becomes apparent that the essential functional groups are the aliphatic and phenolic hydroxyl groups (Figure 2.1). Moreover, the differences in the enzymatic, chemical, and mechanical techniques used for lignin extraction result in structural variations after extraction and isolation. Therefore, the only way to determine the

natural structure of lignin is its isolation from the vegetal matrix without causing any structural change.[26] However, preparing pure samples of unchanged lignin is not an easy endeavor, and consequently, determining the structure of lignin is more challenging than other biopolymers.[27,28] New analytical methods based essentially on spectroscopic techniques such as NMR, mass spectrometry, infrared, or UV have led to a creditable advance in the elucidation of the lignin structure.[29,30]

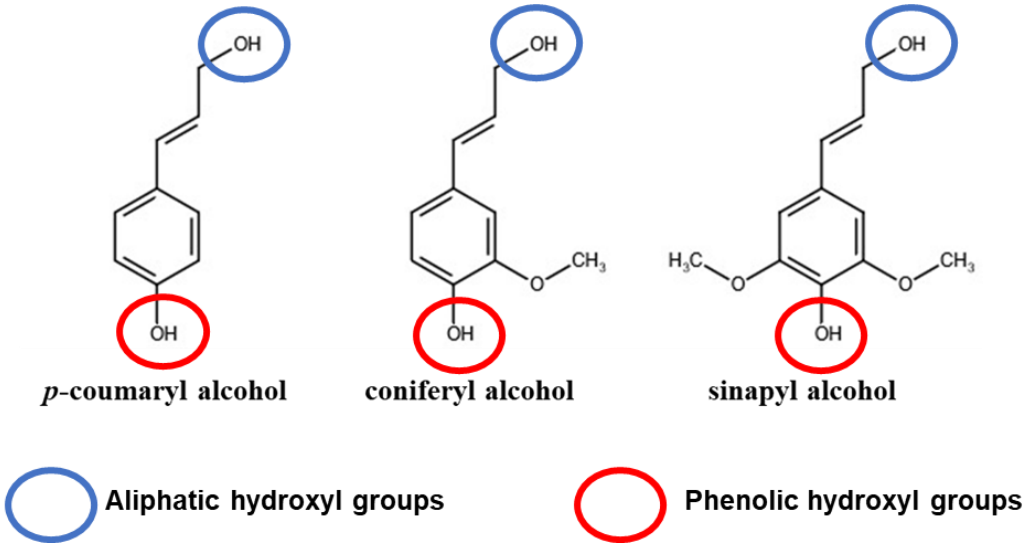


Figure 2.1. Reactive hydroxyl groups in various lignin monomers.

2.2. Extraction of Lignin from Biomass in a Basic Medium

The extraction of lignin from biomass depends mainly on breaking the lignin-polysaccharide linkages in a basic medium. This basic lignin-polysaccharide hydrolysis can

act on two types of links: the esters and ether bonds present in the lignin biomass.[32] It should also be understood that the hydrolysis of the ether (C-O-C) bonds cannot be done just by using a strong base alone. This hydrolysis requires access to high temperature and high pressure too. The saponification of ester bonds is also very fast under similar conditions of heat and pressure.[33,34] The mechanisms of degradation of such ether (C-O-C) and ester (O-CO-) bonds are illustrated in Figure 2.2.

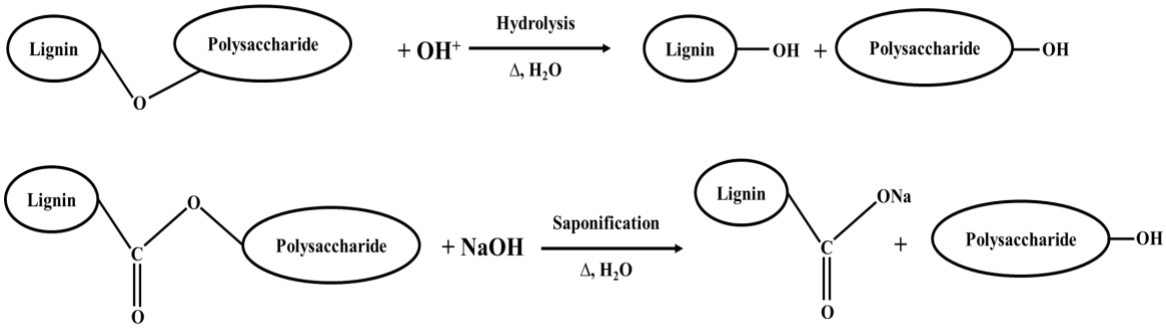


Figure 2.2. Cleavage of ester and ether bonds between lignin and polysaccharides in basic medium.

There are several types of methods that use basic hydrolysis to release lignin for the biomass. These alkaline processes have been defined as producing processed, modified Lignin (PML).[35]

2.2.1. Kraft Extraction Process

The Kraft extraction process depends on the formation of sodium hydroxide from sodium sulfide.[35] Sodium sulfide is obtained by heating sodium sulfate. In the papermaking process, sodium sulfide degrades to form sodium hydroxide, NaOH, and H_2S.[36] The mixture works on plant material at a temperature of 175°C and a pressure of about 5 bar for 2 to 5 hours.[39] Lignin and hemicellulose are subsequently released in the reaction medium, and various sulfur compounds present with the lignin interact to produce soluble thiolignin in the medium. Lignin is then obtained by precipitation in the presence of concentrated organic acids.[35,37] It should be noted that the Kraft process is one of the most widely used methods for extracting cellulose from different types of wood. It usually produces thick stock for printing paper and related products. Lignin Kraft paper obtained through the sulfate cooking process and the extracted lignin accounts for about 80% of the total global lignin production.[38]

2.2.2. The Soda-Anthraquinone Process

Another critical industrial procedure used in lignin production is the soda lignin process, which uses either soda treatment or soda-anthraquinone treatment.[40] This soda-based cooking method is mainly used in the treatment of annual crops and hardwoods.[41] The main difference compared to the Kraft process is the sulfur-free medium of cooking liquid. The use of anthraquinone as a catalyst provides an additional method for alkaline extraction of lignin while protecting cellulose (Figure 2.3).[42]

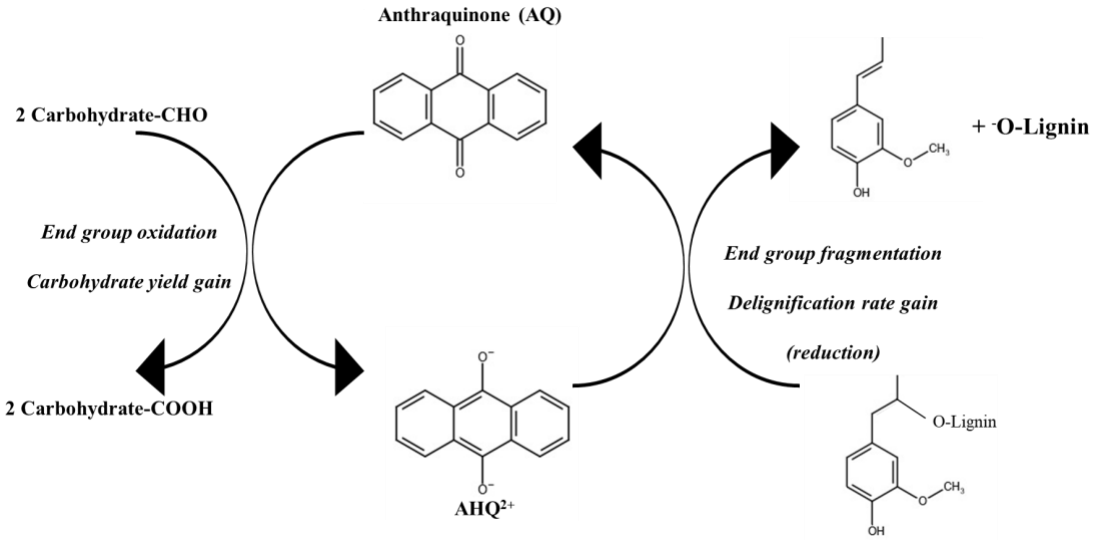


Figure 2.3. Redox cycle of anthraquinone in alkaline lignin extraction.

2.2.3. The Björkman Ball Milling Process

In this review, the so-called milled wood lignins (MWL) have been classified as PMLs.[43] Björkman proposed the original MWL extraction method, which is the most common procedure for isolating lignins from wood.[44] The wood or plant samples are first milled in either a ball mill or knife mill in toluene for 150 h at high temperatures.[45] Lignin is then extracted with a dioxane/water mixture (9:1, v/v), followed by evaporation of the solvent and a subsequent purification.[46] The yields obtained for the MWL method are usually relatively low (20-30%) compared to the rather high yields reported by Björkman.[47]

It is evident from the literature that MWL extraction has been extensively used for the elucidation of the native lignin structure. Lignin researchers consider that the MWL method

is the method of choice that gives the best specimen to analyze. They also believe that these extracted lignins are "less structurally modified" by the mechanical ball milling procedure.[43] Still, concerns exist over the similarity between MWL extracted and native lignin due to structural alterations occurring during the ball-milling.[44,45] These structural changes include increases in the carbonyl and phenolic hydroxyl contents, as well as decreases in molecular weight and cleavage of aryl ether linkages.[45] Recent studies show that phenolic β-O-4' structures in lignin increase continuously at the expense of etherified β-O-4' structures.[43,45,47]

2.3. Extraction of Lignin from Biomass in an Acidic Medium

Ester and ether linkages present in the extracted lignin can be hydrolyzed in an acid medium.[48] Similar to alkaline hydrolysis, the cleavage of the ether bond can be carried out at a high temperature and high pressure in an acidic medium too. This acid hydrolysis is initiated by the protonation of the oxygen atom of the C-O bond, which makes it susceptible and easily broken by the action of water.[49] The following scheme exemplifies this type of bond cleavage in the lignin-polysaccharide biomass (Figure 2.4).

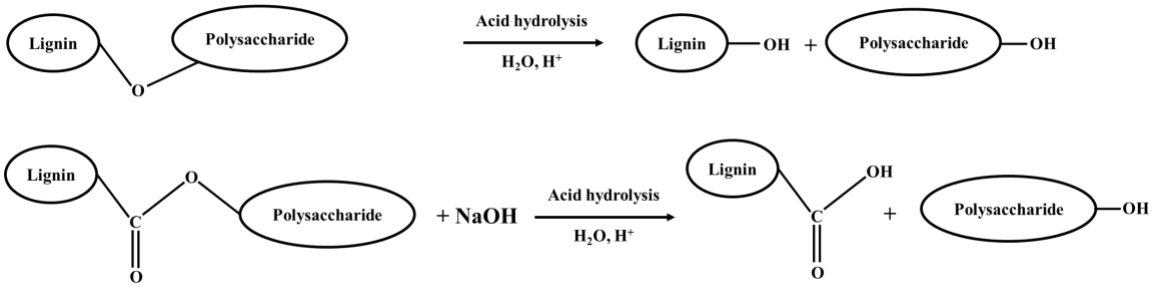


Figure 2.4. Cleavage of ester and ether bonds between lignin and polysaccharides in an acid medium.

2.3.1. The Sulphite Process

Besides the Kraft Pulping process, another important industrial extraction process is the sulfite process. This method is currently more in use than the Kraft pulping process. The sulfite process is initiated by the treatment of the wood with sulfur dioxide and hydrogen sulfite. The technology is based on the oxidation of sulfur-to-sulfur dioxide, which is then directly hydrolyzed to produce sulfuric acid.[42] This process enhances the attachment of sulfonic acid groups to the lignin backbone, which is also why sulfite lignins (called lignosulfonates) are water-soluble.[50] These specific side reactions can be controlled by modifying the pH levels. Plant biomass is introduced in the reaction mixture under high pressure at 120°C to 160°C for several hours. Cleavage of ether and ester bonds releases lignin, but since it decomposes simultaneously with bisulfite ion (HSO_3^-), it results in lignosulfonate formation.[50,51]

2.3.2. The Steam Explosion Process

The steam explosion process, also called self-hydrolysis, includes heating an acid-saturated plant material (H_2SO_4 or SO_2) between 160°C and 260°C by adding saturated high-pressure steam (10 to 50°C). This continues for a few seconds to a few minutes, followed by a sudden relaxation due to the atmospheric pressure.[52,53] As a result of this explosion and return to atmospheric pressure, hydrolysis of hemicellulose and extensive destruction of cellulose take place. Thereafter, lignin dissolves when processing cellulose fibers in the presence of sodium hydroxide. Finally, precipitation of lignin is achieved in an acidic environment, after which the precipitate is filtered and dried.[50]

2.4. Extraction of Virgin Released Lignins (VRLs) by Solvolysis

Several new methods for the large-scale laboratory extraction of lignin have been described. These can be divided into two major methods. The first is used to produce cellulose solely and considers lignins as non-desired by-products.[54] The second extraction process is used to recover lignins. The second process depends mainly on the mechanical treatment of the vegetal raw material.[55] Following the mechanical destruction of the biomass, the raw material is extracted by solvolysis. It is important to understand that when lignin oligomers are released from the biomass without any further treatments or modifications, the lignin extract has chemical structures very similar to those present in the native biomass. Moreover, solvolysis is one of the best separation techniques to produce both pure lignin and cellulose. For these reasons, solvolysis is becoming the most preferred method to obtain VRLs. In addition, this method allows the use of a wide choice of organic

solvents/water combinations.[51] The solvolysis extraction method depends on the solubilization of lignin biomass in an organic solvent. The solvolysis extraction method can proceed in either basic or acidic medium to cleave both ether and ester bonds lignin-polysaccharide linkages.[56] The main purpose of solvolysis is to solubilize the maximum amount of lignin in an organic solvent without modifying or fragmenting it. The following are some examples of the solvolysis method in organic solvents, which are usually combined with water molecules.

2.4.1. The Methanol Solvolysis Process

Methanol extraction involves cooking of the plant material in methanol (50%) in the presence of sodium hydroxide (30%) and anthraquinone (<1%) at 160°C under high pressure for 2 hours. After acidification and subsequent evaporation of the liquor, a precipitate of low molecular weight lignin is formed along with recovered methanol.[57]

2.4.2. The Pepper Method: Lignin Extraction with Dioxane

Pepper lignin is obtained by extraction with 1,4-dioxane (90%) containing hydrochloric acid (0.2M) at 90°C for 10 h. The lignin fraction is obtained by precipitating with ethyl oil in the extraction liquid. This extraction method causes lignin to become partly fragmented and partly recombinant, suggesting a structure similar to its natural structure. [59,60]

2.4.3. The Alcell Process: Lignin Extraction with Ethanol

The dissolution of the vegetal biomass takes place in ethanol (40-60%) at approximately 150°C with 1% concentrated acid (HCl, H_2S0_4) and a pressure of 3 MPa. After the solvents evaporate, lignin settles by acidifying the black liquid (Figure 2.6).[58] The lignin oligomers obtained usually possess low molecular weight, and their solubility depends on the concentration of ethanol used.

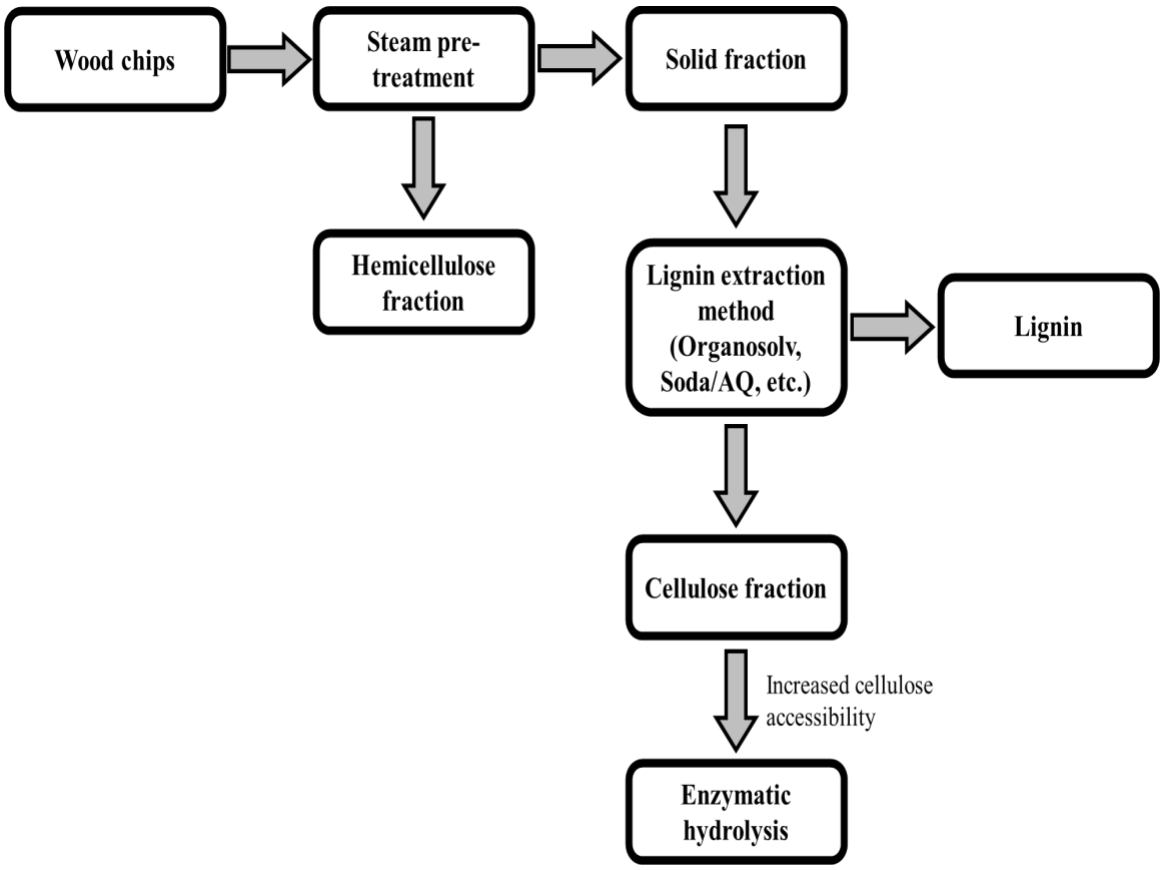


Figure 2.5. Extraction of lignin in aqueous ethanol acid.

2.4.4. The Milox Method: Lignin Extraction with Formic Acid Containing Performic Acid

This method of solvolysis extraction involves the use of formic acid containing peroxyformic acid. This extraction proceeds in two stages. [23,62] The first stage consists of the production of the calculated amount of peroxyformic acid by reacting formic acid with hydrogen peroxide, which is followed by the second step of biomass extraction. This combination of performic/formic acid is strong enough to break the bonds between lignin and polysaccharides. Using such a procedure permits the separation of lignin at atmospheric pressure at a temperature of about 107°C in 4 to 5 hours. Following filtration of the precipitated cellulose, the addition of water to the filtrate allows recovery of the precipitated lignin. The reaction conditions used in the Milox process promote the esterification of hydroxyl groups and the cleavage of β-O-4 bonds of lignin acrylate.[63]

2.4.5. The Acetosolv and Acetocell Processes: Lignin Extraction with Acetic acid

The acetosolv process depends on the extraction of lignin biomass by destroying the vegetal material in acetic acid (90%) containing 0.1% hydrochloric acid. [24.64] Similar to the Milox procedure described above, this process produces peroxyacetic acid in the medium. The total reaction time is 5 hours at about 80°C at atmospheric pressure. Lignin oligomeric mixture is recovered by precipitating in water after filtration. The main advantage of this solvolysis method is the recycling of acetic acid by distillation. Moreover, the Milox method is associated with a low rate of recombination and a high level of free hydroxyl

groups. [64,65] The acetocell counterpart of the acetosolv differs from the original in that it uses a lower amount of acetic acid (85%) without the use of a catalyst.[66]

2.4.6. The Formacell and the CIMV Processes; Lignin Extraction with Formic Acid and Acetic Acid Mixture

The Formacell process is important because the lignin extracted is more in quantity comparing with Milox, Acetsolv, and Acetocell processes, all of which partially follow the same principles but with much milder temperature and pressure conditions. This process uses a mixture of acetic acid / formic acid/water in various ratios up to 190°C under high pressure.[67,68] It releases a significant amount of lignin from the biomass.

Meanwhile, in the CIMV process, lignin is extracted using a mixture of formic acid, acetic acid, and water (30/55/15 v/v/v) for about 3 hours at atmospheric pressure and a temperature of 105-110°C.[68] The final step consists of filtering the hydrolysis mixture to obtain the precipitated cellulose fibers. In turn, the extraction liquor is diluted with water to enhance the solubilization of the hemicelluloses and precipitate the lignin, which is then separated by filtration.[69] CIMV-derived lignin has reactive properties similar to polyphenols. In this CIMV process, acetic acid is used as a solvent to remove the lignin and hemicelluloses part of the biomass. Meanwhile, formic acid is the chemical reagent used as a catalyst to break the ether and ester linkages of lignin-bound polysaccharides. It should be noted that in the CIMV process, the solvents are recycled by evaporation under reduced pressure (by distillation under a pressure of 8 bar). The CIMV organosolv pulping process has high efficiency. For example, CIMV extraction, when applied on wheat straw, allows

the recovery of more than 90% of the original lignin.[68] The two described solvolysis processes can be used at an industrial scale to provide both paper pulp and unmodified VRLs.

2.4.7. Extraction of VRLs Using Softer Sequential and Progressive Pulping Processes

VRL non-modified lignin can also be extracted from the vegetal biomass by a series of sequential steps. For example, barley straw lignin can be obtained by several successive steps using dioxane, acidic dioxane, dimethyl sulfoxide, and aqueous 8% NaOH.[70] Similarly, bamboo lignin can be extracted from the biomass by using a gradient of ethanol/aqueous alkaline solution mixture.[71]

2.4.8. Microwave-Assisted Extraction of Lignin

Lignin oligomers can also be obtained by a combined microwave-assisted extraction/degradation process.[72] This process has many advantages over conventional heating technologies, such as accelerated chemical reactions resulting in shorter reaction times under mild non-catalytic conditions.[73] This procedure is performed in a microwave oven at 800 W in a Teflon microwave bottle containing the biomass and 20 mL of solvent (MeOH or EtOH). The flasks are closed, and the mixture is heated under constant stirring for 20 minutes at a different temperature ranging from room temperature to 80, 120, or 160°C. Increased pressure is observed inside the closed flask due to the high processing temperatures above the boiling point of both solvents.[73,74]

2.4.9. Ultrasound-Enhanced Extraction

The ultrasound-enhanced extraction method is a more efficient way of extracting lignin than traditional extraction methods.[75] This method uses sodium hydroxide solution as a solvent. Its efficiency depends on several factors, such as extraction temperature and solvent/biomass concentration. Studies have shown that the extraction of lignin from palm oil using the ultrasound-assisted method was more efficient than the classic methods of lignin extraction. Furthermore, the extraction reaction temperature can be reduced, and still, lignin can be extracted. With all these advantages, ultrasound-assisted extraction is preferred for a broad range of lignin extraction applications.[76]

2.4.10. Extraction of Lignin Using Ionic Liquids

Extraction of lignin with ionic liquids (ILs) has been explored as a promising green method of lignin extraction from lignocellulosic biomass.[77] ILs, designated as 'green solvents', are liquids that possess strong electrostatic forces between constituent ions, which leads to low evaporation and flammability and high chemical and electrochemical stability. However, the solubility of lignin in ILs is mainly affected by the nature of the constituent anions. Particularly, ILs contain large, non-coordinating anions, which of course, are not suitable solvents for lignin dissolution. Hart et al. showed that while ionic bond strength was not a critical factor, the dissolution of lignins in ILs requires a threshold level of hydrogen bonding.[78]

2.5. General Methods to Determine the Molecular Mass of VRL Extracted Lignin

Having studied the different methods of lignin extraction, we will now focus on the structural analysis methods used for lignin. A major shortcoming of the structural investigation of extracted lignins is that analytical measurements such as density, crystallinity, and molecular mass are performed on heterogeneous mixtures that are not pure. Consequently, all published analytical measurements are always interpreted by deduction and are approximations.[75] The following sections present some molecular weight determination methods as gel permeation chromatography (GPC), light scattering, vapor pressure osmometry and ultrafiltration. [76,77]

2.5.1. Gel Permeation chromatography (GPC)

Gel permeation chromatography (GPC) is the most commonly used method for determining lignin molecular weights. This method involves passing the lignin mixture through a GPC column packed with a gel. Cross-linked polydextran gels with varying pore sizes are employed as the stationary phase in these columns.[78] It is also possible to perform GPC in high-pressure systems using cross-linked rigid polystyrene gels. These allow for a reduction in the time of analysis and can considerably enhance the resolution when compared to normal GPC.[79]

Although GPC has been widely used in biochemistry for the separation of complex mixtures of biomolecules, it is not a purification tool from an organic chemistry

perspective. GPC gives broad-shaped peaks, in which lignin is a mixture of series of oligomers with varying molecular weights, which indicates a distribution of molecular weights. However, detecting the absolute molar mass of lignins by GPC is a challenging task as lignins have a strong tendency to agglomerate in solution. This is especially true in the case of lignins extracted using industrial Kraft, soda-anthraquinone, and sulfite methods which favor polymerization of the extracted lignins, leading to high molecular mass materials which differ substantially from native lignins.[77] Moreover, the interpretation of GPC data is based on comparison with calibration standards, which can be linear polystyrene standards. However, it has been shown that the characterization of molecular mass distribution by GPC and MALDI-MS are not comparable.[80] Furthermore, it has been shown that weight average molecular weights (Mw) and number average molecular weights (Mn) for wheat, hemp, and flax lignins are hardly comparable.[81] In general, mass spectrometric techniques are preferred over GPC because in these methods, the actual weight of the lignins is measured instead of the hydrodynamic radius of the molecules.

2.5.2. Vapour Pressure Osmometry

Vapour pressure osmometry (VPO) is a widely-used technique to determine number-average molecular masses lying between 100 u to 10,000 u. However, the VPO method loses its sensitivity at higher masses.[75] This method relies on the measurement of the decrease in the vapor pressure of the solvent at a given temperature in dilute polymer solutions. A substance of known molecular mass is used to calibrate the instrument used for VPO. It should be highlighted that VPO is the method of choice to calculate the number-average molecular weights of lignins and lignin products.[82]

2.5.3. Light Scattering

Low-angle laser light-scattering photometers are used to determine the average molecular mass of soluble lignins.[83] However, these measurements may be compromised if aggregates are present in the solution, or if the fluorescence or absorbance of lignin in the solution is variable.

2.5.4. Ultrafiltration

Another method used to determine the molecular size distribution of lignin samples is based on membrane-based separation dependent on molecular size. Ultrafiltration can be used to measure a broad range of molecular sizes ranging from 1000 to 300,000 u. This method depends on the filtration of the lignin solution through a series of membranes. The obtained fraction yields are calculated by either weighing the separate lignin fraction or by ultraviolet light absorption.[84] The main feature of this method is that it is not sensitive to impurities. It has been reported that lignins usually permeate through the ultrafiltration membranes without being obstructed by inorganic salts or sugars.

2.6. Chemical Degradation and MS Detection of Lignins

One of the main problems with structural elucidation of lignin structures is that all studies depend on the analysis of smaller fragments obtained by degradation. This means that an extensive structural analysis requires the use of more than one degradation method. For these reasons, various degradation methods have been developed, including acidolysis, permanganate oxidation, nitrobenzene oxidation, ozonolysis and hydrogenolysis.[85]

However, all of the following techniques only release a fraction of the polymer for analysis, as will be discussed in the sections below.

2.6.1. Acidolysis and Thioacidolysis Degradation Methods

Acid-catalyzed degradation usually affects the most important ether bonds in lignins, namely the diacylglycerol-β-aryl ethers. This degradation causes the selective cleavage of diacylglycerol-β-ethers and other labile ether linkages. Often, the resulting monomeric and dimeric acidolysis products are derivatized by silylation and are analyzed by gas chromatography. Furthermore, when present, the labile benzylic (and non-benzylic) aryl-ether linkages are cleaved to give a mixture of ketonic monomers, also called Hibbert ketones. These Hibbert ketones are diagnostic of the presence of some types of lignins (Figure 2.6).[86]

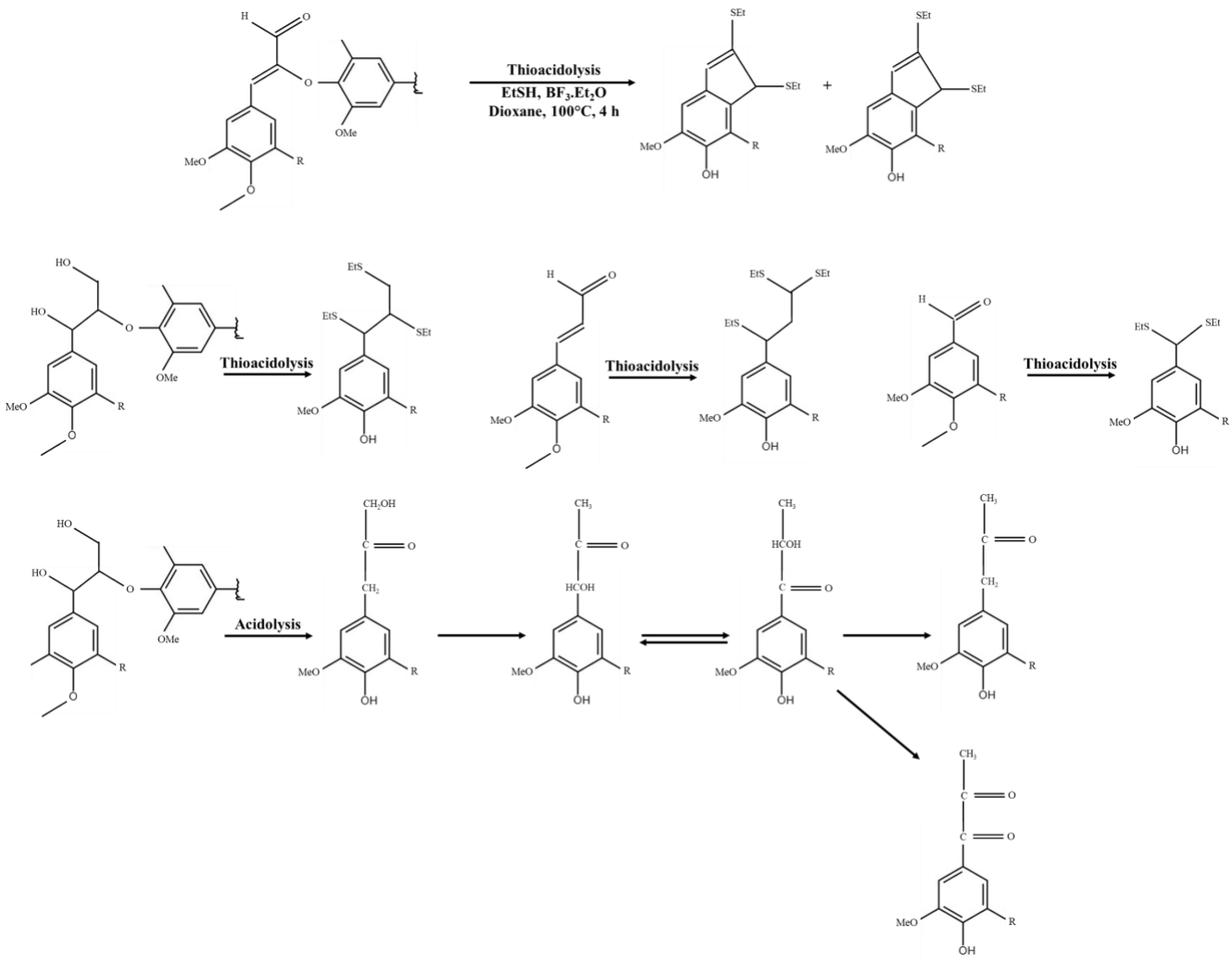


Figure 2.6. Major products resulting from acidolysis and thioacidolysis.

The essential elements detected by acidolysis studies are the following: β-O-4', β-5', β-β,' β-1', 2-aryloxypropiophenone, cinnamic acid, cinnamaldehyde, glyceraldehyde-2-aryl ether, benzoic acid, benzaldehyde, and quinoid types. After sialylation, gas chromatography analysis is performed on the monomeric and dimeric acidolysates.[87]

Acidolysis has now largely been replaced by thioacidolysis (BF_3/C_2H_5SH) treatments which permit the conversion of uncondensed monomers into simple diastereomeric mixtures of 1,2,3-trithioethane phenylpropanoid monomers.[87] In addition, if the lignin samples are originally methylated, this method serves to estimate the number of alkyl-aryl ether bonds in lignin relative to that of free phenolic groups. Furthermore, after the removal of sulfur by reduction with Raney-nickel, the dimeric products can be analyzed by gas chromatography/electron impact mass spectrometry (GC/EI-MS).[88] It is generally accepted by lignin chemists that thioacidolysis is one of the best methods of acid-catalyzed reaction for depolymerization of lignins.[89,87]

The mechanism of thioacidolysis proceeds by cleavage of the arylglycerol-β-aryl ether linkages without any undesired condensation reactions forming new C-C' linkages.[86,88] It has been found that the yields of thioacidolysis products are superior to those obtained with acidolysis. Moreover, the major carbon-carbon bonds existing in these products are related to the original β-5', β- β' and β-1' inter-unit linkages.[87]

2.6.2. Lignin Degradation by Permanganate Oxidation

Lignin degradation can also be achieved by permanganate oxidation of lignin side chains, which degrade into carboxyl groups. As a result of this oxidation, the obtained products usually expose the pattern of substitution on the aromatic rings. It is important to realize that before oxidation, the sample must be alkylated with dimethyl or diethyl sulfate.[90] This derivatization is essential to shield the phenolic aromatic rings from degradation. The products obtained are a mixture of acids that need to be esterified and analyzed by gas

chromatography. The structural information obtained from permanganate oxidation is mostly qualitative, as it generally gives low yields. [91] As always, one of the main drawbacks of this method is that the information obtained accounts only for a part of the total lignin structure.

2.6.3. Degradation of Lignin by Alkaline Nitrobenzene and Cupric Oxide Oxidation

Freudenberg developed the lignin alkaline nitrobenzene oxidation method, which is used to cleave oxidized phenolic aldehydes. This oxidation method can indicate the nominal quantities and relative amounts of the uncondensed phenylpropane monomeric moieties present (p-hydroxyphenyl, guaiacyl, and syringyl units).[92] The oxidation mixture constituents can be determined by quantitatively and qualitatively employing HPLC, GC, and GC-EI-MS.[93]

Another important method of degradation is the alkaline cupric oxide (CuO) oxidation technique, which usually cleaves a wide variety of ether and carbon bonds to release oxidized lignols from the lignin complex polymer. CuO oxidation is another method used for the characterization of lignins in dissolved organic matter, soil, and sediment samples.[94] This oxidation method comprised a solid-phase extraction (SPE) clean-up procedure followed by GC-EI-MS quantification of the obtained monomeric lignin phenols. After the SPE clean-up, the sample is derivatized with a combination of BSTFA (N, O-bis(trimethylsilyl) trifluoroacetamide) and TMCS (trimethylchlorosilane). The method detection limits (MDL) of individual lignin phenols were obtained by spiking low levels (~1 nmol) of standard mixtures into preloaded reaction vessels (Table 2.1).

Table 2.1. Symbols, retention times, characteristic mass fragments, and limits of detection for CuO oxidation products. Reproduced with permission from *Analytical Chemistry*, *84*(1), 459-464.[94]

[a]Retention times are relative to CiAD on an Agilent DB-5 column.

[b]Underlined ions are used for selected ion monitoring (SIM) quantification. Italicized ions were used to assess peak purity.

[c]Determined by replicate analysis (n = 7) of a standard spike (~1 nmol) into 2 mol/L NaOH, 330 mg of CuO, 10 mg of glucose, and 106 mg of $[Fe(NH_4)_2,6H_2O]$ and analyzed as described in the method. By multiplying with the sample standard deviation, MDL was calculated.

Compound	**Symbol**	**RRT_{DB5} [a]**	**Major ions (m/z)[b]**	**MDL (mol/µL)[c]**
Cinnamic acid	CiAD	1	131,*161*,205,*220*	71
Ethyl-vanillin	EVAL	1.106	167,179,*195*,*238*	132
p-Hydroxybenzaldehyde	PAL	0.672	151,*179*,*194*	57

p-Hydroxyacetophenone	PON	0.856	193,*194*,*208*	58
p-Hydroxybenzoic acid	PAD	1.172	193,*223*,267,*282*	49
Vanillin	VAL	0.986	193,194,*209*,*224*	75
Acetovanillone	VON	1.153	193,*208*,223,*238*	71
Vanillic acid	VAD	1.441	223,267,*282*,297,*312*	104
Syringaldehyde	SAL	1.315	224,*239*,*254*	103
Acetosyringone	SON	1.448	223,238,253,*268*	113
Syringic acid	SAD	1.692	253,297,*312*,327,*342*	50
3,5-Dihydroxy-benzoic acid	DiOHBA	1.558	28,*311*,355,370	184

p-Coumaric acid	CAD	1.749	*219*,249,293,*308*	
Ferulic acid	FAD	2.014	*249*,293,308,*323*,338	

CuO oxidation methods give degradation products very similar to the nitrobenzene method. Indeed, both reactions are carried out in alkaline solutions at high temperatures.[93] However, nitrobenzene oxidation possesses an advantage over the cupric oxide procedure as it forms fewer degradation products. On the other hand, the main disadvantage of the nitrobenzene oxidation method is that it gives numerous reduction products that need to be removed before a qualitative and quantitative determination can be carried out. In general, the relatively mild CuO oxidation conditions cleave the β-aryl ether bonds of lignins without altering the propyl side-chain of the lignin moiety.[95]

2.6.4. Degradation of Lignin by Ozonolysis

Ozone reacts with double bonds as well as aromatic rings to generate ozonides. These, when subjected to hydrolysis or reduction, induce the liberation of the cleaved products. Lignin ozonolysis converts the aromatic ring originally attached to the side chains into carboxylic acid groups. However, if the aromatic rings were attached to the side chains via an ether linkage, they will be replaced by hydroxyl groups.[96,97] Consequently, the

extensive ozonation of lignins destroys the aromatic moieties while leaving the side chains intact, which can be recovered as monocarboxylic acids and dicarboxylic acids.[98]

Notably, ozonolysis of lignin works in a manner opposite to permanganate oxidation-based degradation of lignin. This means that permanganate degrades the lignin side chains, while ozonolysis degrades the aromatic rings and the double bonds without affecting the side chains which remain intact as carboxylic acid groups.[96,97] In contrast, during the nitrobenzene oxidation, the aromatic moieties are left intact, and the side chain is shortened to either an aldehyde or a carboxylic acid group. Unfortunately, ozonolysis fails to provide data about the relative frequencies of the various lignin side-chains. As in other methods discussed earlier, product identification is completed by GC-EI-MS analysis.[99,97,100] Overall, the main interest in ozonolysis oxidation arises from its advantage that the stereochemical configuration of the carbon side chains of the lignins is retained. Accordingly, the erythro form of guaiacylglycerol-β-aryl ethers yields the erythronic acid, and threonic acid is derived from the corresponding threo isomer.[96]

2.6.5. Degradation of Lignins by Derivatization Followed by Reductive Cleavage (DFRC)

One of the most popular methods that yield the most information about lignin structure is the thioacidolysis method (discussed earlier), which is followed by the derivatization followed by the reductive cleavage (DFRC) method.[101] DFRC degradation occurs by a clean cleavage mechanism of the β-aryl ether bonds. DFRC is initiated by the derivatization of lignins with acetyl bromide, followed by reductive cleavage of the resulting benzyl

bromides with zinc dust.[102] Following treatment with thioacidolysis, the monomers, which are β-O-4'ether-linked with other H-, G-, and S-units, are released. The obtained fragments can be quantified by GC-EI-MS.[103] This sequence of reactions cleaves the β-aryl ether bonds, liberating the phenylpropane units, which are analyzed as cinnamyl alcohol derivatives. It should be noted that the DFRC method, besides releasing monomeric products, can also release various dimers and trimers, including all forms of the commonly found inter-unit linkages in softwood lignin except β-O-4', that is cleaved during the degradation reaction (β-1', β-β,' β-5', and β-O-4').[104]

2.6.6. Identification of Lignin fragments by Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS)

Pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) is another crucial method used to characterize polymers. In this method, pyrolysis thermally degrades polymers into small fragments that are subsequently separated using gas chromatography and identified using MS.[105]

In Py-GC-MS, the sample is first fragmented at a high temperature in an inert gas atmosphere. Subsequently, a gas chromatographic column is used to separate the fragments. Lastly, an attached mas spectrometer that uses an electron impact ionization source helps identify the fragments. This destructive analysis method can work with a small sample amount, without the need for a separate isolation step. However, this method is only useful for the identification of p-hydroxyphenyl, guaiacyl, and syringyl structures and a few other characteristic structures of lignin polymers.[106]

Degradation of lignin mixture by pyrolysis, which is known to cleave ether and certain C–C' interunit linkages, usually releases a mixture of phenols of relatively simple chemical structures.[105,107] However, the released methoxylated phenol monomers maintain their original substitution patterns, which allows the identification of H, G, and S lignin unit components. These obtained lignin units have propanoic side chains that have either been cleaved completely, shortened to one or two carbon chains, or fully conserved. Moreover, pyrolytic dehydrogenation can result in the formation of new double bonds in the side chains. Overall, pyrolysis degradation of lignin is a useful analytical technique as well as a method that can thermally and chemically transform lignin into high-value base aromatic chemicals.[105]

2.6.7. Thermochemolysis of Lignins with Tetramethylammonium Hydroxide (TMAH) Combined with MS

Recently, it was shown that the co-injection of tetramethylammonium hydroxide (TMAH) during pyrolysis could provide more structural information than conventional pyrolysis.[107,109] TMAH derivatization protects the thermolabile compounds and makes it possible to simultaneously separate both polar and non-polar target compounds chromatographically. Additionally, pyrolysis in the presence of TMAH helps to break the polar bonds and methylate the -COOH and -OH groups on lignins.[107]

After TMAH treatment, the hydroxyl group after methylation of the polar products released becomes more amenable to GC-EI-MS analysis. Furthermore, the TMAH technique circumvents any decarboxylation of the polar moieties and produces phenolic derivatives,

which are not observed during conventional pyrolysis.[108,109] Another interesting observation on the TMAH/pyrolysis method is the effective cleavage of lignin β-O-4' bonds. This means that during pyrolysis in the presence of TMAH, the propyl-aryl ether bonds cleavage occurs and liberates the hydroxyl groups located on the alkyl side chains and aromatic rings, which are then methylated.[108]

Therefore, the TMAH/pyrolysis method is useful for the characterization of the three different lignin types extracted from gymnosperm wood, angiosperm wood, and nonwoody angiosperms plant materials. However, a pitfall of TMAH thermochemolysis has been noted with respect to the softwood lignins containing the cinnamyl end groups.[110] TMAH thermochemolysis is not able to provide the required information on the cinnamyl alcohol end groups of lignins. This contradiction is due to the shortage of the *in situ* formed cinnamyl aldehyde end groups that cannot be converted into the expected cinnamyl aldehyde methyl ethers.

2.7. ^{1}H- and ^{13}C-Fourier Transform Nuclear Magnetic Resonance studies

More rapid and direct detection of all lignin functional groups can be obtained by conducting ^{1}H- and ^{13}C-Fourier Transform Nuclear Magnetic Resonance (FT-NMR) of the whole lignin structure. The benefit of FT-NMR spectroscopy over other spectroscopic techniques, such as infrared (IR), ultraviolet-visible (UV), and Raman spectroscopy, is that it has a much higher resolution, enabling a larger amount of information to be obtained without destruction of the analyte.[111,112] The application of quantitative ^{13}C-FT-NMR for lignin analysis has been an important milestone in lignin chemistry.

2.7.1. 1D-FT-NMR studies for lignin analysis

While lignin mixtures are usually insoluble, some portion of the lignin mixture can be solubilized, and as a result, ^{1}H- and ^{13}C-FT-NMR have been extensively used to characterize the soluble parts of lignins.[113] At the same time, solid-state ^{13}C-FT-NMR of the complete mixture definitely provides quantitative information on some of the key structural features of lignins in plant tissues and isolated lignin samples.[114] It is important to mention that the interpretation of the ^{13}C-FT-NMR spectra rests on the availability of lignin model compounds. This means that for the structural elucidation of lignin mixtures containing intricate structures, the NMR studies are done solely by comparison with known standards. Therefore, even though NMR spectroscopy allows the non-destructive characterization of the lignin macromolecule, this technique is hindered by the difficulty of making unambiguous assignments for each signal, which may be overlapping carbohydrate resonances. As well, NMR spectroscopy does not allow molecular weight determination.

2.7.2. 2D-^{1}H-^{13}C-FT-NMR Studies of Lignins

In the last few decades, a quantitative two-dimensional (2D)-FT-NMR approach has been developed and used for investigating lignin structure.[115,116] Quantitative ^{13}C-NMR is still one of the most used spectroscopic methods of lignin characterization since it is very informative, reliable, and, at the same time, feasible. Quantification of lignin functional groups by using the distortionless enhancement by polarization transfer (DEPT) NMR method is a very complicated approach that requires tedious work to obtain experimental conditions that enable the technique to be quantitative.[117] Meanwhile, 2D experiments,

such as ^{13}C-^{1}H-correlated heteronuclear single quantum correlation (HSQC) and heteronuclear multiple quantum correlation (HMQC) spectroscopies, bring together the higher resolution of ^{13}C-NMR and the sensitivity of ^{1}H-NMR. However, the HMQC technique will not allow the detection of a quaternary carbon.

Nevertheless, these 2D experiments continue to be the ideal technique for revealing the frequencies of the different lignin units and the inter-unit bonding patterns. In addition, HSQC experiments help identify those structural features of lignin which are not generally identified by other structure analysis methods.[115,117]

While NMR is the preferred method for many in lignin research, it is still a low-sensitivity method, which is suitable only for purified compounds. However, since the extracted lignins or cell walls are a complex mixture of various components, NMR analysis cannot properly determine the way in which different units are connected to one another. Nevertheless, NMR analysis is still useful for identifying the frequencies of the various lignin components and bond types. Structural elucidation and authentication are solely based on a comparison of diagnostic resonance signals with chemically synthesized standards.

2.7.3. ^{31}P-FT-NMR Spectroscopy Technique for Lignin Characterization

The ^{31}P-FT-NMR spectroscopy technique to characterize lignins involves the selective phosphorous-tagging of a variety of functional groups present in lignin, followed by a ^{31}P-FT-NMR experiment. The selectivity of this technique permits the analysis of isolated or *in situ* lignins.[118] However, the sensitivity of a ^{31}P-FT-NMR experiment is about 15 times

less than that of a ^{1}H- NMR experiment. Still, the range of ^{31}P chemical shifts (more than 1000 ppm) makes this analysis very powerful for lignin characterization. The first step consists of phosphorylation of all labile protons in lignin samples with 2-chloro-1,3,2-dioxaphospholane (reagent I) or 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (reagent II).[119]

The ^{31}P-FT-NMR analysis of derivatized lignin samples permits the determination of the three principal forms of the lignin phenolic hydroxyl groups (i.e., p-hydroxyphenyl, guaiacyl, and syringyl structures).[119] However, a limiting factor is created by a signal overlap between the syringyl phenolic subunits and substructures containing condensed phenolic groups. This discrepancy limits the distinction and accurate determination of such moieties (Table 2).[120] As such, it should be understood that it is very difficult to establish a global lignin structure using IR and NMR analytical techniques. Yet, the ^{31}P-FT-NMR approach has shown its efficiency for the structural characterization of lignins. This tool has distinct advantages over its carbon and proton counterparts since it allows for rapid quantitative acquisitions of well-defined lignin moieties. This information coupled with that obtained from ^{13}C-FT-NMR data offers a comprehensive, detailed understanding of the complex lignin structures.

Table 2.2. P-NMR signals of various functionalities in lignins after derivatization with reagent I. Reproduced with permission from Taylor and Francis Group, LLC: Boca Raton, FL (Argyropoulos, 2010).

δ (^{31}P) (ppm)	Lignin Functionality
136.5-135.8	OH, group in xylene
136.8-135.2	*Erythro* α-OH in β-*O*-4 of S-units
135.2-135.4	*Erythro* α-OH in β-*O*-4 of G-units
134.5-133.7	*Threo* α-OH in β-*O*-4 of S- and G-units
133.7-133.2	γ-OH in α-carbonyl containing units, cinnamyl alcohols
133.2-132.7	γ-OH in β-*O*-4 units
132.7-132.1	Primary OH (probably phenylcoumaran type)
132.1-131.0	Phenolic OH in S-units
131.6-131.0	Phenolic OH in biphenyl units, cinnamyl aldehydes
130.4-129.7	Phenolic OH in G-units
129.7-129.3	Phenolic OH in G-units and catechol structures
127.1-126.5	COOH groups in aliphatic acids and cinnamic acids

2.8. Soft Ion Mass Spectrometry Analysis

With the arrival of soft mass spectrometric ionization methods such as the Matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS), new concepts began to emerge concerning the structure of lignin. The structure of synthetic lignin obtained from the dehydrogenative enzymatic polymerization has been explored using MALDI-TOF-MS, indicating the presence of a mixture of the following dimeric structures (Figure 2.7).[121]

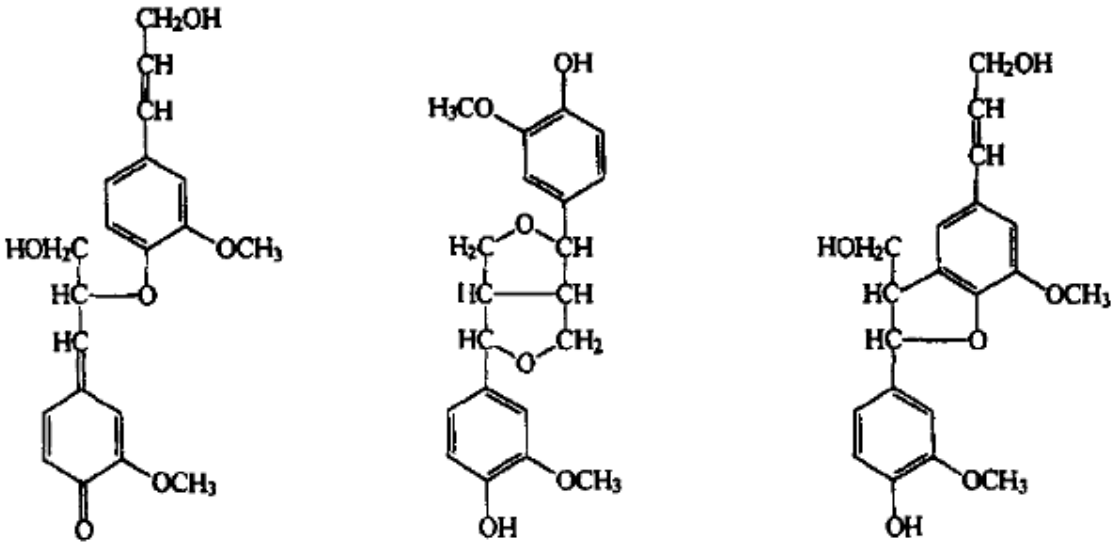


Figure 2.7. Dimer structures obtained from enzymatic dehydrogenation polymerization of coniferyl alcohol. Reproduced with permission from *Rapid Communications in Mass Spectrometry*, 10(10), 1304-1308. (1996).[121]

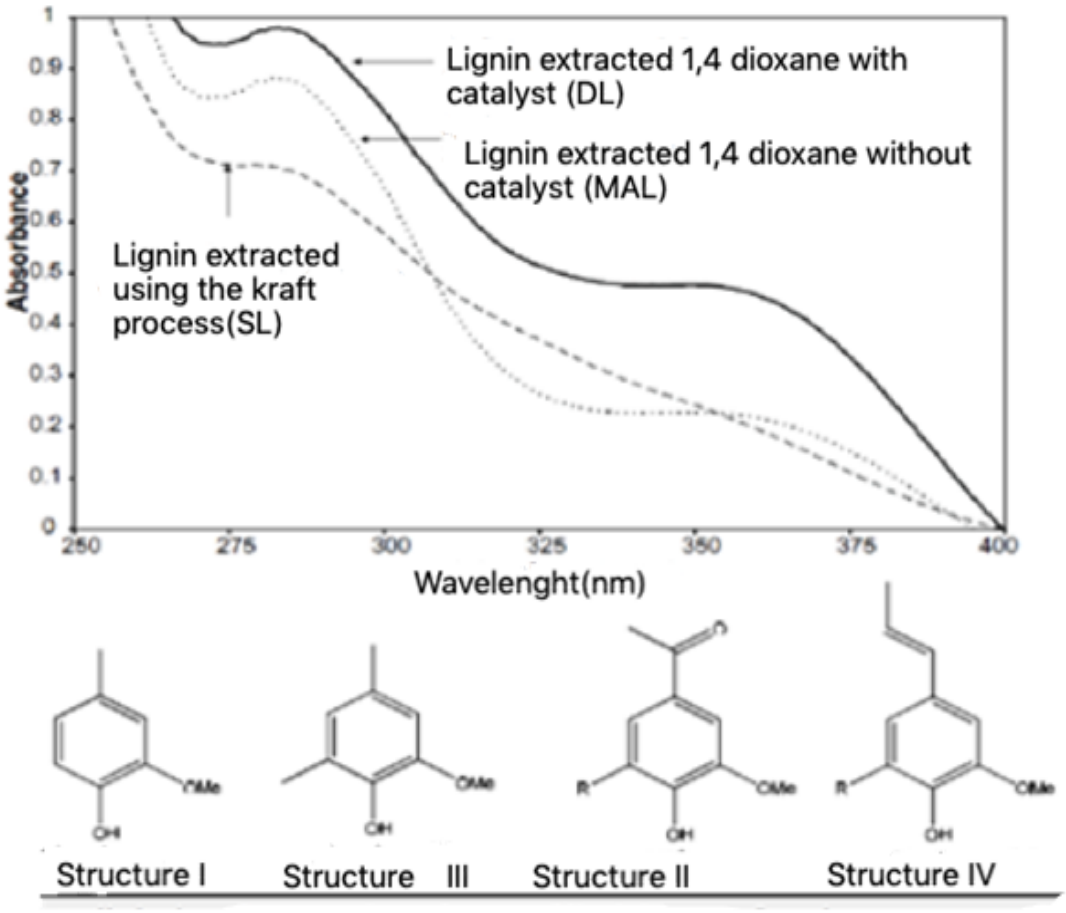
In addition, ESI-MS was conducted on eucalyptus lignins extracted with either 1,4-dioxane or Kraft process. Eucalyptus lignin was found to contain mainly H, G, and S subunits. Another ESI- and atmospheric pressure photoionization mass spectrometry (APPI)-MS study was performed on the wheat straw lignin extracted by the CIMV method. Here, the authors also used low-energy dissociation collisional dissociation (CID-MS/MS) for a structural study.[121,122] The authors were able to establish the presence of a mixture of short linear oligomers.

2.9. Analytical Quantification of Hydroxyl Functional Groups of Lignin

In order to devise a quantification method for diverse functional groups such as aliphatic and phenolic hydroxyl groups, it is important to quantify the hydroxyl and especially phenolic functional groups.[123]

2.9.1. Analytical Quantification of OH Groups by Spectroscopic Methods

A UV spectroscopic study was described to evaluate the phenolic hydroxyl content of different α-lignins and compare the differences in lignin reactivity according to the extraction method used. In this method, the UV absorption at 300 and 350 nm corresponded to unconjugated and conjugated free phenolic units (Figure 2.8).[124,125]



SL=Lignin extracted using kraft process

DL=Dioxane Lignin

MAL= Milled wood alpha Lignin

Figure 2.8. The measurement of the phenolic groups in different lignins using UV spectroscopy. Reproduced with permission from *Holzforschung-International Journal of the Biology, Chemistry, Physics and Technology of Wood*, *37*(3), 143-146.

Phenolic content differed based on the extraction method used to obtain the lignin samples. Kraft lignin has significantly fewer phenolic groups than the lignin extracted with 1,4-dioxane with HCl. It could not be explained in this study why there was a difference in the number of phenolic groups between DL and MAL lignins. It should be noted that MAL is extracted only by solubilization in 1,4-dioxane.

2.9.2. Quantification by Chemical Methods

Quantification of lignin hydroxyl groups can also be performed after undertaking a degradation process or by a chemical transformation of lignin. The quantitative analysis of the total hydroxyl and phenolic lignin groups can be achieved by aminolysis and gas chromatography (GC). Lignin is first acetylated with acetic anhydride in pyridine. This is followed by a quantification method that depends on the determination of acetate functions. Each mole of quantified acetate corresponds to one mole of hydroxyl initially present on the lignin.[125,126]

2.9.3 Total Hydroxyl Quantification of Lignin

The complete analysis of the aliphatic and phenolic hydroxyl groups of the acetylated lignin is performed by a transesterification/saponification reaction to release acetic acid.[127] The released acid is then converted into ammonium acetate and then converted to benzyl acetate (Figure 2.9) which is quantified by GPC.[128]

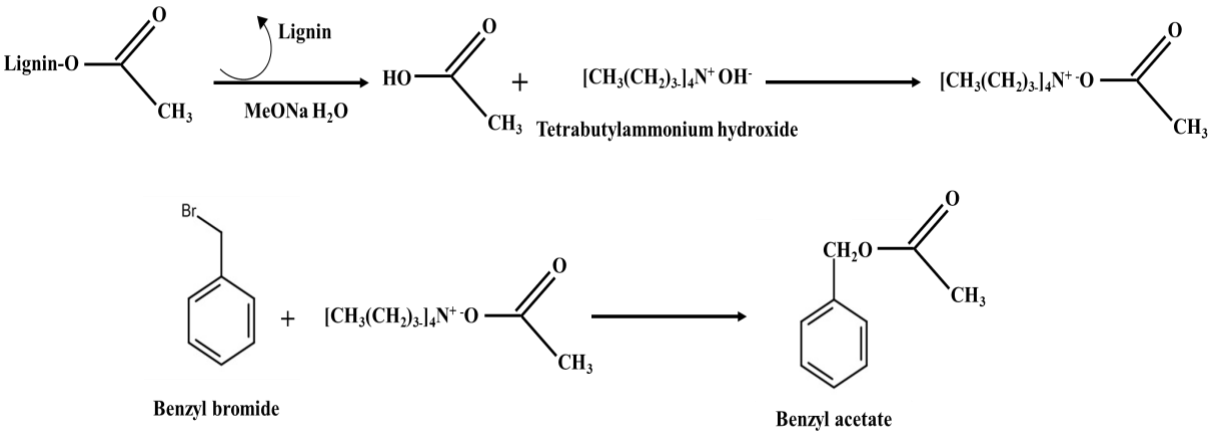


Figure 2.9. Quantification of the lignin phenolic OH groups using acetate.

This method depends on the indirect quantification of phenolic hydroxyls. It depends on the aminolysis of acetylated lignin, which in turn depends on the rate of deacetylation of aromatic acetates in pyrrolidine, which is greater than that for aliphatic acetates.[129] Consequently, the acetylated phenolic groups react with pyrrolidine more rapidly to form 1-acetylpyrrolidine (Figure 2.10).

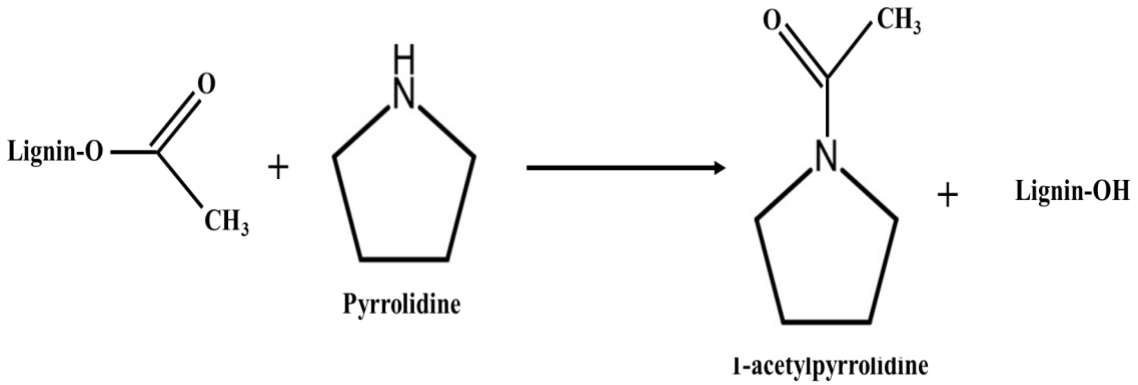
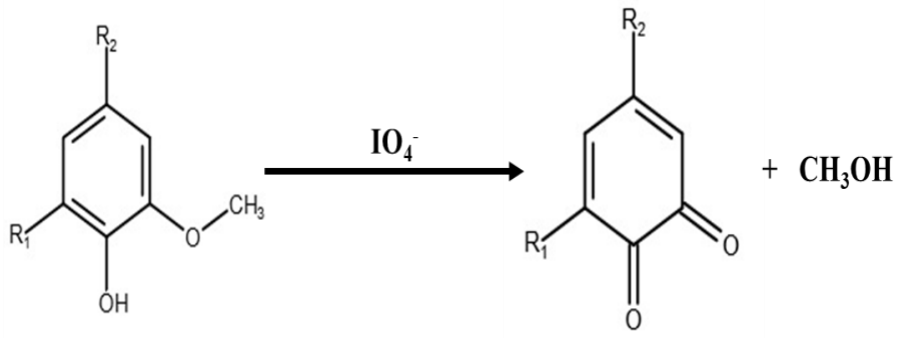


Figure 2.10. Aminolysis of lignin acetylene.

There is another similar method based on the oxidation of guaiacyl and applied to the syringyl residues.[130] This reaction is performed using aqueous sodium periodate. As a result of this oxidation, one molecule of methanol is released, as shown in Figure 2.11.



R_2 = lignin side chain

R_1 = H, OCH_3, H, lignin unit

Figure 2.11. Oxidation of G and S lignin nuclei by sodium periodate.

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Chapter 3: EXTRACTING LIGNIN FROM NEWFOUNDLAND HARDWOODS AND SOFTWOODS USING THE FORMACELL AND BIOEB EXTRACTION METHODS

3.1. Introduction

Wood, one of the most available resources in nature, is structurally quite complex.[1] It consists of three main constituents. The first two are cellulose fibers and hemicelluloses which are complex carbohydrate polymers that are linked together both covalently and non-covalently.[2] The third one, lignin, is a significant chemical constituent of lignocellulosic wood matter and is one of the oldest existing biopolymers in nature. The relative proportions of these three components vary significantly among different wood species.

Cellulose fibers in the lignocellulosic matrix are encompassed by a monolayer of hemicellulose, which in turn lies within a hemicellulose and lignin matrix.[3] Different species of woods, broadly classified as hardwoods (angiosperm trees) and softwoods (gymnosperm trees), contain cellulose, hemicelluloses, lignin, and other extractives in different proportions, as shown in Table 3.1.[4-6]

Table 3.1. Reported chemical composition of wood in tree species.[6]

	Volatile (wt %)	Ash (wt %)	Lignin (wt %)	Cellulose (wt %)	Hemicellulose (wt %)
Softwood	0 - 5	0.5	25 - 35	40 – 45	25 – 28
Pine	0.7	0.5	34.5	40.4	24.9
Hardwood	0 – 5	1	15 - 25	40 – 50	25 – 40
Poplar	1	2.1	25.6	41.3	32.9

Lignin is estimated to account for about 30% of the wood weight. In nature, lignin is particularly important in developing cell walls, especially in wood and bark, because it lends rigidity and does not rot quickly.[7] Lignin also protects against enzymatic attacks by pathogens through its antimicrobial properties.[8] Industrially, lignin is an important raw material in the forestry, agriculture, and bioproduct sectors and shows immense potential in novel value-added industrial applications.[9] Presently, most of the industrially obtained lignin is burnt to produce heat for power.[10] However, value-added applications of lignin, such as in emerging bio-based materials, including composites, adhesives, thermoset resins,

and surfactants, are gaining popularity for commercial uses.[11] These emerging properties make lignin extraction an important commercial avenue.

However, compared to other wood components (cellulose and hemicellulose), lignin is a much more complex polymer.[12] It is formed by a combination of methoxylated derivatives of phenylpropanoid alcohol monomers such as coniferyl, coumaryl, and sinapyl alcohols (or monolignol precursors) (Figure 3.1).[13] The concentration (Figure 3.1) and physicochemical properties (such as functional group abundance and chemical structure) of lignin differ not only between different wood species but also according to extraction methods.[14,15] For example, hardwoods contain two different phenylpropane units (coniferyl alcohol and sinapyl alcohol), whereas softwoods are primarily composed of p-coumaryl and coniferyl alcohol units.[16]

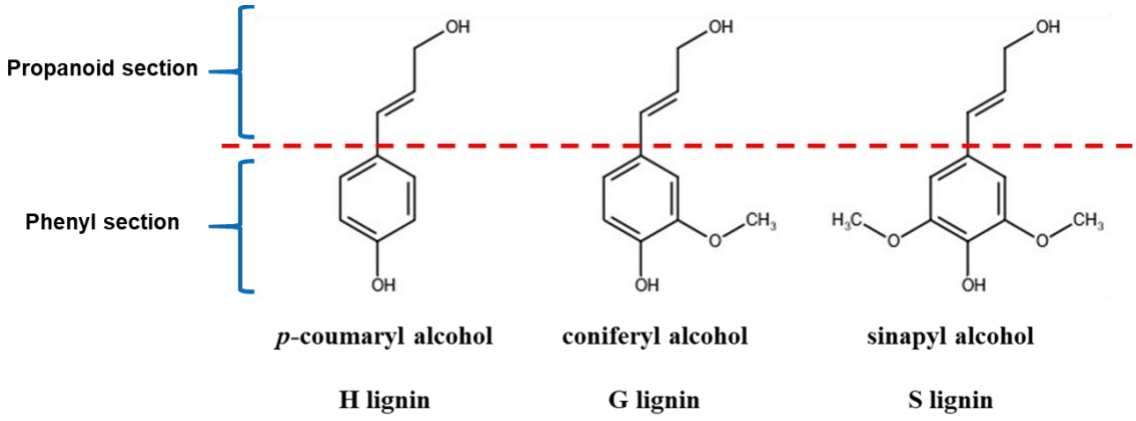


Figure 3.1. Monolignol precursors

In order to separate the different wood components discussed above, wood extractives are commonly dissolved in one or more of the following solvents – alcohols, ether, acetone, water, or organic acids. However, the choice of solvent(s) depends on the kind of wood sample being investigated. In most cases, the solvent works best when it is at a neutral pH. Acidic and alkaline organic compounds generally tend to attack cell wall constituents which is why they are less preferred. Similarly, studies have suggested that an aqueous extraction is best performed with cold water since hot water tends to degrade the cell wall. Ultimately, the extractive is recovered after evaporation of the extraction solution to dryness.

Extraction of lignin from wood vegetal matter involves the formation of a black liquid known as "black liquor" upon cooking of the organic matter.[17] This black liquor contains lignin and other organic constituents of wood that are dissolved in the initially white reaction liquid. Black liquor is characterized by its density, thermal conductivity, surface tension, specific heat, and viscosity. These physical properties of black liquor can be easily determined using various physicochemical features of the waste liquid, including dry solids content and temperature. It is known that with an increase in temperature, the density of black liquor decreases. On the other hand, there is a near-linear correlation between dry solids content and evaporation temperature. Meanwhile, the kinematic viscosity of black liquor is a function of its chemical composition, overall dry solids content, and temperature.[17] At high temperatures, the viscosity of black liquor decreases as the constituent polymers degrade. Further, the density of black liquor undergoes a steep increase when the proportion of dry solids is greater than 30%.[17] Moreover, the thermal

conductivity of black liquor decreases with an increase in dry solids content and increases with an increase in temperature.[17] Another physical property of black liquor – specific heat – increases with a rise in temperature. On the other hand, an increase in dry solids content results in a rapid decrease in the specific heat.[17]

Various extraction processes have been established to isolate lignin from lignocellulosic matter in the black liquor discussed above. Some examples include soda extraction (sodium hydroxide), kraft extraction (sodium hydroxide and sodium sulfide), organosolv extraction (ethanol, organic acids, etc.), and lignosulfonate extraction (sodium hydroxide and bisulfite acid).[18] Organosolv extraction is known to yield high-quality lignin, albeit at higher costs compared to most other methods.[18] However, a comprehensive study that compares multiple lignin extraction methods under different reaction parameters and for multiple wood species is absent from the literature. In the present study, two different lignin extraction methods, namely, Formacell (formic acid, acetic acid, and water) and BioEB (formic acid method), have been compared for their efficacy in 6 different species of wood. The Formacell and BioEB methods have been chosen for this analysis because (1) they are two of the greenest and most environmentally friendly lignin extraction methods, (2) the acids used in these methods are inexpensive and easily available, and (3) the acids can be recycled after the experiments are completed. It should also be noted that the BioEB method is a new lignin extraction method developed by Dr. Guo-Hua Delmas and Dr. Michel Delmas in France. As such, studies evaluating its efficacy and optimum parameters are absent from the literature. Three of the wood varieties chosen for the present study were softwood species (balsam fir, pine, and spruce), and the other three were hardwood species

(maple, oak, and birch). Parameters such as temperature and cooking time were compared to identify the most optimum lignin extraction condition for these two methods among the conditions tested. Furthermore, the composition of the extracted Newfoundland oak VRL was investigated by using mass spectrometric methods.

3.2. Methods

Different methods have been reported for the extraction of lignin from the black liquors obtained after destructuring of vegetal wood. In this study, we have used and compared two solvolysis methods for destructuring wood. Technical Association of the Pulp and Paper Industry (TAPPI) standards were followed in all extraction procedures.

3.2.1. Wood sources

For lignin extraction, the source materials used were fresh sawdust from softwood species balsam fir, pine and spruce (black), and hardwood species birch, maple and oak (Figure 3.2). All samples were collected from the Memorial University Botanical Garden. Brief descriptions of each of the tree species from which wood samples were collected have been provided below:

3.2.1.1. Balsam Fir

Balsam fir (*Abies balsamea*) is a softwood coniferous tree very common across North America, particularly the northern USA and Canada.[19] Newfoundland is a well-known location where Balsam fir trees grow in Canada, in addition to Labrador, northern Quebec and Ontario, central Alberta, and other areas.[19] It is typically a small or medium-sized tree

popularly used for the production of lumber and pulpwood.[19] In the forest, balsam fir is known to often co-occur with other tree species like black spruce, white spruce, aspen, and birch, some of which were used in the present study and have been discussed below.[19]

3.2.1.2. Pine

The pine tree, which commonly finds application in tanning, has been known for a long time in the Middle East, Europe, and North Africa and is also found in eastern and southwestern Canada.[19,20] White pine or *Pinus strobus* is also considered to be an important source of medicinal compounds.[21] Moreover, extracts from its inner bark is known to be abundant in tannins.[21]

3.2.1.3. Black Spruce

Black spruce (*Picea mariana*) is another common tree in northern regions of North America, including Canada.[19] It is found in lowland areas as well as mountainous regions. Black spruce wood is strong, lightweight, and yellowish-white in color.[19] Like balsam fir, black spruce also finds applications in pulpwood and lumber, in addition to being used in Christmas trees.[19]

3.2.1.4. Birch

Birch (such as paper birch or *Betula papyrifera*) is a common species of hardwood trees found in Canadian regions such as Newfoundland, British Columbia, western Alberta, etc.[19,22] Birch, which grows in proximity to trees such as balsam fir, is a deciduous tree

generally small- to medium-sized.[19,23] It finds diverse applications in pulpwood and plywood as well as beer and wine production.[23]

3.2.1.5. Maple

Maple (such as sugar maple or *Acer saccharum*) is a widely observed hardwood species in Newfoundland, Canada.[22] Maple trees are often found in close proximity to pine trees.[19] Along with other trees like oak (discussed below) and ash, maple belongs to a category of high-density hardwood trees because of the high density of components in their wood.[19]

3.2.1.6. Oak

Oak is a tree in the genus of the beech family, which has nearly 600 species. Northern red oak is commonly found in northern, eastern, and central Canada.[24] Studies have shown that oakwood is highly resistant to attacks by insects and fungi.[25] This resistance is imparted by the relatively high tannin content of this hardwood tree.[25]

Figure 3.2. Wood species used in the present study.

3.2.2. Sample Preparation

Two logs, each 30 cm long, were collected from the bottom of each tree and debarked manually. They were then crushed to obtain small white chips. The wood chips were oven-dried at 50°C for 24 h to reduce their moisture content to less than 18%. Dried chips were ground to 4 mm diameter particles which were subsequently transferred to a 2 mm sieve. The particles were then vacuum-packed and stored in a freezer at -20°C for up to 6 days until they were used for analysis (Figure 3.3).

Figure 3.3. Dried wood samples.

3.2.3. Extraction of Lignin from Wood

The methods of extraction of lignin from wood components can be broadly classified into two categories: (1) methods that preferentially dissolve lignin, and (2) methods that preferentially dissolve the non-lignin components of wood.[26] The first category of techniques includes methods such as the Brauns method in which lignin components are precipitated out after treatment with cold water, ether, and ethanol under varying reaction conditions.[26] Other techniques in this category include the milled wood lignin and cellulolytic enzyme extraction methods. In the second category, there are methods like Klason extraction wherein alcohol-benzene mixture is first used to remove non-lignin components wax, resins, fat, and gum, after which a sulfuric acid-based hydrolysis treatment is done to remove polysaccharides, ultimately leaving behind lignin matter.[26] Other methods in this category include the Willstätter, Periodate, and Cuproxam extraction processes.[26]

In addition to extraction from wood, lignin can be extracted from black liquor too. Two such methods were used in this study, as described below:

3.2.3.1. Formacell (CIMV) Lignin Extraction Method

Firstly, the Formacell (formic acid HCOOH/acetic acid CH_3COOH/water H_2O) method at atmospheric pressure was used for the delignification of lignocellulosic raw materials. This organic acid method offers good separation of major lignocellulosic components (cellulose, hemicelluloses, and lignin) via hydrolysis and the subsequent dissolution of lignin hemicellulose fragments in ‘black liquor’ (Figure 3.4).

Figure 3.4. Black liquor.

In this process, acetic acid acts as the dissolution solvent for hemicelluloses and lignin, while formic acid serves as the catalyst for the cleavage of lignin – carbohydrate complexes. The possible recycling of the organic acids at the end of the process creates an excellent opportunity for an eco-friendly strategy.

Accordingly, the present study sought to evaluate the effect of the cooking time and temperature needed to dissociate and destructure the vegetal wood matter. For the Formacell extraction method, three independent variables (solvent ratio, time, and temperature) were evaluated based on preliminary experiments. The cooking temperatures evaluated were 60°C, 80°C, and 110°C, while the cooking times used were 1h, 2h and 3h.

Moreover, two different formic acid/acetic acid/water ratios were used: 55/30/15 (v/v/v) and 50/40/10 (v/v/v).

For the Formacell extraction, 5 g of the biomass sample (sawdust) was transferred to a glass reactor, and a mixture of formic acid/acetic acid/water was added. For all 18 treatment conditions evaluated (3 temperatures × 3 cooking times × 2 solvent ratios), the material was first soaked at 50°C for 30 min, and then the temperature was increased to the final cooking temperature (60°C, 80°C or 110°C). The solutions were cooked with stirring for 1h, 2h, and 3h (for each temperature condition). The liquid/dry matter mass ratio was 25/1.

At the set time points, reactions were stopped by removing the flasks from the heating plates and allowing them to cool at room temperature. At the end of each treatment, pulp and black liquor were separated by filtration. The pulp was filtered with a vacuum filter funnel (500 mL, 95 mm diameter) assembled with a fritted disk (40-100 μm pore size; Robu Glasfilter-Geräte GmbH). The vacuum was maintained until black liquor was no longer observed.

Following filtration, the pulp was washed twice with the same cooking mixture and finally with distilled water. The organic solvents were recycled by evaporation under reduced pressure and by distillation under a pressure of 8 bars. Extraction liquor was added with water to solubilize the hemicellulose and precipitate the lignin extracted by filtration. In this process, acetic acid serves as the solvent for lignin and hemicellulose. In contrast, formic acid serves as a catalyst to break the ether and ester bonds of polysaccharides bound

to lignin. The reaction time and efficiency of the procedure allow lignin to be extracted in a weakly degraded and poorly esterified form.

3.2.3.2. BioEB Method or Formic Acid-Based Extraction of Lignin from Black Liquor Lignocellulosic Biomass

The second lignin extraction method evaluated in the present study was the BioEB method, wherein formic acid/water is used for delignification (Figure 3.5).[27] Dried wood chips (90% dry matter) from 5 g of biomass sample were finely crushed, ground, and suspended in a solution of formic acid in water (85% w/w) in the biomass to the formic acid ratio (w/w) of 1:5. The mixture was stirred at medium speed with a mechanical stirrer with an inox- anchor for homogenous mixing.

Thereafter, two temperature conditions and two cooking times were used in this method. The temperatures used were 80°C and 90°C (controlled using a thermometer). For each temperature, the chemical reaction was allowed to continue for 2 h and 3 h. At the end of the treatment, the reaction mixture was cooled to room temperature, and the precipitated raw cellulose was filtered. The black liquor (fraction 1) was saved as it contains the lignin fraction.

The precipitated cellulose was washed with formic acid (concentration 85% to 99% w/v), then pressed and filtered with a Buchner funnel to remove the residual black liquor (fraction 2).[28] The cellulose was then washed with warm water (40-50^{o}C), pressed, and filtered until the pH of the filtrate was neutralized. The black liquor fractions 1 and 2 were then mixed

together to obtain the main black liquor, which was subsequently concentrated under vacuum at 60°C until a black syrup was obtained (dry matter content 50-60% w/w).

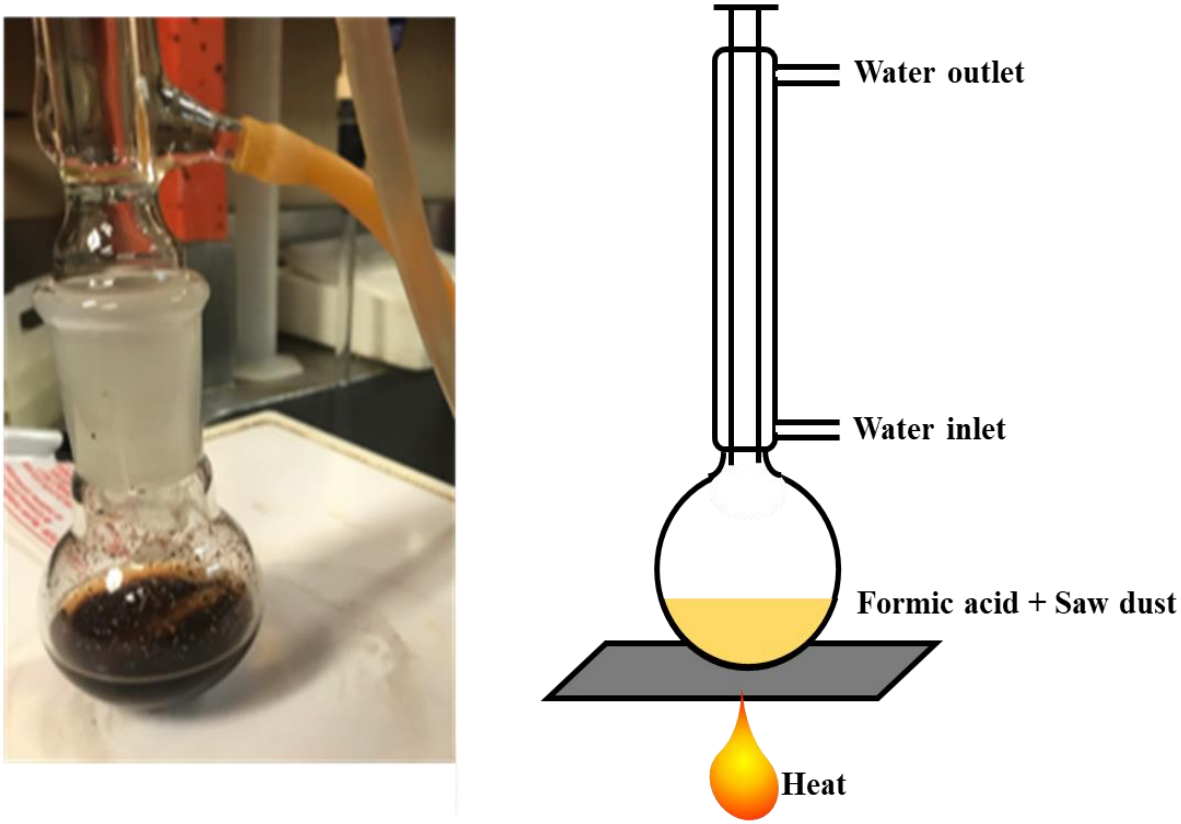


Figure 3.5. Lignin extraction using the BioEB method, wherein wood dust was introduced in a formic acid and water mixture for lignin extraction.

3.2.3.3. Formic Acid Recycling

In the second phase (formic acid recycling), the pH of the solution at room temperature was determined, and the solution was transferred to a 2L round flask (Figure 3.6). Rotavapor was used (at a low pressure of ~0.2 mBar) to concentrate the liquor and recover formic acid condensates. The mixture was heated at around 40^{o}C initially, and the temperature was increased to 60^{o}C when the flow of formic acid condensates slowed down. When a very viscous syrup was visually confirmed, the Rotavapor was stopped, and the syrup was allowed to cool to 30-40^{o}C. The pH of the syrup was determined at this temperature. Finally, warm water was added to the syrup, and the mixture was centrifuged and filtered to separate the precipitated lignin particles from the hemicellulose fraction. The lignin fraction was washed with warm water and filtered until the filtrate was neutralized. The resulting lignin cake was dried at a maximum temperature of 50°C until it had 90-95% of dry matter. Thereafter, it was finely crushed. The hemicellulose fraction was concentrated under vacuum (up to 50°C, 100 mBar) until a viscous brown syrup was obtained. The used formic acid was collected back by using the Rotavapor.

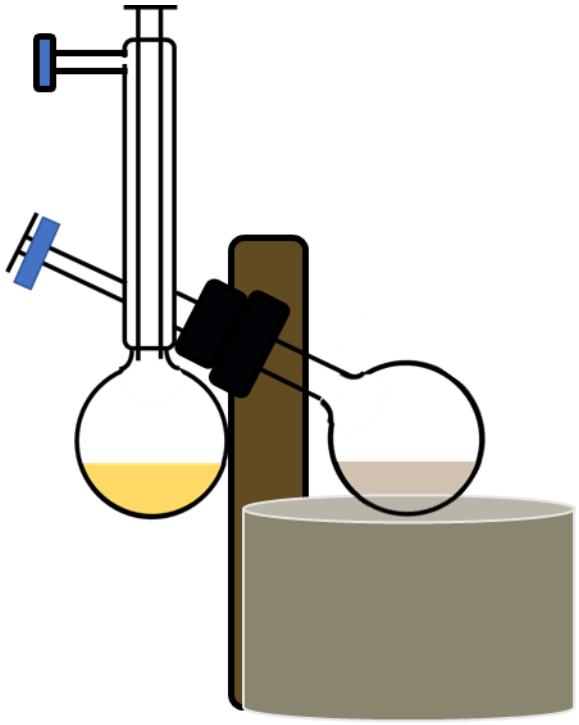


Figure 3.6. Rotavapor used for formic acid recycling.

3.2.4. Lignin Yield Calculation

For both extraction methods, lignin was isolated from about 5 g of the dry weight of sawdust (W). The acid-insoluble lignin content (or yield) of each extraction method and condition was calculated in the samples by using the following equation:

$$\text{Lignin (w/w \%)} = (A/W) \times 100 \quad \text{– Equation 1}$$

where A = weight of lignin (g)

W = oven-dry weight of extractive-free sawdust (g)

3.3. Results

3.3.1. Formacell Extraction

The dry weights of lignin obtained from the Formacell method have been summarized in Table 3.2 (55/30/15 (v/v/v)) and Table 3.3 (50/40/10 (v/v/v)). It was observed that among the conditions tested, the best lignin yields were obtained at a cooking temperature of 80°C and a cooking time of 3 h for both the solvent ratios. Please note that the concentration reaction was at the highest rate at 80°C at 3 h of treatment. For this reason, the reaction for this particular time point (t = 3 h) was not continued beyond the temperature of 80°C for both the solvent ratios tested. Therefore, in Tables 3.2 and 3.3, the columns for 110°C are left unpopulated.

Interestingly, hardwood samples had higher lignin dry weight at this optimum condition (oak > birch > maple) than softwood samples (pine > balsam fir > spruce).

Table 3.2. Lignin dry weight (in g) as obtained from the Formacell extraction method at formic acid/acetic acid/water = 55/30/15 (v/v/v). All data have been presented as mean ± standard deviation (n = 3 for each condition). The best yield conditions are highlighted in red.

		1 h			2 h			3 h	
Wood kind		**60ºC**	**80ºC**	**110ºC**	**60ºC**	**80ºC**	**110ºC**	**60ºC**	**80ºC**
Hardwood	Oak	1.00 ± 0.17	1.00 ± 0.12	1.10 ± 0.09	1.10 ± 0.15	1.60 ± 0.08	1.70 ± 0.19	1.20 ± 0.07	2.20 ± 0.31
	Maple	0.98 ± 0.14	1.00 ± 0.11	1.00 ± 0.08	1.20 ± 0.10	1.30 ± 0.17	1.40 ± 0.13	1.20 ± 0.21	1.50 ± 0.26
	Birch	0.97 ± 0.07	1.10 ± 0.04	1.20 ± 0.19	1.10 ± 0.16	1.30 ± 0.09	1.40 ± 0.18	1.40 ± 0.26	1.90 ± 0.09
Softwood	Spruce	0.88 ± 0.06	0.90 ± 0.11	0.93 ± 0.18	0.90 ± 0.10	0.92 ± 0.03	0.94 ± 0.08	0.92 ± 0.12	0.95 ± 0.06
	Pine	0.90 ± 0.10	0.95 ± 0.16	1.00 ± 0.09	0.95 ± 0.11	1.00 ± 0.13	1.10 ± 0.20	1.00 ± 0.07	1.20 ± 0.11
	Balsam fir	0.97 ± 0.10	1.00 ± 0.18	1.00 ± 0.04	1.00 ± 0.08	1.10 ± 0.12	1.10 ± 0.05	1.00 ± 0.15	1.10 ± 0.08

Table 3.3. Lignin dry weight (in g) as obtained from the Formacell extraction method at formic acid/acetic acid/water = 50/40/10 (v/v/v). All data have been presented as mean ± standard deviation (n = 3 for each condition). The best yield conditions are highlighted in red.

		1 h			**2 h**			**3 h**	
Wood kind		**60ºC**	**80ºC**	**110ºC**	**60ºC**	**80ºC**	**110ºC**	**60ºC**	**80ºC**
Hardwood	Oak	0.77 ± 0.06	0.95 ± 0.10	1.00 ± 0.18	0.98 ± 0.11	1.40 ± 0.23	1.50 ± 0.13	1.20 ± 0.05	1.60 ± 0.22
	Maple	0.78 ± 0.02	1.00 ± 0.09	1.10 ± 0.04	1.00 ± 0.02	1.20 ± 0.16	1.30 ± 0.06	1.10 ± 0.10	1.40 ± 0.17
	Birch	0.94 ± 0.08	1.10 ± 0.03	1.20 ± 0.02	1.00 ± 0.06	1.30 ± 0.11	1.40 ± 0.20	1.20 ± 0.07	1.50 ± 0.10
Softwood	Spruce	0.67 ± 0.07	0.69 ± 0.01	0.70 ± 0.09	0.70 ± 0.11	0.77 ± 0.04	0.79 ± 0.11	0.75 ± 0.10	0.80 ± 0.03
	Pine	0.77 ± 0.07	1.20 ± 0.22	1.00 ± 0.16	1.00 ± 0.12	1.20 ± 0.15	1.20 ± 0.10	1.10 ± 0.09	1.30 ± 0.06
	Balsam fir	0.88 ± 0.05	0.89 ± 0.01	1.00 ± 0.14	0.90 ± 0.04	0.95 ± 0.10	1.00 ± 0.02	1.00 ± 0.05	1.20 ± 0.13

Overall, the formic acid/acetic acid/water ratio of 55/30/15 (v/v/v) yielded a higher lignin concentration than the 50/40/10 (v/v/v) ratio. Taking together all the variables tried, it can be said that among all conditions tested for Formacell extraction, lignin yield was the highest with 55/30/15 (v/v/v) formic acid/acetic acid/water ratio, temperature 80°C, and cooking time 3 h (Figure 3.7). The percentage lignin (w/w) yield, as calculated according to equation 1, was in the 30-44% range for hardwoods and 19-24% for softwoods.

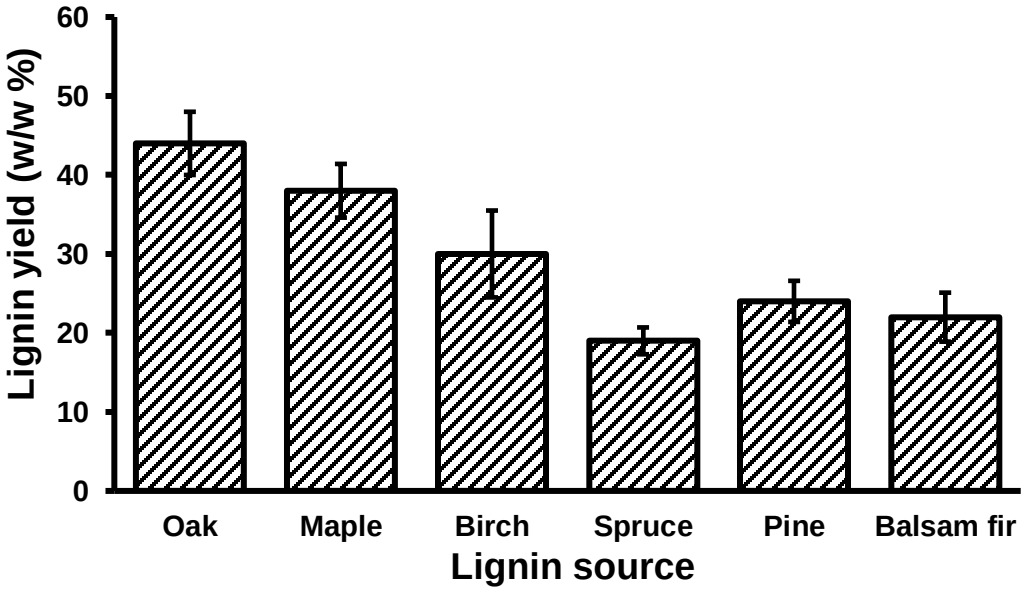


Figure 3.7. Lignin yield (w/w %; n = 3) obtained using the Formacell method (formic acid/acetic acid/water = 50/30/15) with a cooking time of 3 h at a temperature of 80°C.

3.3.2. BioEB Extraction

The dry weights of lignin obtained from the BioEB method have been summarized in Table 3.4. Among all treatment conditions tested for all six wood species, the BioEB method yielded the highest lignin content after treatment at 90°C for 3 h (Table 3.4). Among hardwoods, the sequence of lignin content was oak > birch > maple (similar to the trend observed in the Formacell method), and among softwoods, this sequence was spruce > pine > balsam fir. The percentage lignin (w/w) yield, as calculated according to equation 1, was in the 38-46% range for hardwoods and 28-32% for softwoods (Figure 3.8).

Table 3.4. Lignin dry weight (in g) as obtained from the BioEB (formic acid/water) extraction method. All data have been presented as mean ± standard deviation (n = 3 for each condition). The best yield conditions are highlighted in red.

		1 h		2 h		3 h	
Wood kind		**80°C**	**90°C**	**80°C**	**90°C**	**80°C**	**90°C**
Hardwood	Oak	1.41 ± 0.16	1.49 ± 0.21	1.70 ± 0.25	1.90 ± 0.20	1.90 ± 0.09	2.30 ± 0.16
	Maple	1.36 ± 0.11	1.43 ± 0.15	1.60 ± 0.14	1.70 ± 0.17	1.70 ± 0.08	1.90 ± 0.05
	Birch	1.39 ± 0.13	1.54 ± 0.23	1.70 ± 0.04	1.90 ± 0.08	1.80 ± 0.13	2.00 ± 0.18
Softwood	Spruce	1.02 ± 0.05	1.21 ± 0.11	1.20 ± 0.10	1.40 ± 0.03	1.50 ± 0.19	1.60 ± 0.11
	Pine	0.98 ± 0.02	1.07 ± 0.10	1.10 ± 0.05	1.20 ± 0.02	1.30 ± 0.09	1.40 ± 0.02
	Balsam fir	0.99 ± 0.04	1.08 ± 0.11	1.10 ± 0.10	1.20 ± 0.06	1.20 ± 0.15	1.30 ± 0.08

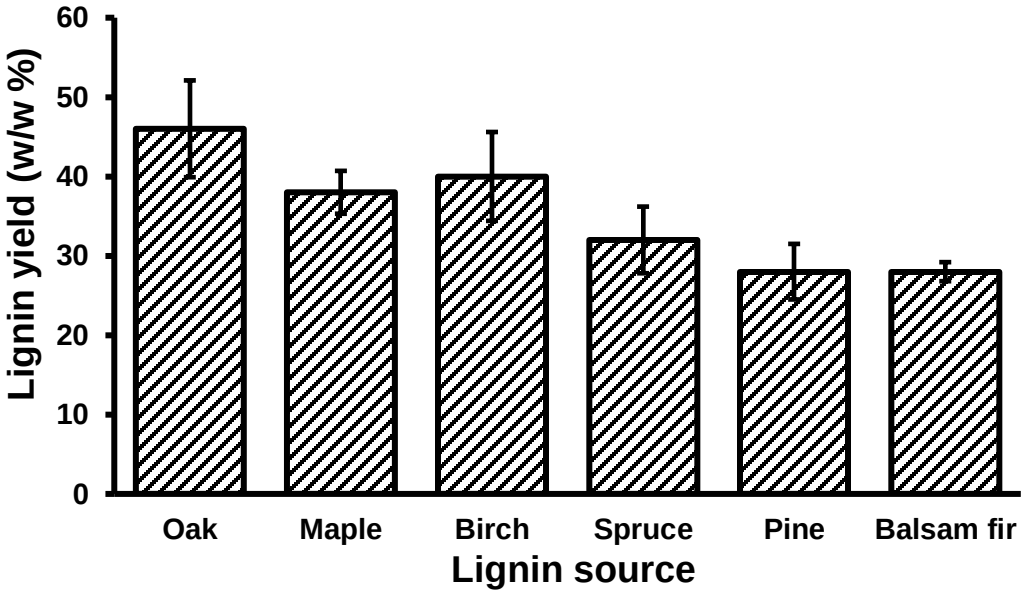


Figure 3.8. Lignin yield (w/w %; n = 3) obtained using the BioEB method with a cooking time of 3 h at a temperature of 90°C.

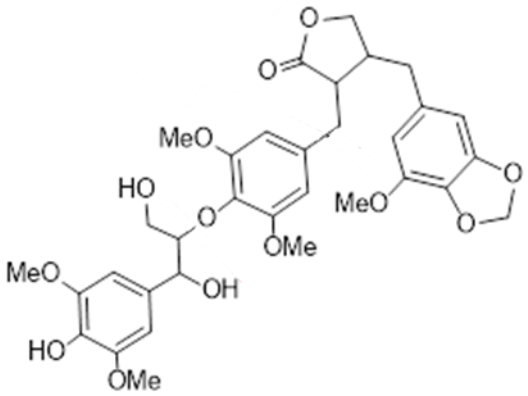
3.3.3. Mass spectrometric analysis of Oak VRL

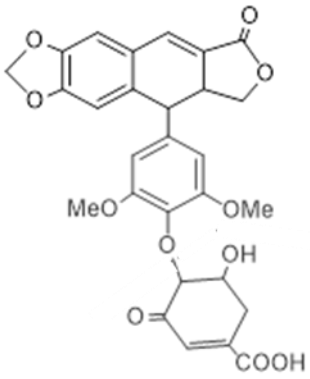
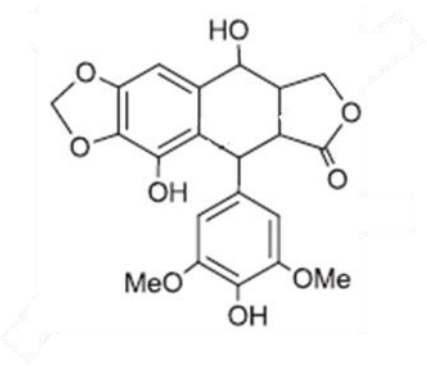
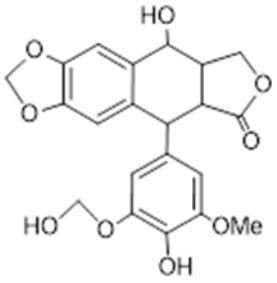
The second part of this study sought to identify the components of an extracted oak wood VRL mixture. Mass spectrometric methods revealed oak wood VRL to be a diverse and complex mixture of a variety of oligomeric compounds, as discussed in the forthcoming sections.

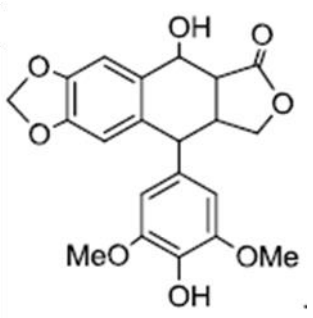
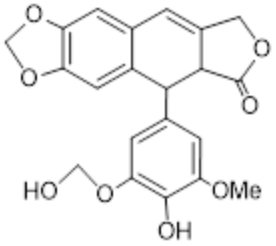
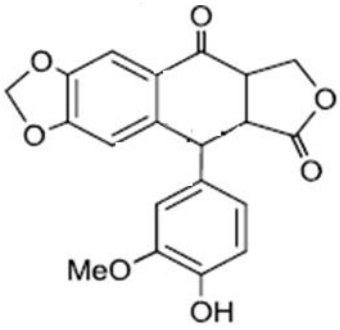
3.3.3.1. Positive Ion Mode APPI-QqTOF-MS and Low-Energy CID-MS/MS

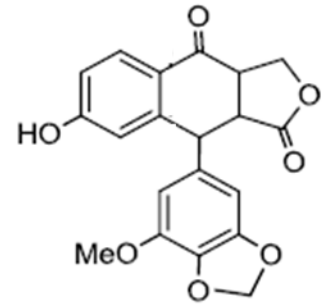
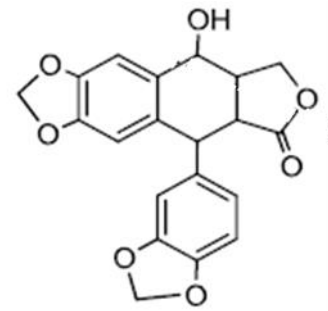
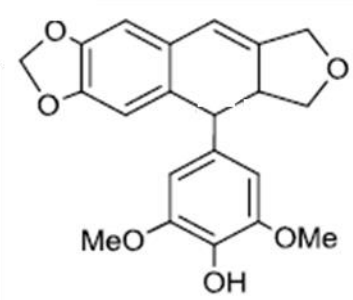
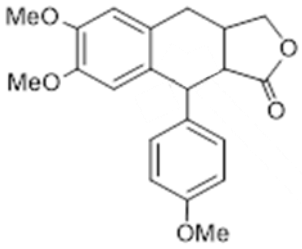
The extracted Oak VRL complex mixture was investigated by positive ion mode APPI-QqTOF-MS (Figures B.18a and B.18b). The process was performed following the method described in the Appendix (B.1). This study led to the identification of eight major protonated lignin oligomers: neolignan cedrusin, five different aryltetralin lignans dimers, one lignan-dehydroshikimic acid complex, and a lignan trimer (Table 3.5). These identified structures were supported by low-energy CID-MS/MS analyses and/or fragmentation mechanisms, as shown in some selected schemes from this work (Appendix B).

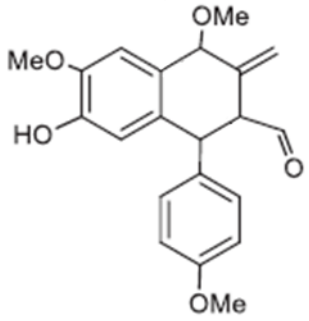
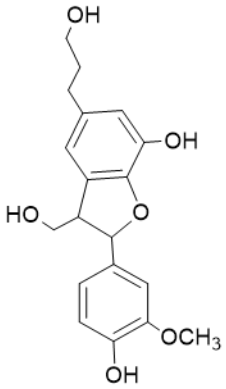
Table 3.5. Lignin oligomers identified in the positive ion mode.

S.no.	m/z	Lignin Oligomer (chemical formula)	Structure
1	643.16	$C_{33}H_{38}O_{13}$	 A lignan trimer containing a syringlyglycerol unit linked to the dibenzyl butyrolactone lignan

2	537.14	$C_{28}H_{24}O_{11}$	 Dehydrated form of an isomer of the lignan 4’-demethylepipodophyllotoxin linked to dehydroshikimic acid Moiety
3	417.11	$C_{21}H_{20}O_9$	  An aryltetralin lignan derivative

4	401.11	$C_{21}H_{20}O_8$	 An isomer of the lignan 4'-demethylepipodophyllotoxin
5	399.10	$C_{21}H_{18}O_8$	 Oxidised lignan 4'-demethylepipodophyllotoxin
6	369.10	$C_{20}H_{16}O_7$	

			   Aryltetralin lignan derivatives
7	355.16	$C_{21}H_{22}O_5$	

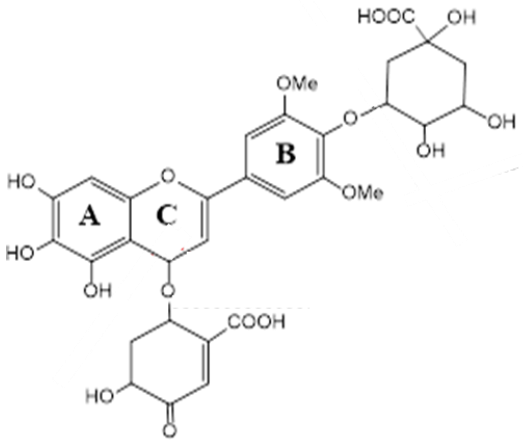
			 Aryltetralin lignan derivatives, containing methylated coumaric acid and methylated coniferyl alcohol
8	347.13	$C_{19}H_{22}O_6$	 Neolignane cedrusin

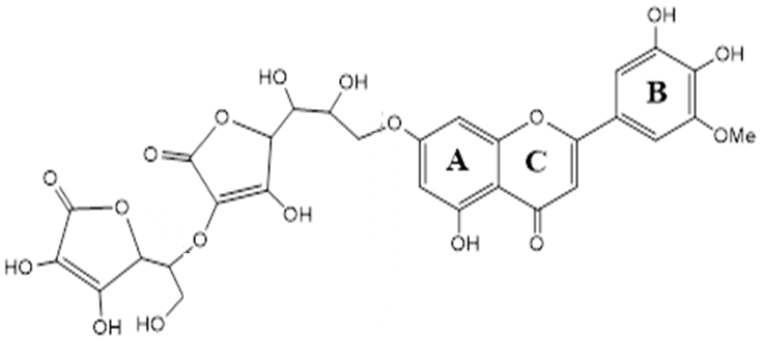
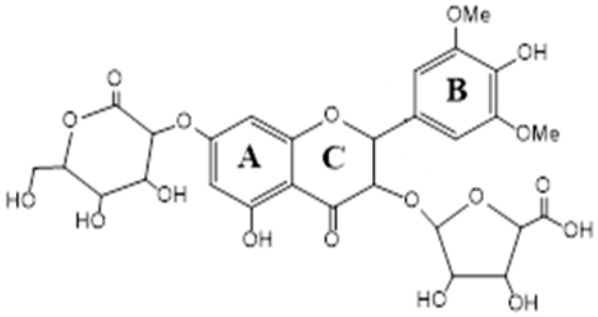
3.3.3.2. Negative ion mode MALDI-MS/MS

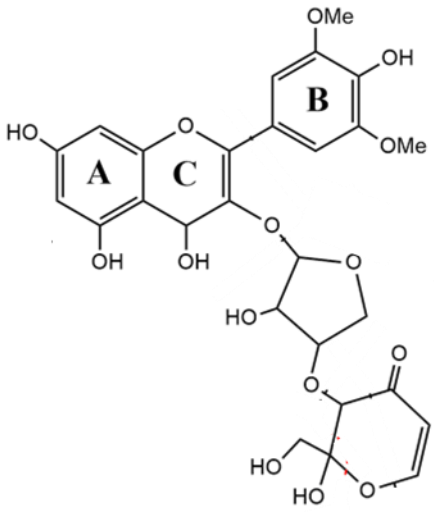
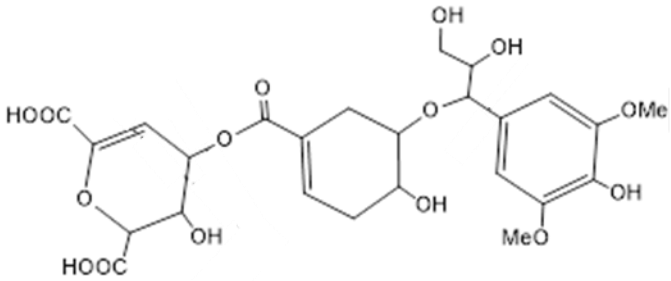
Newfoundland oak VRL was further analysed using mass spectrometry in the negative ion mode. The process was performed following the method described in the Appendix (B.2). The negative-ion mode MALDI-MS/MS of the VRL extracted from the Oak wood showed the complexity and the diversity of the lignin mixture. From this study, several types of compounds were identified: seven tricin derivatives, five syringyl lignin derivatives, two flavonolignin derivatives, and six miscellaneous compounds such as luteoferol,

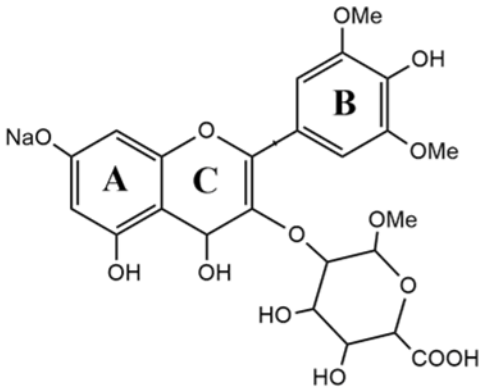
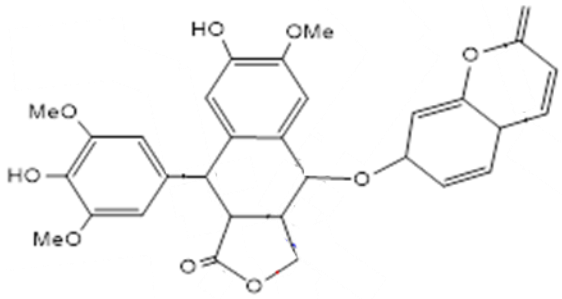
lariciresinol isomer, 5-hydroxy guaiacyl derivative, syringyl -$C_{10}H_{10}O_2$ dimer, trihydroxy benzaldehyde derivative, and aryl tetralin lignan derivative (Table 3.6). Interestingly, most of the detected compounds were carbohydrate and/or shikimic acid complexes. These identified structures were supported by tandem mass spectrometry (MS/MS) and/or their fragmentation mechanism, as shown in some selected schemes from this work (Appendix, Figures B.2-B.17 and Schemes B.1-B.16). These identified compounds support the abundance of syringyl lignin units in the oak VRL sample and the association of lignin with carbohydrate in the wood network.

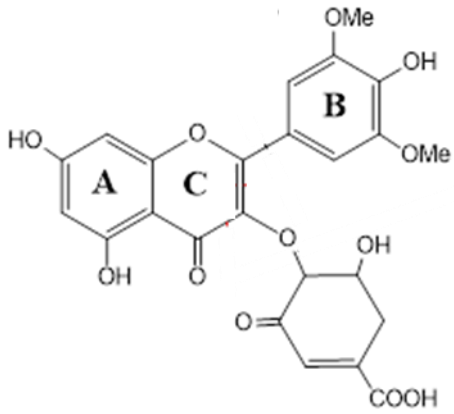
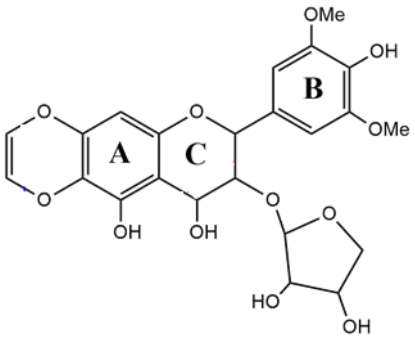
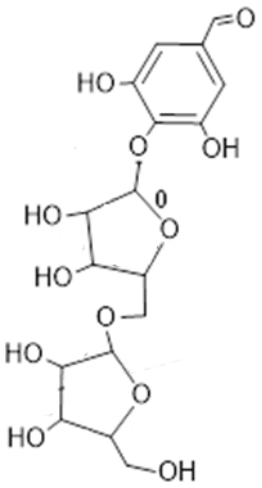
Table 3.6. Lignin oligomers identified in the negative ion mode.

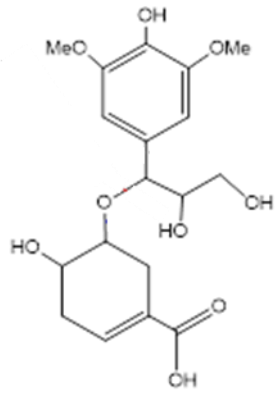
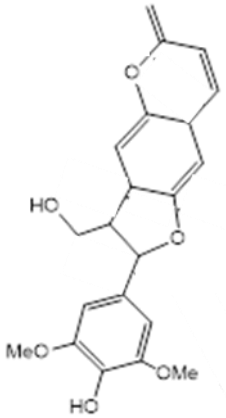
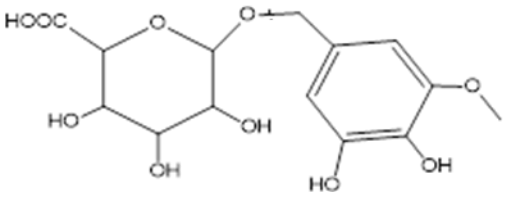
S.no.	m/z	Lignin Oligomer (chemical formula)	Structure
1	675.03	$C_{31}H_{32}O_{17}$	 Quinic acid-deoxyshikimic acid

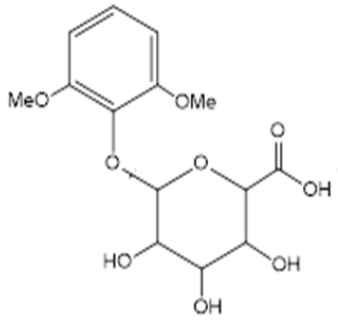
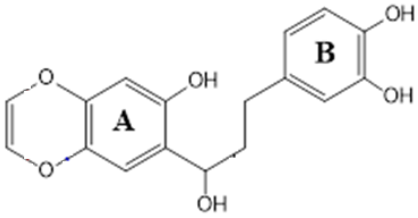
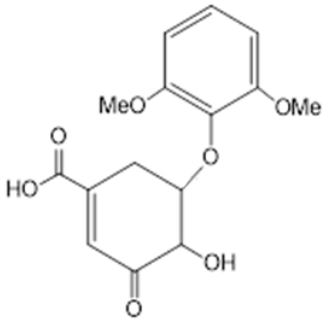
2	661.04	$C_{29}H_{26}O_{18}$	 Ascorbic acid - glucascorbic acid
3	653.05	$C_{28}H_{30}O_{15}$	 Disaccharide hexose lactone - penturonic acid

4	590.90	$C_{27}H_{28}O_{15}$	 Disaccharide tetrose - ketohexose derivative
5	568.93	$C_{25}H_{30}O_{15}$	 Deoxyshikimic acid - daucic acid

6	558.97	$C_{24}H_{26}O_{14}$	 Methylated hexuronic acid
7	545.97	$C_{31}H_{30}O_9$	 Aryl tetralin lignan structure containing syringyl and guaiacyl units, and $C_{10}H_{10}O_2$ lignin derivative

8	499.03	$C_{24}H_{20}O_{12}$	 Dehydroshikimic acid
9	491.05	$C_{23}H_{24}O_{12}$	 A tricin derivative with an extra hydroxyl group on ring A, linked to a tetrose sugar.
10	417.07	$C_{17}H_{22}O_{12}$	

			Trihydroxy benzaldehyde attached to the disaccharide dipentose
11	382.96	$C_{18}H_{22}O_9$	 Deoxyshikimic acid
12	368.97	$C_{21}H_{22}O_6$	 Syringyl lignin unit attached to $C_{10}H_{10}O_2$ lignin derivative
13	345.02	$C_{14}H_{18}O_{10}$	 3,4-dihydroxy-5-methoxy benzyl alcohol (5-hydroxy guaiacyl derivative) linked to hexuronic acid

14	329.04	$C_{14}H_{18}O_9$	 Hexuronic acid
15	315.05	$C_{17}H_{16}O_6$	 A degraded flavonolignans or flavonosugar derivative
16	307.04	$C_{15}H_{16}O_7$	 Dehydroshikimic acid

3.4. Discussion

In the present study, delignification of wood was conducted while (1) varying the amounts of formic acid, acetic acid, and water, (2) changing the cooking time, and also (3) altering

the temperature of the reaction mixture. Each of these factors was studied with regard to the yield or percentage of pulp or residual lignins. It was found that the yield of lignin extraction increased slightly as the pH values decreased. Moreover, lignin yield from softwoods was higher than from hardwoods. Increasing the proportion of formic acid in the acid mixtures reduced the amount of acid-insoluble lignin, irrespective of the cooking period. Earlier studies have shown that an increase in formic acid concentration in the acid mixture improves delignification and decreases waste material.[29] The amount of water in the acid mixture was maintained at 20% by volume. Low water proportion in the acid mixture could cause a decrease in delignification. Therefore, a threshold amount of water is essential for optimal pulping. Also, when water and organic acids are mixed, hydrolytic changes take place in the organic acids in the solution (formation of solvate acids), which in turn facilitates the decomposition of plant matter in the acidic environment.

In concentrated organic acid solutions, molecules are linked to one another by hydrogen bonding. Hence, protons are not easily available. The addition of water first breaks these bonds between the molecules of the organic acids and then helps in the dissociation or ionization of formic acid and acetic acid, which then results in the release of protons. Many studies have been done on the mechanism of delignification using acetic acid on model lignin molecules. Initially, carbonium ions are created with acid-catalyzed cleavage of the alpha-aryl ether bond in arylglycerol-1, 3-aryl ether, a basic structural unit of lignin (Figure 3.9). Many carbonium ions lose three protons immediately in order to develop vinyl ethers, which are then hydrolyzed. However, most of the ions undergo intramolecular and intermolecular nucleophilic attacks via an aromatic ring to create condensation products.

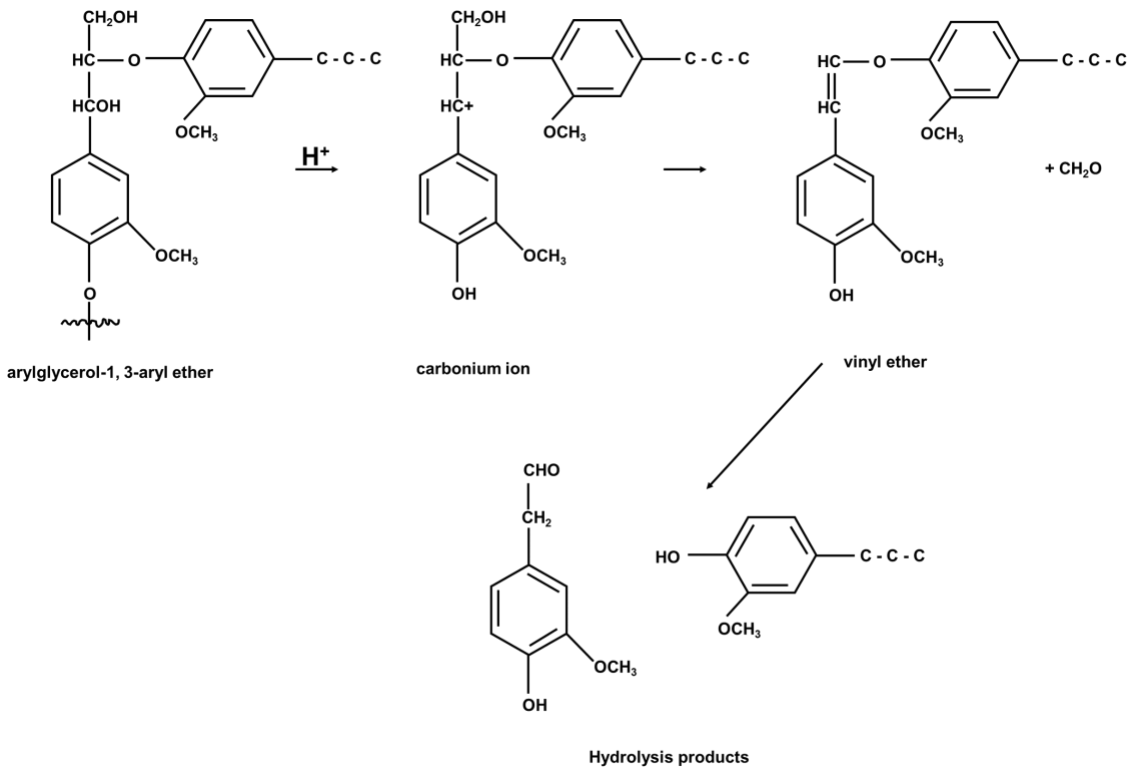


Figure 3.9. Basic reaction scheme for acid-catalyzed degradation of lignin.

An overarching comparison between the two extraction methods used in the present study reveals that the lignin yield of the BioEB method was higher than that of Formacell method (for both hardwoods and softwoods). There were, however, some similarities between the Formacell and BioEB methods. In both methods, it was observed that the extraction of lignin from hardwood was much easier than from softwood. Moreover, the amount of hardwood lignin that was extracted at the tested conditions was higher than that of softwood. These results are interesting because typically published data in the literature suggests an opposite trend: softwoods tend to have a higher lignin content than hardwoods (Table 3.1). However, it should be noted that hardwood lignocellulosic mass is less crosslinked than softwood, and hardwoods can be softened more easily than softwoods.[28]

Moreover, the glass transition temperature of softwood lignin is typically higher (138-160°C) compared to hardwood lignin (110-130°C).[30] These data could possibly explain why at the temperatures tested (all less than or equal to 110°C), more lignin yields were obtained in hardwoods than softwoods.

When comparing the lignin yields for each individual wood species with corresponding data reported in literature, the BioEB method once again proved to be better than the Formacell method (Table 3.7). Specifically, the lignin yields obtained for all three hardwood species by both Formacell and BioEB methods in the present study were higher (30-46%) than previously reported concentrations obtained using Klason extraction (21-27%). In hardwoods, the BioEB method performed slightly better than the Formacell method. Among the three softwood species, the Formacell method performed more poorly (19-24%) than previously reported yields using Klason method (27-29%). However, the BioEB method resulted in lignin yields (28-32%) that were comparable or slightly better than the reported concentrations (27-29%).

Table 3.7. Comparing the best lignin yields obtained in the present study with reported lignin content from literature.

Wood type	**Formacell (Present study)**	**BioEB (Present study)**	**Klason lignin content reported in literature (72% sulfuric acid used for lignin extraction)[31]**

Hardwood	Oak	44%	46%	27%
	Maple	38%	38%	22%
	Birch	30%	40%	21%
Softwood	Spruce	19%	32%	27%
	Pine	24%	28%	27%
	Balsam fir	22%	28%	29%

In the present study, it was also found that the amount of formic acid recycled in the end is approximately the same as that used for the extraction. The recycling of organic solvents such as formic acid provides the added advantage of eco-friendliness, sustainability, as well as promotion of the low-carbon economy.[32] Formic acid is a low-cost chemical and is easy to be recovered by distillation for reuse. Also, this process can be operated at a low 3temperature and atmospheric pressure. It appears that formic acid alone is capable enough to break the covalent bonds between lignin and polysaccharides. The implementation of this process allows the separation of lignin at atmospheric pressure at a temperature of around 80-90°C for 3 h.

3.5. Conclusions

The first part of this study sought to compare the efficacy of two different lignin extraction methods, namely the Formacell method and the BioEB method. Various parameters were tested, including solvent concentration, temperature, cooking time, and wood species (hardwoods and softwoods). Among all conditions tested for Formacell extraction, lignin yield was the highest with 55/30/15 (v/v/v) formic acid/acetic acid/water ratio, temperature 80°C, and cooking time 3 h. Meanwhile, among all treatment conditions tested for all six wood species, the BioEB method yielded the highest lignin content after treatment at 90°C for 3 h. Overall, the lignin yield of the BioEB method was higher than that of Formacell method (for both hardwoods and softwoods).

However, in both the methods, it was observed that the extraction of lignin from hardwood was much easier than from softwood, which could be explained by the lower crosslinking and easier softening, and lower glass transition temperatures of hardwood lignin compared to softwood. An additional advantage associated with the BioEB method is that it allowed for a near-100% recovery or recycling of the formic acid used in the reaction, making it a more sustainable method of lignin extraction compared to the Formacell method. Overall, the results of this part of the study could be applied to inform and improve industrial lignin extraction processes to obtain better yields in the most optimal manner. In particular , the recently developed and previously unexplored BioEB method resulted in considerably better lignin yields for all wood species (hardwoods and softwoods) investigated in the

present study. This knowledge provides a basis for the exploration of the BioEB method for lignin extraction/removal in industrial applications.

The second part of this study sought to identify the components of an extracted oak wood VRL mixture. Mass spectrometric methods revealed oak wood VRL to be a diverse and complex mixture of a variety of oligomeric compounds. This is among the first studies to have identified many of the large number of chemical components in extracted lignin. Future studies should focus on identifying the role of each component in determining lignin properties.

References for Chapter 3

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Chapter 4: INDUSTRIAL APPLICATIONS OF LIGNIN

Lignin consists of a series of linear aromatic oligomers which can be used to replace petroleum products in biobased industrial developments. Extracted lignin obtained from forestry and agriculture sectors is an important raw material that shows immense potential in novel value-added applications. Indeed, presently lignin value-added applications for industrial uses are becoming the trend of the future.[1] It has been shown that the pulp and paper industries, in conjunction with other newer avenues such as the production of ethanol from lignocellulosic raw materials, produce approximately 150 million tons of lignin annually. Unfortunately, the extracted lignin is burnt to produce power.[2] Several value-added applications of extracted lignin have been developed of late, but a comprehensive discussion of these applications in a single review is absent from the literature. A knowledge of these new value-added applications can significantly enhance the scope of the field of lignin research, as well as help guide similar lignin-based innovations in other industrial sectors. This chapter discusses the latest developments related to value-added uses of extracted lignin oligomers for the preparation of novel bio-chemicals and various bio-based materials, including biobased composite materials, bio-adhesives, biobased thermoset resins, bio-surfactants, with the added advantage of eco-friendliness, sustainability as well as in promoting the low-carbon economy.

4.1. Heat Energy and Power

Most of the lignin produced worldwide is combusted to generate heat and electricity, with 1 kg of dry lignin producing approximately 25 MJ of energy. This has found several industrial applications. For example, Luo and Abu-Omar used a mixture of lignin and coal as a fuel for a pulping boiler.[3] Khitrin et al. demonstrated that combustion of lignin with coal improves the boiler efficiency by 38% compared to the combustion of coal solely, plus it lowers the carbon emission by 60% (Scown et al., 2014).[3,4,5]

4.2. Pyrolysis and Syngas Production

Lignin pyrolysis is the thermal treatment and/or degradation of lignin. The temperature and time of the pyrolysis process affect the kind of products that can be obtained from specific biomass. Consequently, the resulting products could be solid biochar, bio-oils, or gases. The most important approach for lignin valorization is gasification. This process produces syngas which is a mixture of hydrogen (H_2), carbon monoxide (CO), and a very small amount of CO_2. Syngas has been used in different industrial processes, such as the synthesis of methanol to produce dimethyl ether (DME) (Figure 4.1) and the synthesis of liquid hydrocarbons (Fischer Tropsch process) to produce green diesel (Figure 4.2).[6,7] Additionally, syngas can be used in heating, cooking, and generating electricity.[6]

$$CO_{(g)} + 2H_{2(g)} \rightarrow CH_3OH_{(g)} \qquad \Delta H_0 = -90.4 \text{ kJ/mol}$$

$$2CH_3OH_{(g)} \rightarrow CH_3OCH_{3(g)} + H_2O_{(g)} \qquad \Delta H_0 = -23.0 \text{ kJ/mol}$$

Figure 4.1. Synthesis of dimethyl ether from syngas.

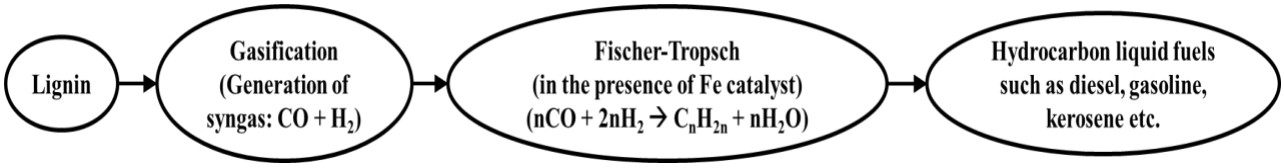

Figure 4.2. The use of lignin-derived syngas to produce diesel.

4.3. Lignin as a Binder

Water-soluble lignin salts are frequently utilized as binders in water-based printing inks, coal briquettes or ceramics, briquetting of mineral dust (fines, shavings, turnings), and wood-related materials such as plywood or particle boards.[8] Moreover, binders derived from lignin can be utilized in silicon anode for lithium-ion batteries.[8]

4.4. Lignin as Cement

Lignin-derived cement has shown economic efficiency in several studies.[9,10] Low levels of lignin can improve concrete strength and ease grinding while decreasing the effect of

moisture and acid rain on external walls. For example, charged lignin sulphonate has been used to cause repulsion between cement particles, which increases their dispersion power in the water. This lignin additive helps in decreasing the amount of mixing water needed and hence, increases the strength of concrete.[9,10]

4.5. Carbon Materials

Lignin's high carbon content makes it an ideal low-cost source to produce carbon fibers, which are widely used in sports, aerospace, and industry.[11] For example, Kraft lignin from different types of wood (soft and hardwood) has been used in the production of lignin-based carbon fibers.[11,12]

Generally, lignin-based carbon-rich materials have been used in advanced materials, energy storage, electrodes, and absorbent materials. It should be noted that energy efficiency, reduced environmental impact, and lignin abundance are the driving forces for developing lignin-based carbon materials. However, lignin heterogeneity and thermoplasticity remain major obstacles to producing high-quality lignin-based carbon materials.[13-15]

The conversion of lignin to carbon fibers usually involves five main steps. The first one is the purification and/or modification of the isolated lignin to optimize the final carbon fibers properties. The second step is the spinning process which forms lignin fibers. One example is melt spinning, which is a process in which lignin is melted and moved through a spinneret containing small holes. Solid fibers are formed when the melt exiting the spinneret is left to cool. The third step is the oxidative thermostabilization of the resulting lignin fibers by air and temperature of 200-250°C. This treatment increases the lignin fibers strength

through crosslinking to avoid their fusion in the fourth step. The fourth step is the carbonization treatment (inert atmosphere and 500-1000°C), which produces lignin-based carbon fibers. The last step is an extra step to modify and/or optimize the properties of the resulting lignin-based carbon fibers. This can be accomplished by either graphitization (>1200°C) for structural purposes or activation and/or functionalization for a target application (Figure 4.3).[16]

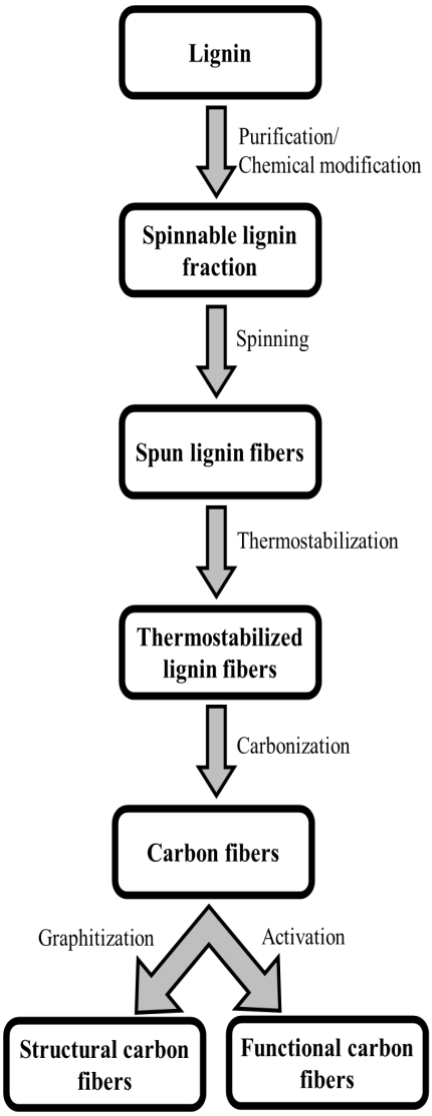

Figure 4.3. Manufacturing of lignin-based carbon fibers.

4.6. Oxidized Products

High-value products like aldehydes, vanillin, vanillic acid, aromatic acids, aliphatic acid, and cyclohexane can be derived from lignin oxidation. It should be noted that the phenolic groups of lignin ease its oxidation; however, mild oxidants like nitrobenzene, metallic

oxides, and hydrogen peroxide are often used to preserve the aromaticity of the resulting products.

Several methods have been established for lignin oxidation. The first method is organometal catalyzed oxidation, such as the use of cobalt salen ([Co(salen)]) complexes for the oxidation of several lignin model compounds such as the benzofuran shown in Figure 5.4. The mechanism for this kind of reaction usually starts with the formation of phenoxy radicals that react with molecular oxygen and form several oxidation products (Figure 4.4).[17] The second method is biomimetic oxidation, such as the use of metalloporphyrin derivatives (biomimetic systems), which can activate hydrogen peroxide for lignin oxidation (Figure 4.5).[17] In this method, the products are usually formed from the oxidation of side chains, phenolic hydroxyl groups, aromatic moieties, and cleavage in the side chain. The third method is enzyme-based oxidation, such as laccase enzyme, which can oxidize phenolic lignin group to reactive phenoxy radicals, which initiate the lignin depolymerization and/or oxidation (Figure 4.6).[17,18,19]

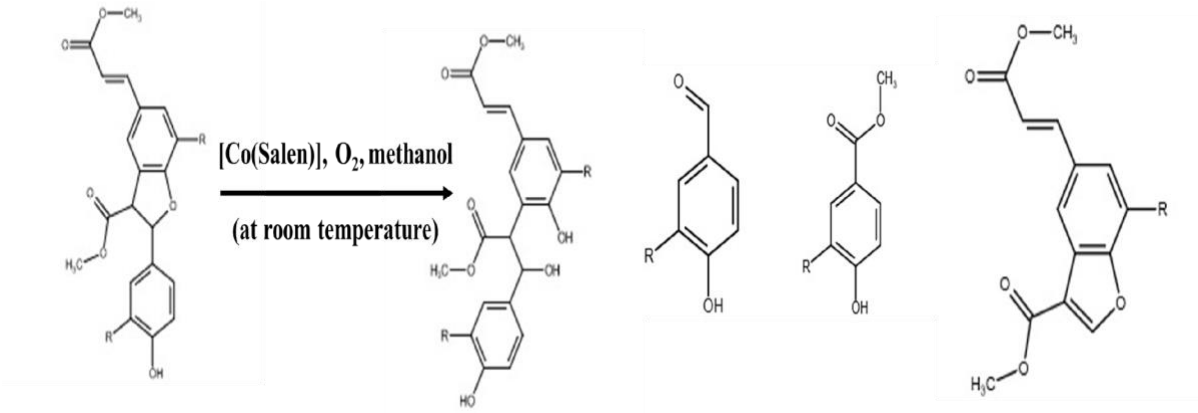

Figure 4.4. Oxidation of lignin model compounds using [Co(salen)] complexes.

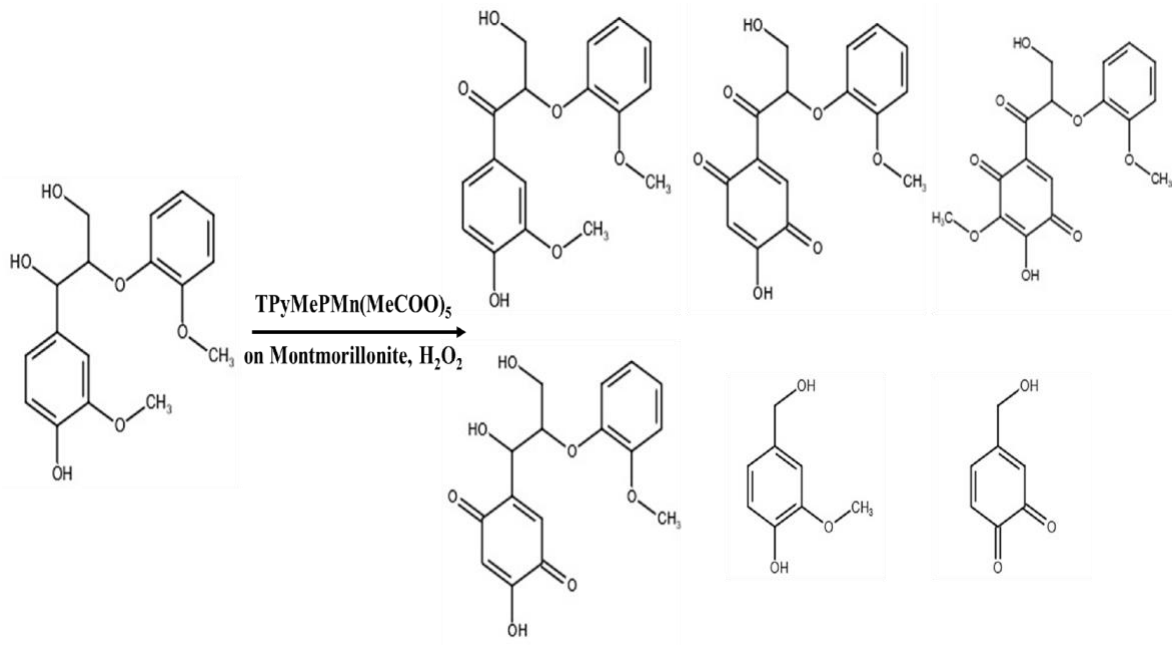

Figure 4.5. Lignin biomimetic oxidation with a Metalloporphyrin derivative.

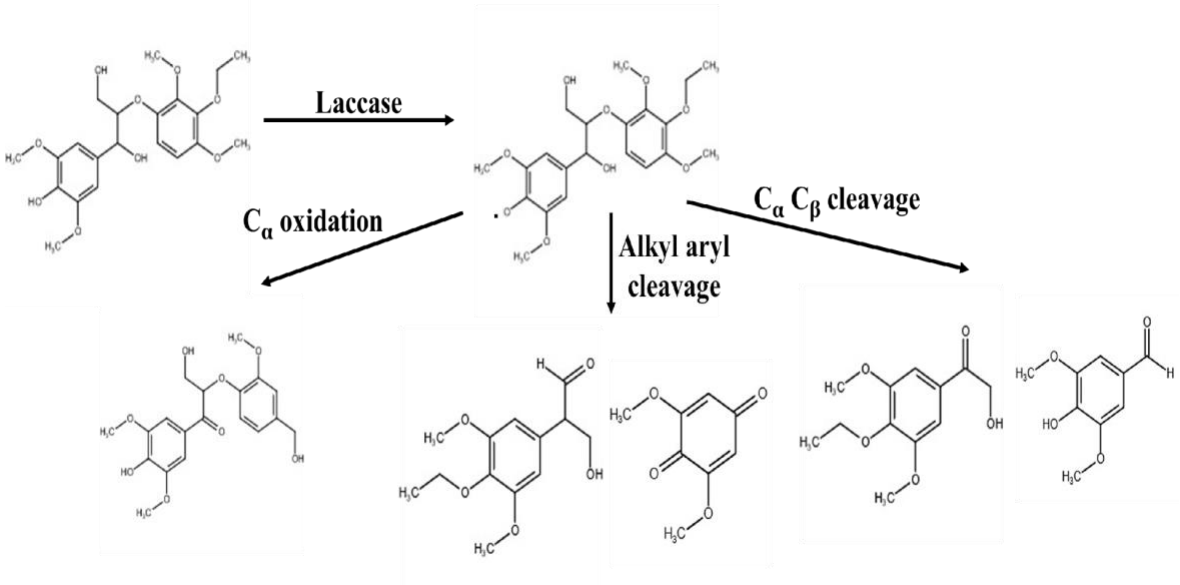

Figure 4.6. Oxidation of lignin model compound with laccase enzyme.

4.7. Hydrocarbon Compounds

Catalytic hydrogenolysis has been used to make hydrocarbons from different lignin samples.[19] This process depolymerizes lignin through the breakage of C-O and C-C bonds (Figure 4.7) to produce valuable hydrocarbons such as benzene, toluene, and xylene.[20] It should be noted that due to the complexity of the lignin structure, various catalysts may be used to achieve efficient depolymerization.[19]

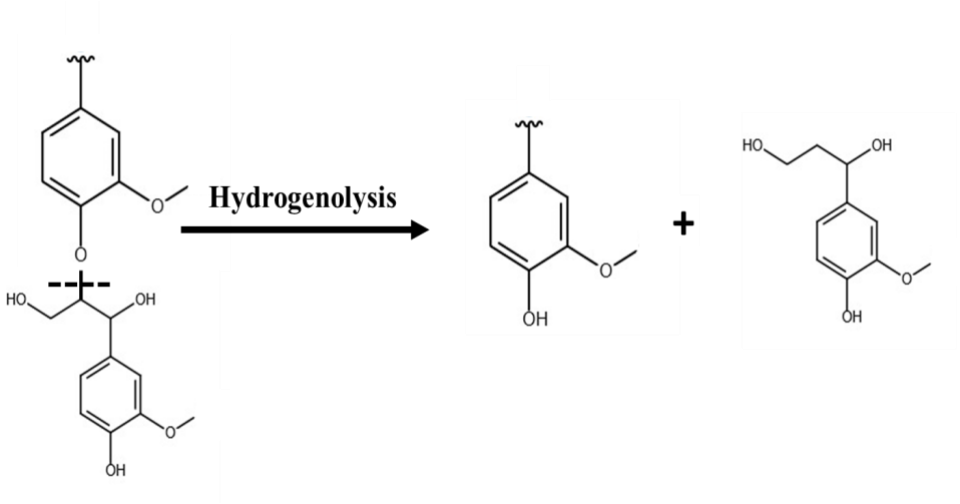

Figure 4.7. Lignin hydrogenolysis reaction.

4.8. Lignin-based Polyurethane

Lignin can be utilized in the synthesis of environmentally friendly biodegradable polyurethane. Polyurethanes have been used in several applications such as the shipbuilding industry, automotive industry, furniture insulation, and packaging industry.[23] Conventional non-biodegradable polyurethane is synthesized through the reaction between a diisocyanate and a diol. However, the polymerization of natural lignin with the resulting polyurethane will make it more environmentally friendly due to its biodegradability.[22] In general, there are two approaches for the production of lignin-based polyurethane. The first is a one-step approach that involves the copolymerization of lignin with a diisocyanate (Figure 4.8).[21] The second one is a two-step approach that involves the formation of a conventional polyurethane polymer followed by its polymerization with lignin. It should

be noted that some lignin-based polyurethanes have shown better foam properties with respect to their corresponding conventional polyurethanes.[21-24]

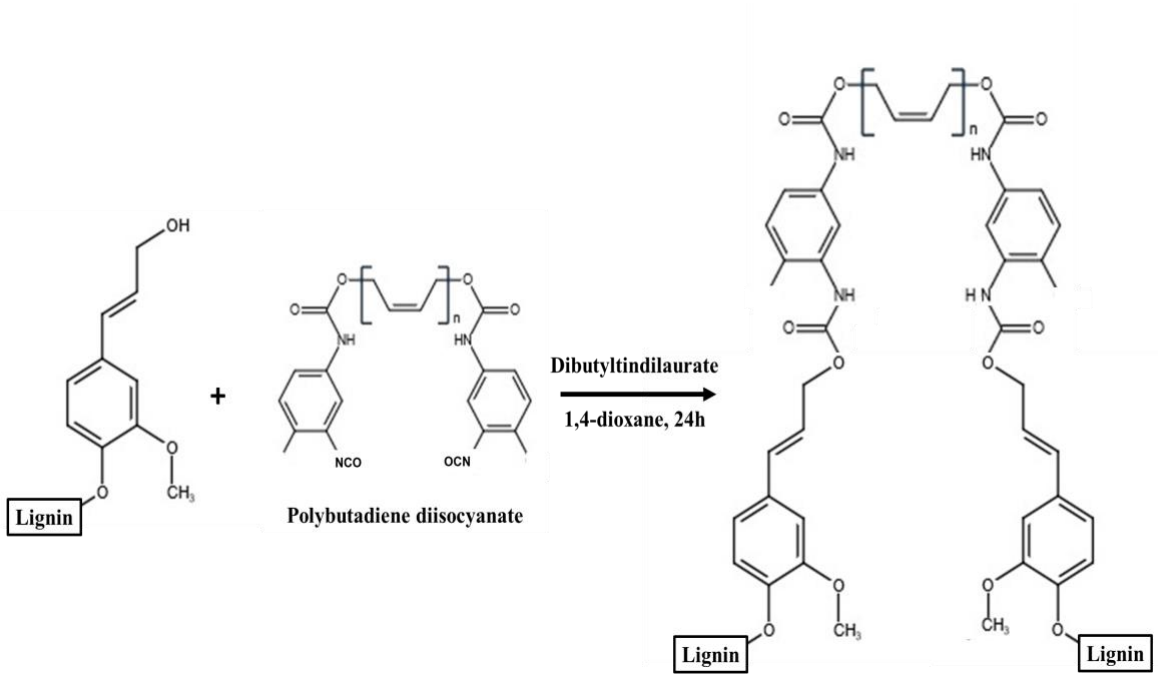

Figure 4.8. Synthesis of lignin polyurethane.

4.9. Biomedical Applications

Lignin has several promising biomedical applications. For example, lignin nanotubes (LNTs) and lignin nanowires (LNWs) are considered smart biobased nanocarriers for the delivery of DNA and therapeutic agents.[25] Also, lignin can provide protection against many diseases like cancer. Additionally, lignosulfonates antioxidant activity allowed them

to be introduced in drugs to stimulate the immune system of the body.[26,27] Lastly, it has been reported that lignin-based hydrogels can be used in drug delivery, wound care, tissue engineering, and contact lenses.[28] It should be noted nanosized lignin has been used in hydrogel technology due to its biobased nature, biocompatibility, biodegradability, enzymatic degradation, and safety.[25-28]

4.10. Fire Retardant

Lignin can act as a natural flame retardant. It was reported that lignin could decrease the flammability of polymers such as polypropylene, PBS, ABS, and PET.[29] These lignin-based polymers showed reduced flammability and smoke release. For example, adding 30 wt% of CP-Lignin to polybutylene succinate (PBS) reduces its flammability and improves its biodegradation. The CP-Lignin is synthesized from cyanuric chloride (CNC), 2,6,7-Trioxa-1-phosphabicyclo-[2,2,2]octane-4-methanol-1-oxide (PEPA), and lignin, as shown in Figure 4.9.[29,30]

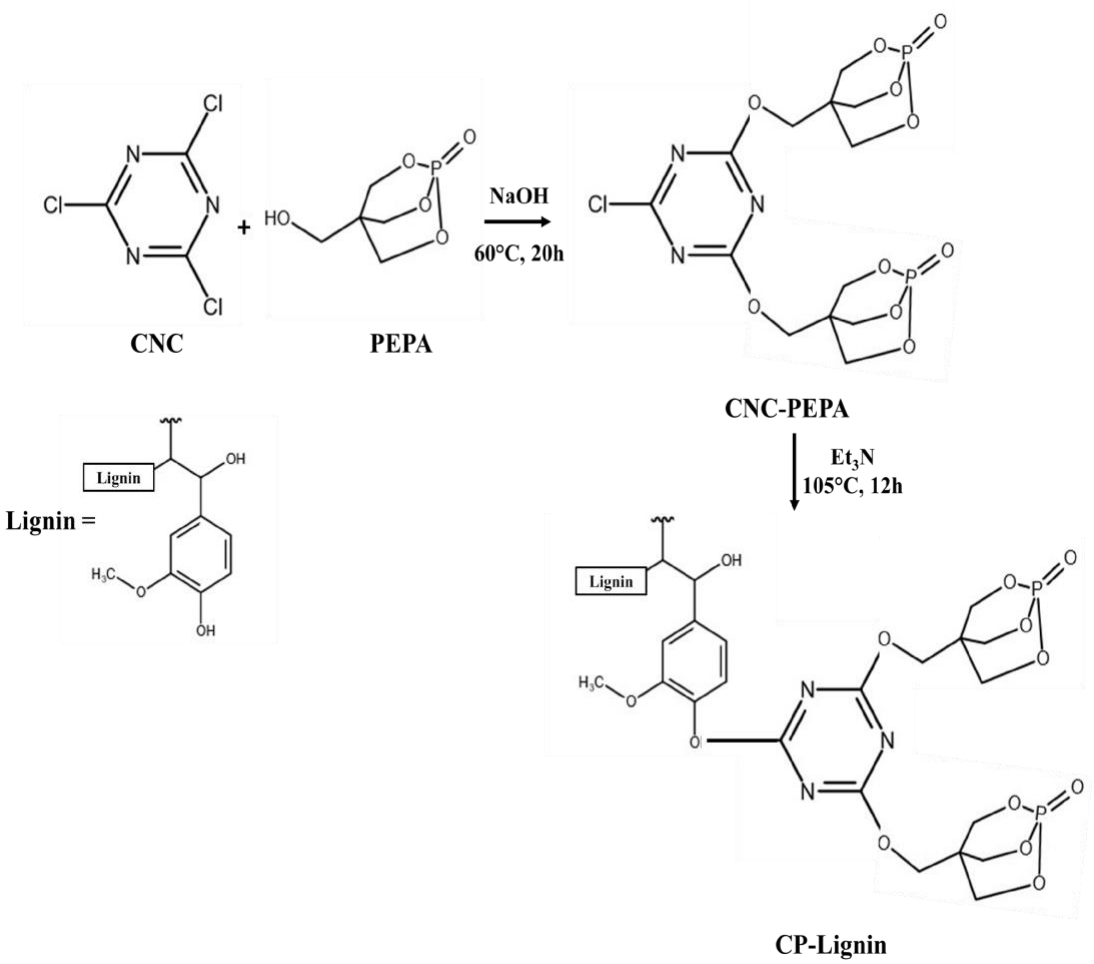

Figure 4.9. Synthesis of the flame-retardant CP-lignin.

4.11. Lignin as a Sequestering Agent

Chemical modification of lignin structure can change its properties to be used in the removal of heavy metals.[31,32] For example, lignosulphonates can trap harmful metals from soils efficiently through complex formation. Also, lignosulphonate-g-acrylic acid hydrogels can adsorb contaminants and dyes from wastewater. It should be noted that

oxidative modifications of lignin increase the number of carboxylic acids and/or hydroxyl groups (Figure 4.10).[31] These modifications increase lignin hydrophilicity and capability of complex formation with various toxic metals such as Cd^{2+}, Pb^{+2}, etc.[31-33]

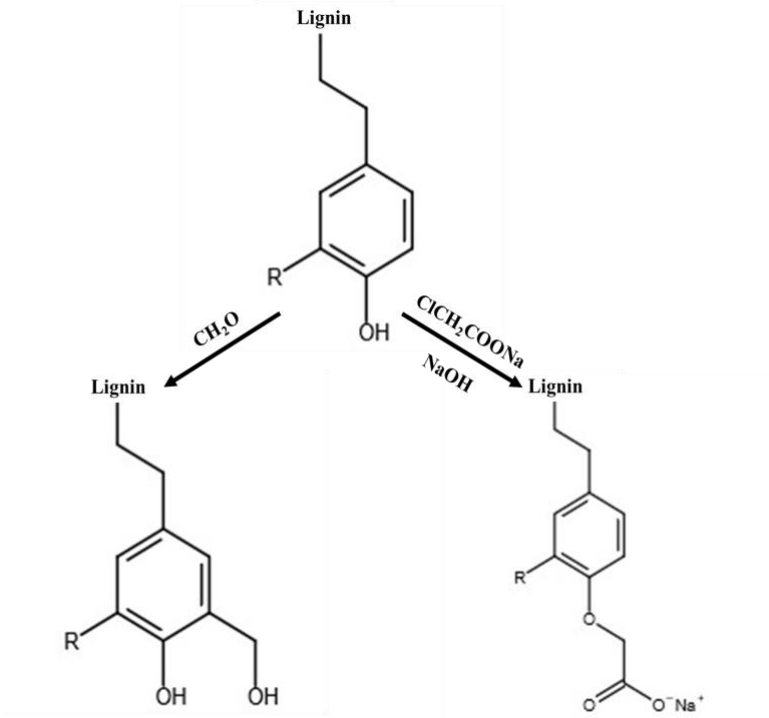

Figure 4.10. Modification of lignin with carboxyl and hydroxyl groups.

4.12. Energy Storage

It has been reported that lignin used in energy storage devices improves their efficiency and reduces their toxicity (making them eco-friendly). For example, lignosulfonate has been used as an additive in the negative paste of a lead-acid battery, which increases its efficiency and life cycle.[34] Also, lignin has been used in making environmentally friendly electrochemical capacitors and super-capacitors.[36,38] Moreover, a mixture of lignin and conducting polymers have been used for solar cells and fuel cells.[34-41]

4.13. Application of Lignin in Aquaculture

Hydrolysis lignin is the left-over lignin material from industrial conversion processes such as bioethanol or biogas production from agricultural waste (plant material).[42] Hydrolysis lignin has over 60 % (w/w) of lignin, in addition to remnant cellulose and monosaccharide and oligosaccharide by-products.[42] Because of this composition, hydrolysis lignin has recently been explored for improving fish feed utilization in aquaculture.[43] The cost of fish feed in aquaculture is a major challenge in the area.[43] Meanwhile, the use of lignin in animal diets reportedly improves the digestion and utilization of feed in animals, thereby helping to reduce feed costs. Therefore, Colombo et al. studied if using hydrolysis lignin as a feed for Atlantic salmon affected their growth performance and feed conversion ratio.[43] Their results suggest that hydrolysis lignin could prove to be useful in improving the growth performance of fish in aquaculture.

4.14. Lignin Products - Life Cycle Assessment Future Work

To check the safety of lignin commercial products, its environmental impacts should be investigated. Life cycle assessment (LCA) is a powerful tool that can be used to assess the environmental impact of a product. LCA assesses the impacts created by a product during all its stages, from production to disposal. Due to the presence of a vast number of lignin applications and/or products, it is crucial to certify that these products have the lowest impact on the environment before being commercialized. Examples of these impacts are greenhouse emissions, ozone depletion, smog, acidification, eutrophication, and fossil fuel depletion. To date, there are very few LCA studies that have been conducted on lignin-based products, with the majority focusing on phenolic compounds and fuel.[44]

For example, Corona et al. compared the environmental impacts of adipic acid produced from two sources: fossil fuel and lignin.[45] This study showed that lignin-based adipic acid has a low environmental impact compared to conventional fossil-based adipic acid. The bio-based adipic acid showed a carbon emission 78% lower than conventional adipic acid. In the same manner, Montazeri and Eckelman showed that lignin-based catechol is better than fossil-based catechol in terms of global warming potential, ecotoxic effects, and fossil fuel depletion, respectively.[46] Similarly, LCA of different lignin-based products discussed in this thesis will be an interesting project for future work.

4.15. Summary

This chapter discussed the latest developments related to value-added uses of extracted lignin oligomers for the preparation of novel biochemicals and various bio-based materials, with the added advantage of eco-friendliness, sustainability, as well as promoting the low-carbon economy. Lignin use has graduated immensely from the days when lignin was only used as fuel to generate heat energy and power. Today, lignin is used to produce syngas and green diesel. It is also used as binders in printing inks and ceramics and as a source of numerous chemicals such as aldehydes, aromatic organic acids, and organic solvents like benzene, toluene, and xylene. As an additive, lignin is known to improve the strength of concrete, enhance the biodegradability and mechanical properties of polymers like polyurethane, and reduce the flammability of polymers. At the same time, lignin derivatives, like lignosulfonates, find applications in sequestration of harmful chemicals, development of batteries and supercapacitors, and as antioxidants in the biomedical industry. In the biomedical sector, lignin has also been explored for the delivery of therapeutic agents as nanocarriers or through hydrogels. Recently, lignin has also been used in aquaculture to improve the feeding performance of fish. Thus, lignin is a multi-dimensional wood component that has numerous applications in diverse industries. This paves the way for the development of additional innovative applications of lignin in industry, while also emphasizing the need to develop high-yielding lignin extraction methods, such as the ones discussed in Chapter 3.

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Chapter 5: SYNTHESIS OF CROSS-LINKED LIGNIN-EPOXY RESINS

5.1. Introduction

Specific applications of lignin may require it to have certain unique physicochemical characteristics. This is especially true when lignin is used as a component in a copolymer with thermosetting plastics.[1,2]

Lignin is considered a suitable polymer for incorporation in thermosetting plastics because of its rigidity and brittleness as well as the presence of multiple types of functional groups.[2] This latter property also makes it possible to readily modify the physicochemical and mechanical properties of lignin. For example, the viscoelastic behaviour of lignin can be modified by synthesising hydroxyalkyl lignin derivatives. One study found that hydroxypropylation of lignin led to a significant reduction in its glass transition temperature.[1] Hydroxyalkylation also makes lignins more soluble[1] while also improving their mechanical properties[3]. It further facilitates the copolymerization of lignins with monomers of common thermosetting plastics.[1]

In one study, hydroxyalkyl lignin derivatives were copolymerized with ethylene oxide, propylene oxide and butylene oxide, with the overall lignin content of the copolymers ranging from 50 % to 70 %.[1]

The synthesis of epoxy lignin derivatives to form resins or plastics has also been explored in literature.[2] Epoxy resins are plastics that find wide-ranging applications in the adhesive, appliance and coating industries, among others.

Like hydroxyalkylation, epoxidation of lignins can also enhance their mechanical properties. For example, one study found that increasing the amount of lignin in lignin-epoxy resins increased the mechanical stiffness and ultimate tensile strength of the material.[2]

In another study, lignin-polyether-copolymer-epoxide/amine resins were synthesised and it was found that this copolymer had distinct vitrification properties.[4] It vitrified quicker when the lignin content was high and vice versa.[4] Epoxidation of lignin also increases polymer viscoelasticity and Young's modulus.[5] Similarly, lignin-epoxy resins were found to have improved adhesion strength, making a strong case for using lignin-epoxy resins as adhesives.[6] Lignin-epoxy resins are also good sources for the production of biopolyesters, and show high potency for replacing petroleum-derived polyesters.[7]

Thus, the introduction of epoxide in lignin can modify physicochemical properties of both the resulting lignin derivative and its copolymers with thermosetting plastics. There are various methods that can be used to introduce epoxy groups in lignin, two of them being the use of epichlorohydrin (ECH) and polyethylene glycol diglycidyl ether (PEGDGE).[8] Different epoxy sources (ECH and PEGDGE) can result in significant variations in the physicochemical properties of the lignin-epoxy resins formed. However, to the best of our knowledge, a holistic study of these variations comparing multiple epoxidation methods is

absent from the literature. Thus, the primary objective of this study was to synthesise, and study the effect of reaction parameters on the production of, cross-linked lignin epoxy resins (with ECH and PEGDGE) and acetylated cross-linked VRL-(PEGDGE) resins using oak VRL.

5.2. Methodology for Preparing Lignin-Epoxy Resins

Lignin-epoxy resins were synthesised following a method described previously by Gioia et al.[9] Briefly, oak lignin VRL (300 mg) was added to 45 mL of an aqueous solution of acetone (50 % v/v) in a round bottom flask. Thereafter, sodium hydroxide (moles 3× the number of equivalents of active hydroxyl groups in the VRL) and epichlorohydrin (ECH; moles 20 times the number of equivalents of active hydroxyl groups in the VRL) or polyethylene glycol diglycidyl ether (PEGDGE) were added to this mixture. The resulting mixture was magnetically stirred for 5 h at a temperature of 55°C. Subsequently, the mixture was diluted with 40 mL water and its pH was set at 3.5 using 0.1 M HCl. This resulted in the formation of a precipitate. The precipitate was separated from the rest of the mixture by filtration using a glass filter of pore size 4. It was then washed with deionized water (2×) and further dissolved in acetone. Thereafter, deionized water was used to precipitate the product. Finally, the aqueous dispersion was lyophilized, and a brown powdery substance was obtained.

The general scheme of formation of the lignin epoxy resins is shown in Figure 5.8, in which ECH and PEGDE were used for the epoxidation reaction on the C-4 hydroxylate group, which is created by alkaline hydrolysis of the phenyl coumaran unit.

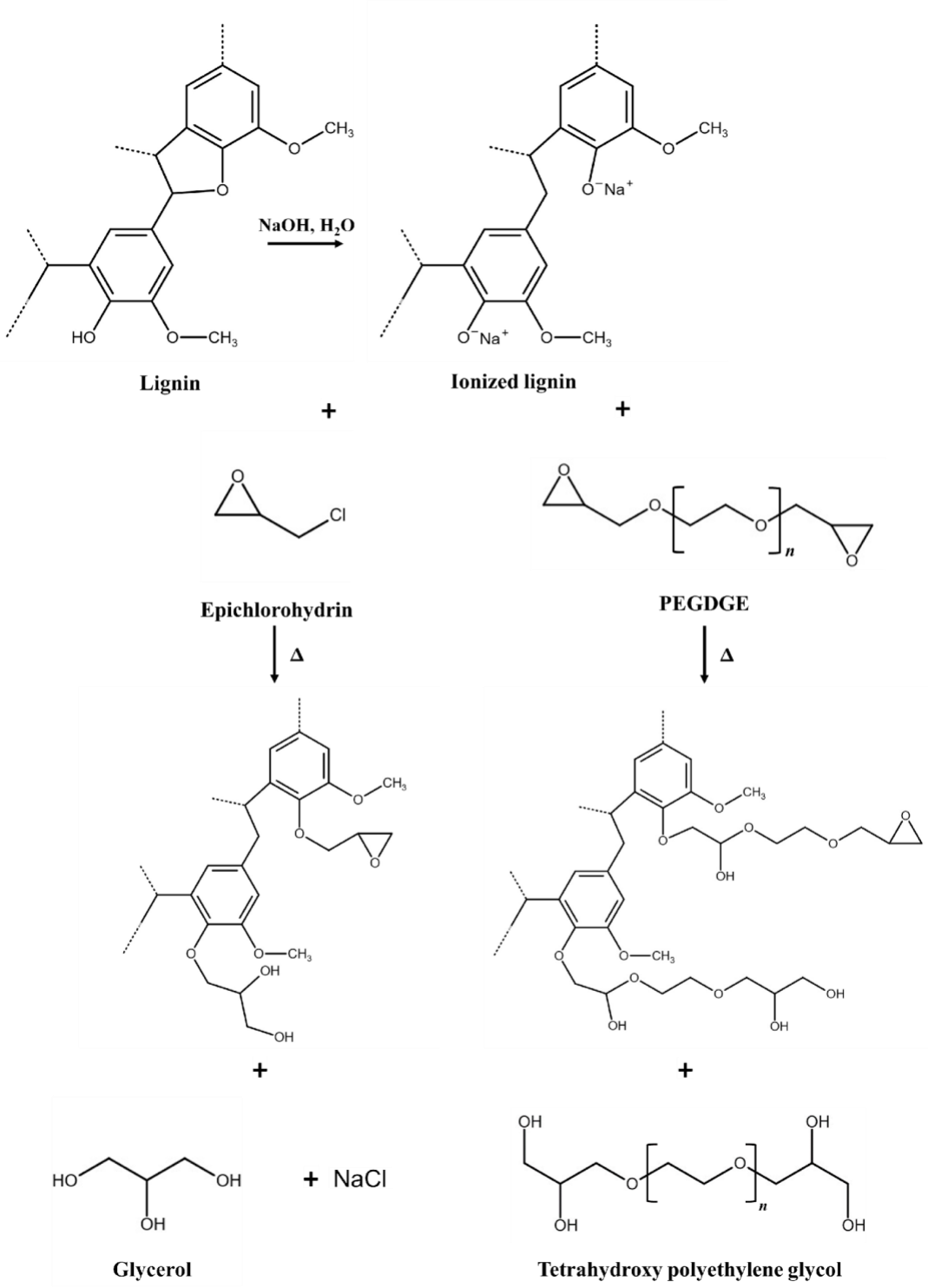


Figure 5.1. Synthesis of the Cross-linked Lignin-Epoxy Resins.

The product obtained is a black solid, which is insoluble in all types of solvents. The filtered solid is washed extensively with water. Please note that VRLs are mixtures of infinitely possible combinations of straight chains oligomers. Therefore, it is virtually impossible to calculate the exact number of moles of VRLs being used in a given reaction. This is why when we say that we have used equimolar quantities of, for example, epoxide and VRLs, we mean that we have used equal weights of epoxide and VRLs.

5.3. Results

This sub-section examines the reaction of oak VRL with ECH and PEGDGE to synthesize the straight-chain lignin-epoxy resin.

5.3.1. Reaction Time

ECH is an extremely versatile reagent that contains an epoxide functional group and is used to manufacture epoxy resins. On the other hand, the ECH-PEGDE copolymer possesses two epoxide functional groups (Figure 5.2).[10]

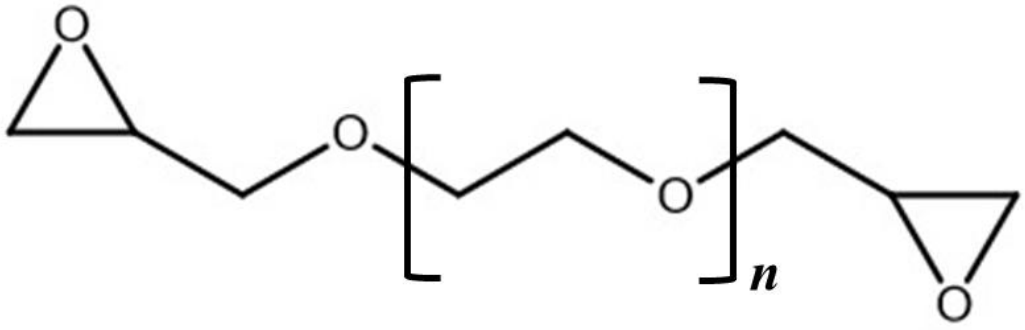


Figure 5.2. Polyethylene glycol diglycidyl ether (PEGDGE)

In general, the treatment of epichlorohydrin in an aqueous solution of sodium hydroxide (pH > 12) resulted in the formation of an emulsion due to the low solubility of the formed glycidol. The reaction medium became monophasic after three hours of reaction. This was reflected by the formation of released glycerol, which was completely soluble in the medium, as has also been reported previously (Figure 5.3).[11]

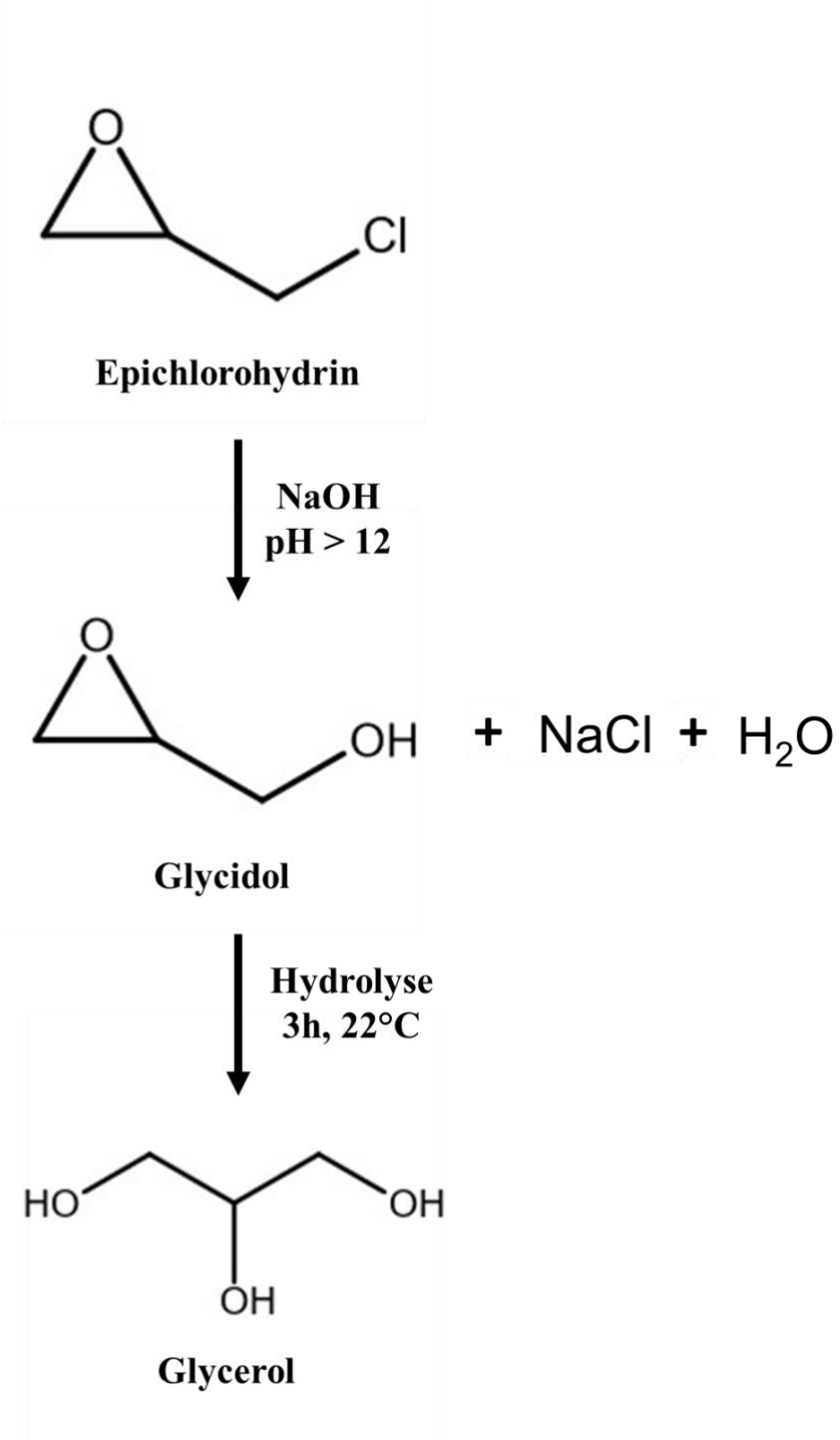


Figure 5.3. Hydrolysis of epichlorohydrin in a basic aqueous medium.

Similarly, PEGDGE was also hydrolyzed in a basic medium to form the derivative of tetrahydroxy polyethylene glycol or "tetrahydroxy-PEG" (Figure 5.4). However, the hydrolysis of PEGDGE was much slower than that of ECH, and it took almost two hours to be completed, as has also been reported previously.[12]

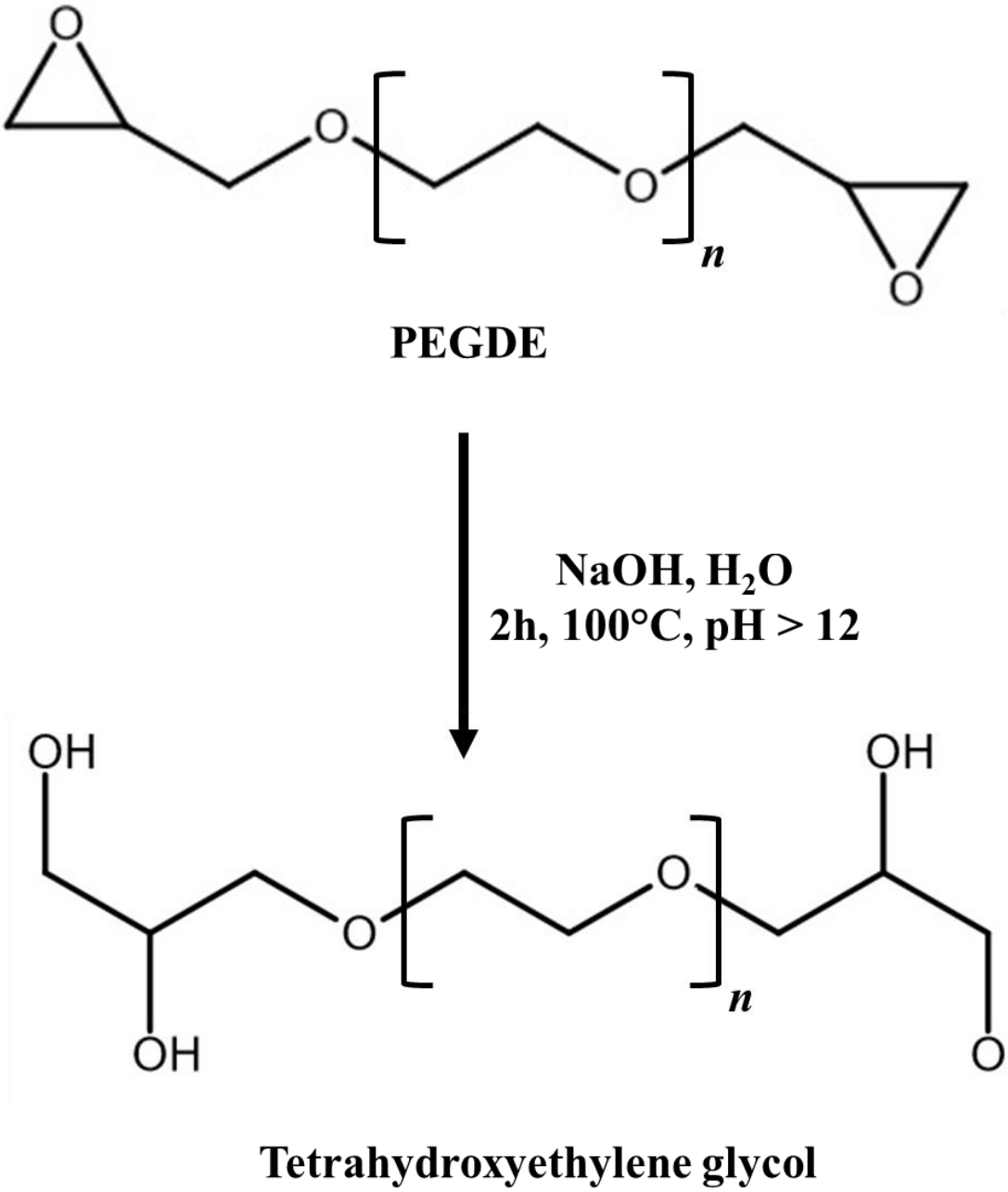


Figure 5.4. Basic hydrolysis of PEGDGE.

5.3.2. Optimizing the Reaction Conditions and Verifying the Product

Considering that hardwood lignin contains around 6% of phenylcoumaran structures[13], we decided to investigate the epoxidation reaction of the Newfoundland oak lignin. In general, hardwood lignin contains several functional groups as shown in Figure 5.5.[14]

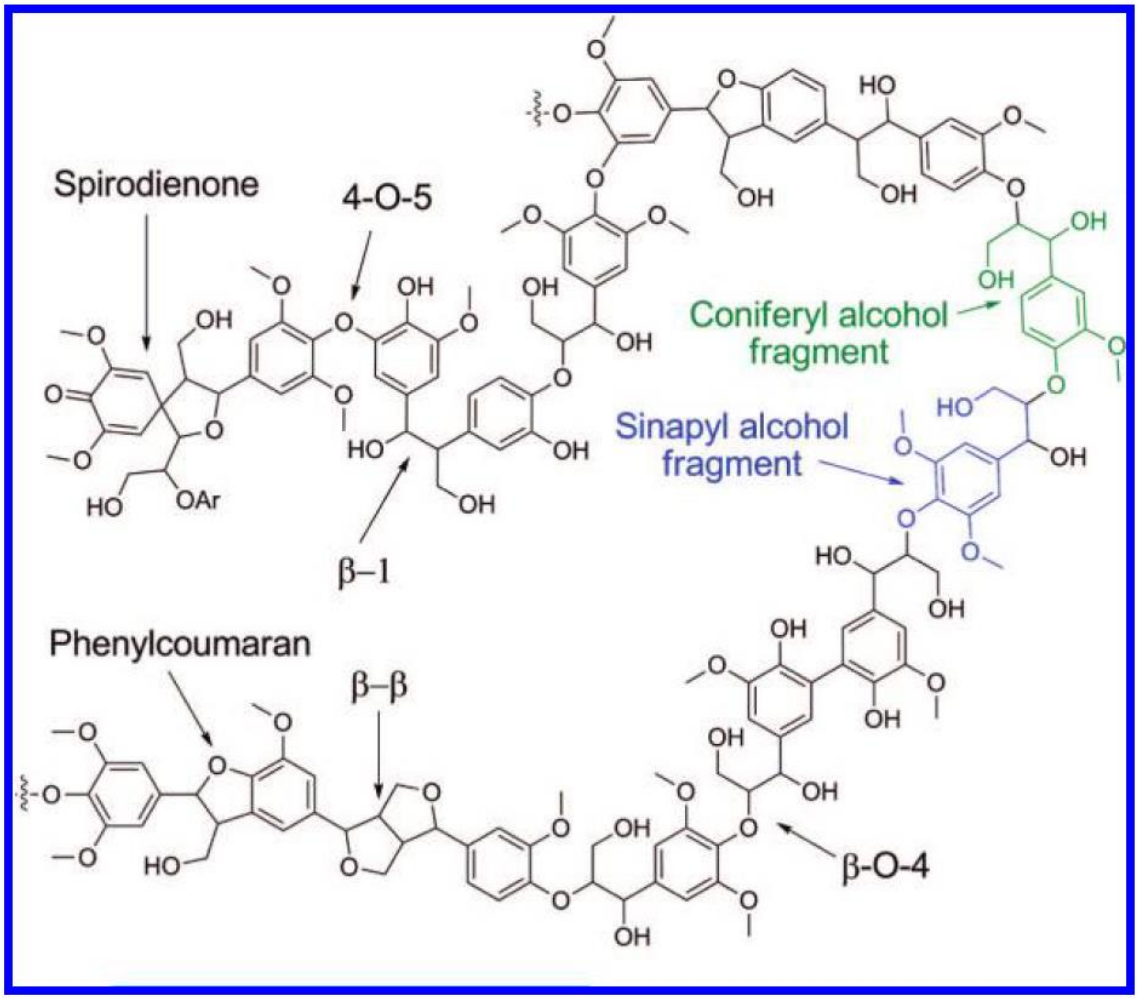


Figure 5.5. General structure of hardwood lignin. Reproduced with permission from *Chemical Reviews*, *110*(6), 3552-3599.

The reactions of oak VRL with both ECH and PEGDGE epoxide donors seem to be driven by the nucleophilic substitution of chlorine atom of the epichlorohydrin with the phenyl functional group of the lignin with the production of HCl, as shown in Figure 5.6.[15, 16]

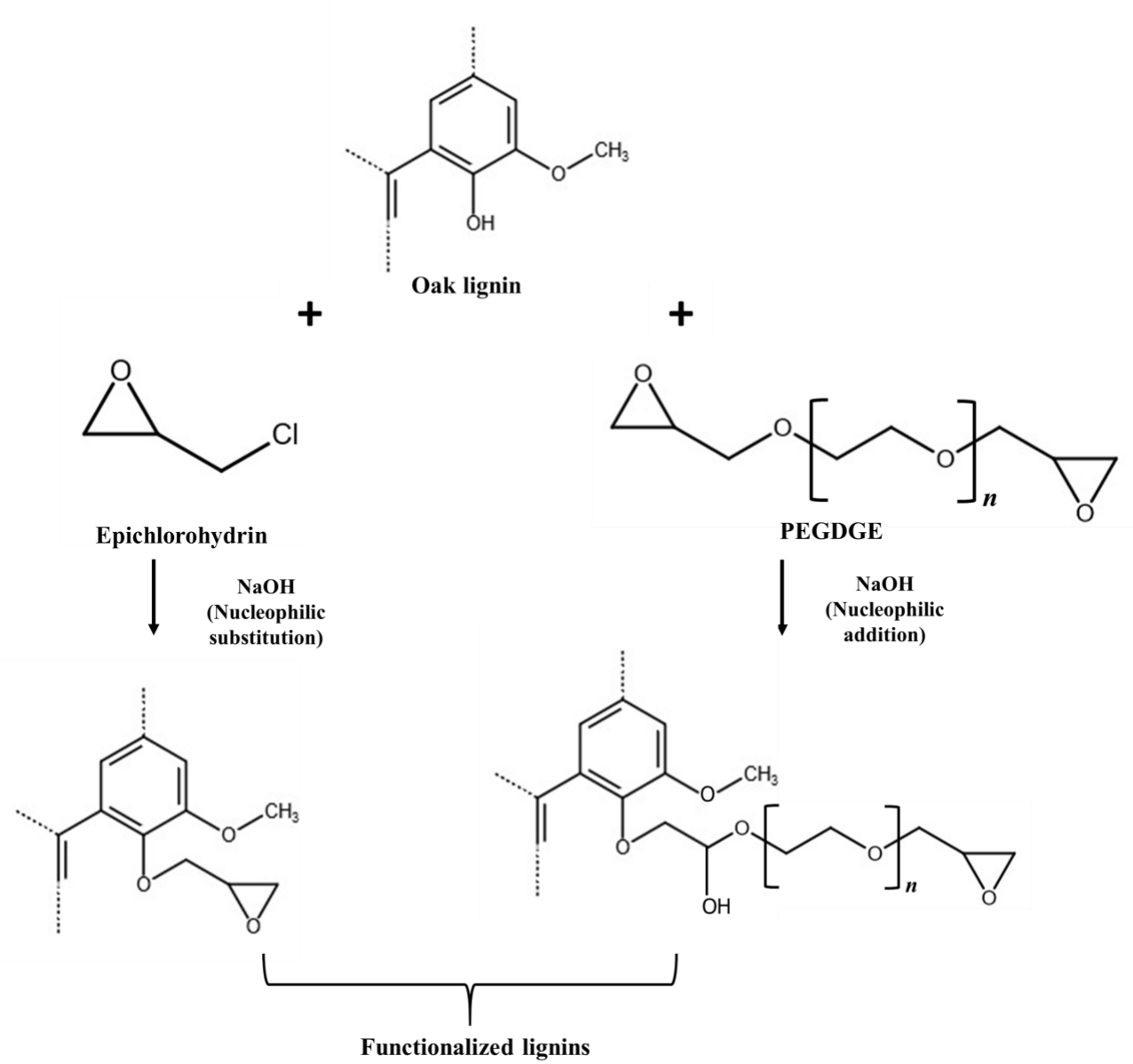


Figure 5.6. Proposed reaction mechanism of the lignin-PEGDGE Resin.

We noticed that the best conditions of this epoxidation reaction occurred when oak VRL was totally solubilized in an aqueous medium with a pH > 11.5. When the oak VRL was dissolved in an aqueous solution of sodium hydroxide (pH≈12), and the resulting solution was subjected to evaporation, we obtained a solid.

When the solid was analyzed by ^{13}C-NMR and compared with the original VRL, we noticed an enhanced conversion of the phenolic -OH groups into sodium phenolates.

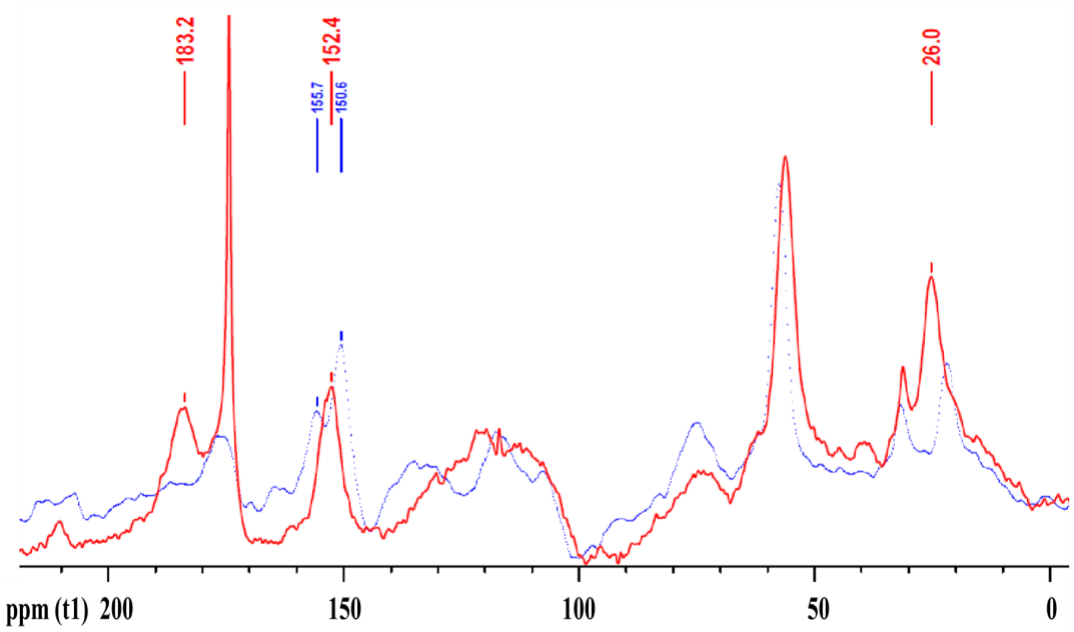


Figure 5.7. ^{13}C NMR spectra of the solid of Oak Lignin (in blue) and Ionized Oak Lignin (in red).

Accordingly, the ^{13}C-NMR spectrum of the ionized VRL (sodium phenolate) shown in red is substantially different from that of the original oak lignin (Figure 5.7). The diagnostic signal at 152.4 ppm indicates the presence of the C4 carbon of sodium phenolate. It should be noted that all VRL C-4 resonances, which were initially etherified in the form of the phenyl coumarin bicyclic structures, are hydrolyzed in alkaline media to produce C4 phenolates (Figure 5.8).

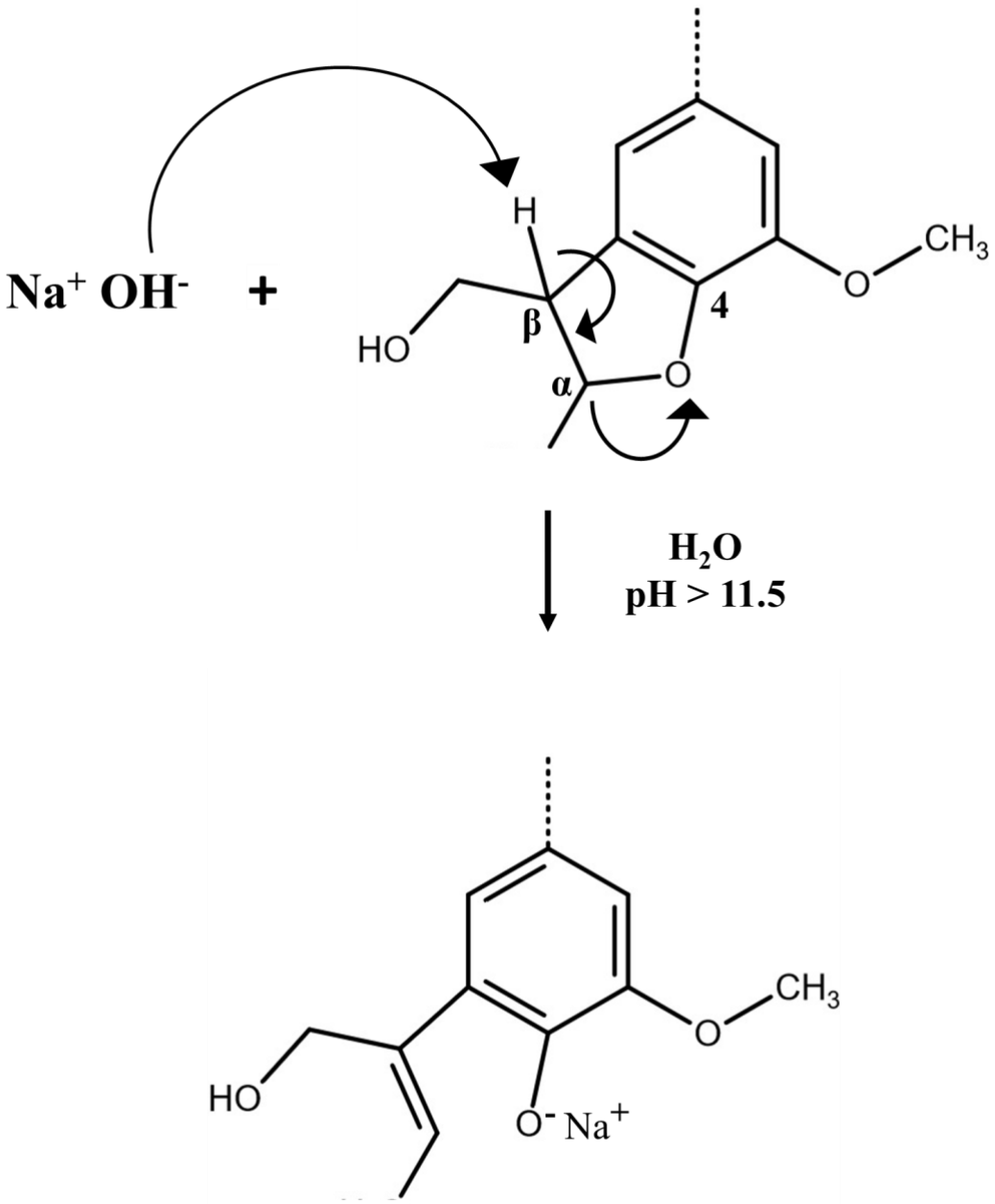


Figure 5.8. Opening of an etherified phenol in C4 in basic medium.

It was noted that the ^{13}C-NMR spectrum of the solid ionized oak lignin VRL contained all the organic functions of the extracted native oak lignin VRL, except they resonated at slightly higher chemical shifts. We attributed the signals at 183.2 ppm and 26 ppm to carbonyl C=O groups of esters or carboxylic acids and aliphatic carbons. In general, all original functional group resonances possessed slightly higher chemical shifts.

After NMR-based verification of the reaction product, an investigation of the formation of cross-linked epoxy resins was conducted on oak VRLs. We studied the epoxidation reaction more closely by varying several reaction parameters, including the type of epoxide used, temperature, and the molar ratios of reagents to reactants. The following sub-sections indicate how we have used different conditions for the synthesis of VRL cross-linked epoxy resins.

5.3.2.1. Changes in Temperature of the Epoxidation Reaction

Epoxidation reaction was conducted at different temperatures using equimolar quantities of 1:1 of the epoxide and VRLs. Gel formation dynamics of the reaction were monitored. As such, gelling is considered to be completed when the reaction medium cannot be stirred. The gelling time of the reaction medium is shown in Figure 5.9.

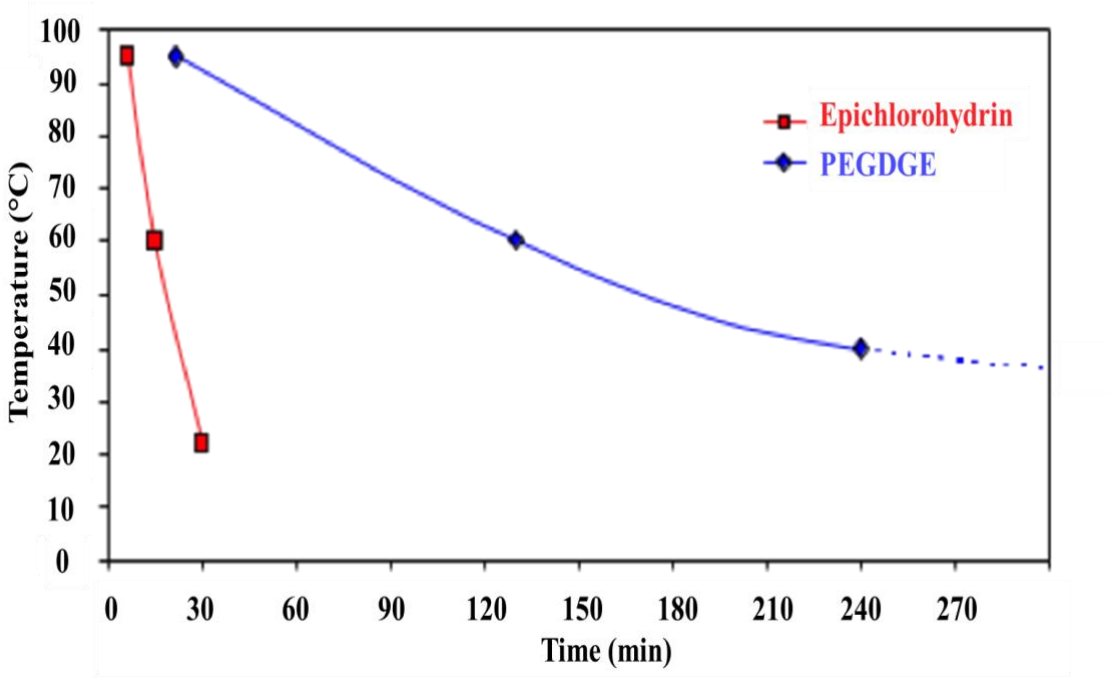


Figure 5.9. Gelation time of the reaction medium as a function of the temperature and the epoxide used.

It was observed that for epoxidation using ECH, gelation was affected rapidly at 22°C in less than 30 minutes. However, we found that the epoxidation reaction was slower with PEGDGE. Accessing phenolate anions of the oak lignin on the epoxide functional groups is likely to be more difficult for PEGDGE than for ECH. This can be explained by the presence of steric hindrance between the glycol chains of the PEGDGE and the structure of the oak VRLs.

Epoxidation can also occur on other free phenolic and aliphatic hydroxyl groups of the oak VRLs, thus forming a three-dimensional network. This is reflected by the rapid increase of the viscosity and the gelling of the reaction medium. Furthermore, we noticed that the rate of gelation / crosslinking increased rapidly when the reaction medium was heated to 95°C. For this reason, we established that cross-linking was better achieved at room temperature (≈18°C) for ECH, whereas at 60°C with PEGDGE.

5.3.2.2 Altering the VRLs to Epoxide Molar Ratio

It was established that when the VRL to epoxide ratio was changed, we were able to obtain better cross-linked VRL-Epoxide Resins, which contain more grafted epoxide groups. Henceforth, the gelling time increases in proportion to the amount of epoxide added (shown in Figure 5.10).

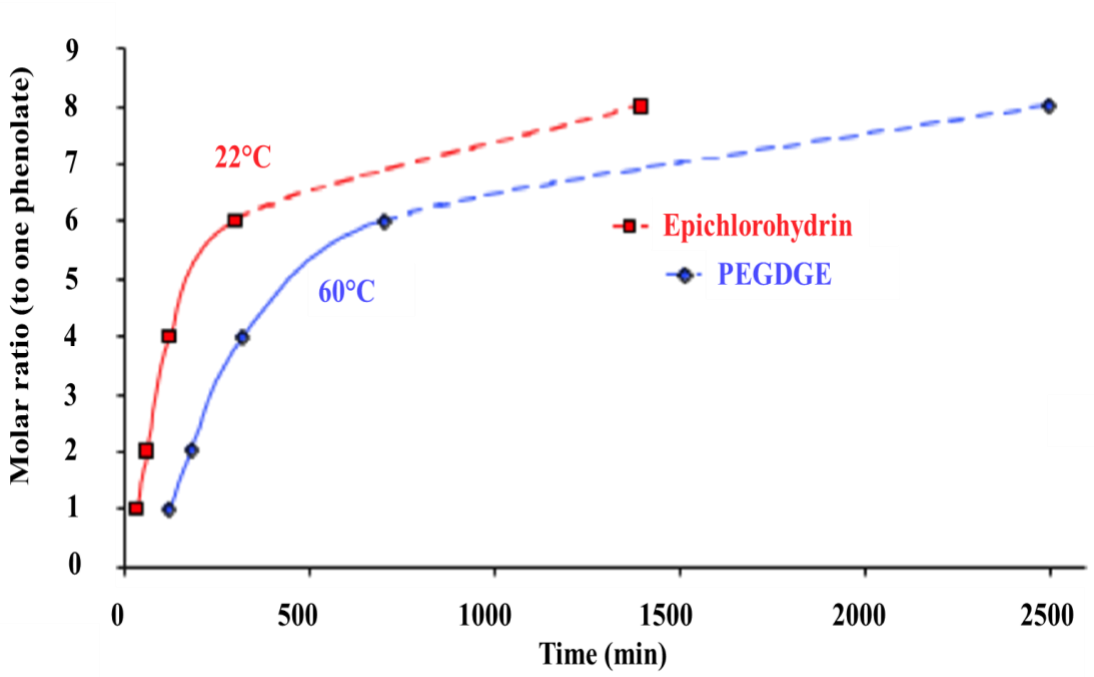


Figure 5.10. Change of VRL/Epoxide Ratio and Gelation Time.

A comparison of the physical properties of the cross-linked lignin epoxy resins synthesized with ECH and PEGDGE is shown in Table 5.1.

Table 5.1. Cross-Linked VRL-Epoxy Resins Physical Properties.

Phenol to epoxy ratio	**ECH**	**PEGDGE**
1:1	Black and friable solid	Strong black flexible and very resistant

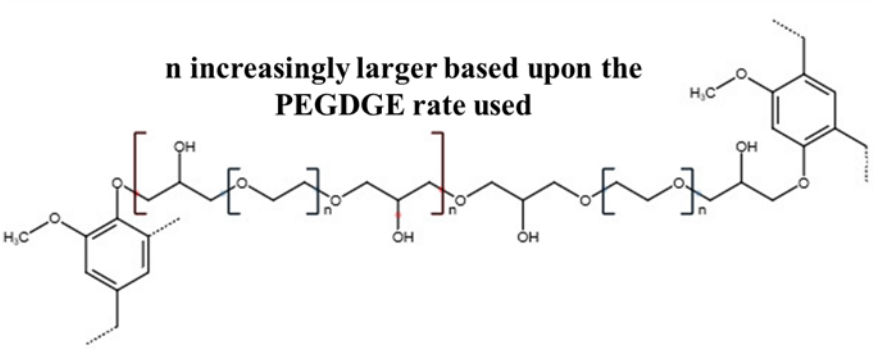
1:2	Black and friable solid	Strong and supple black solid
1:4	Black and friable solid	Strong black elastic and brittle
1:6	Black and brittle solid	Sticky black solid and brittle
1:8	Black and brittle solid	Black liquid very viscous and sticky

5.3.2.3. Synthesis of Acetylated Cross-Linked VRL-PEGDGE Resins

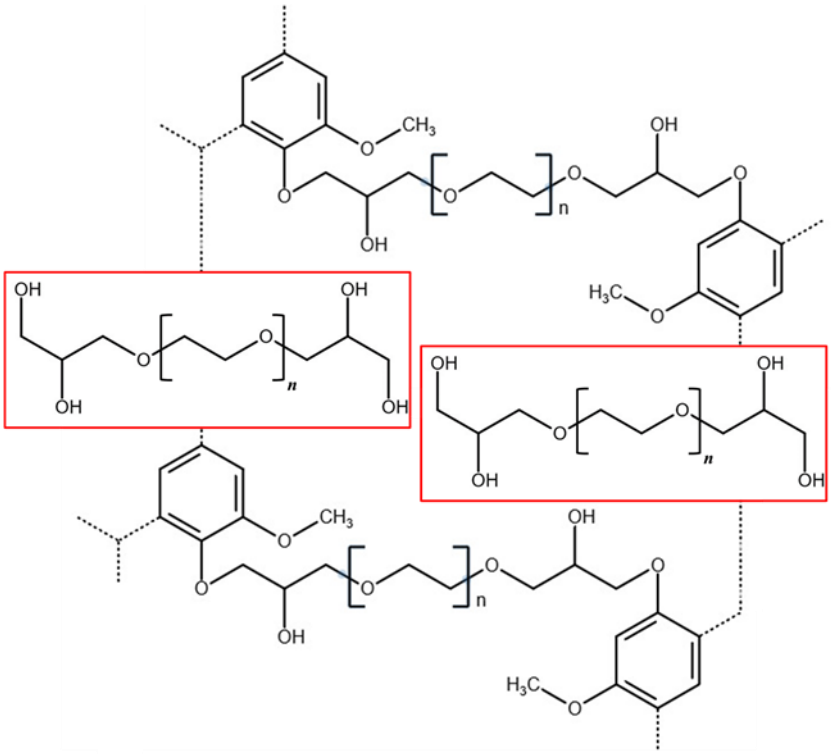
We found that acetylation of the cross-linked VRL-PEGDGE resin could be carried out at room temperature with an excess of acetic anhydride and pyridine for 72 hours. The success of the acetylation process was checked by the comparing the IR spectrum of the acetylated cross-linked VRL-PEGDGE resin with the non-acetylated cross-linked lignin PEGDGE resin. The acetylated sample showed the presence of the characteristic acyl peaks at 1734. 8 cm^{-1} (C=O) and 1220.7 (C-O), which were completely absent in the non-acetylated sample.

Upon extraction, the acetylated cross-linked VRL-PEGDGE resin was soluble in dichloromethane. The dichloromethane extract was washed with aqueous solutions of hydrochloric acid and water. During the synthesis of this cross-linked VRL-PEGDGE resin, we observed that when the ratios PEGDGE resin were higher with respect to the VRL, the resins obtained became more flexible. This later became a sticky paste when the VRL to

PEGDGE ratio was 1:8. At this point, the cross-linked VRL-PEGDGE resin contains several tetrahydroxy-PEG molecules (linker), which could act as a template for more introduction of VRL on both sides of this linker epoxy chain to afford a polyblend type epoxy resin. In addition, the self-polymerization of PEGDE can also occur, and consequently, the length of the PEG chain will increase, giving a more complex polyblend structure. These two hypotheses have been schematized in Figure 5.11.



Lengthening of PEG-type chains in lignin resin



Tetrahydroxy PEG molecules intercalated in lignin resin

Figure 5.11. Proposal of two different structures of lignin PEGDGE resins depending on the phenol epoxy ratio.

Please note that in this thesis, when the reaction between PEGDGE and oak VRL was performed with a ratio of 1:6 (VRL/PRGDE) for 8 hours at room temperature, the precipitate obtained was deemed to be the cross-linked VRL-PEGDGE resin. However, when the dichloromethane solution was assessed by infrared spectroscopy, it was found to contain the open epoxide product of the original PEGDGE identified as the tetrahydroxy-PEG. It is important to mention that all cross-linked VRL-PEGDGE resins obtained had almost similar molecular masses, regardless of the epoxide amount used.

Overall, it can be concluded that the alkaline ionized oak VRL mixture reacts with the PEGDGE epoxide donor by nucleophilic addition of the epoxide functions of PEGDGE on the ionized phenate groups of the VRL mixture. Hence, the phenolic functions are grafted onto the epoxide PEG-type groups. In addition, the PEGDGE molecules can polymerize with each other and yield increasingly lengthy PEG-type chains.

5.4. Conclusions

In this study, epoxy resins of oak lignin VRL were synthesized using ECH and PEGDGE. Resin formation with ECH required lower gelation times, whereas that with PEGDGE required higher gelation times. However, the mechanical properties of VRL-PEGDGE resins were better than VRL-ECH resins. The lignin-epoxy resins formed with ECH were friable or brittle at all phenol to epoxy (that is, VRL to ECH/PEGDGE) ratios tested, whereas those formed with PEGDGE were strong, flexible and resistant from 1:1 to 1:4

ratios, and became highly viscous and adhesive at lower ratios (1:8). Further, acetylation of VRL-PEGDGE resins made the product soluble in dichloromethane, suggesting a change in physicochemical properties. The differences in the physicochemical properties of the lignin-epoxy resins formed with ECH and PEGDGE suggest that PEGDGE may be a better choice for synthesizing lignin-epoxy resins in industry, as it allows for a more dynamic range of properties that can be easily altered using varying amounts of the epoxy source.

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Chapter 6: LIGNIN DEGRADATION BY MICROORGANISMS: A REVIEW

6.1. Introduction

Lignin is an aromatic biopolymer which is found abundantly in the vascular tissues of plants [1]. It is one of three primary biopolymers, the other two being cellulose and hemicellulose, which together form a natural structural bio-composite to provide rigidity to the plant cell wall and consequently provide mechanical strength to trees [2-4]. Additionally, lignin imparts hydrophobicity to the plant vascular tissues, allowing for water transportation across a plant's height [3, 4]. Furthermore, lignin possesses antimicrobial properties that protect plants against pathogenic bacteria [3, 4]. It also protects the structural polysaccharides in plants, namely cellulose and hemicellulose, from microbial enzyme-mediated hydrolysis [5, 6].

The concentration of lignin in plants varies based on the type of the plant. For example, grasses are composed of 15-25% lignin by weight, while hardwoods contain 19-28% lignin by weight [4, 7]. Meanwhile, softwoods are composed of the highest amounts of lignin, ranging from 24% and 33% by weight [4, 7]. In addition to its direct availability in plants, lignin is formed as a by-product in the pulp, paper and bioethanol industries [8]. In fact, the amount of lignin produced annually in the pulp industry itself is as high as 30 million tonnes [8]. Lignin and lignin-containing materials, whether obtained directly from plants or

indirectly from industrial biowaste, are capable of serving as important raw materials in a variety of other commercial applications [7-12]. Most of these applications require pre-treatment of lignin-containing materials, or partial or complete degradation of lignin.

Microbes, including fungi and bacteria, offer a number of environment-friendly and efficient approaches to degrade lignin, many of which may have commercial potential. However, a comprehensive review detailing the various mechanisms of microbial biodegradation of lignin as well as potential applications in an industrial setting is absent from the literature. Therefore, this review focuses on the mechanisms of natural degradation of lignin by microorganisms. To that end, forthcoming sections discuss the chemical building blocks of lignin, its various industrial applications, the principles of lignin biodegradation, and the possibilities of microbial processing of lignin biowaste in industries that produce lignin as a by-product.

6.2. The Building Blocks of Lignin

Chemically, lignin is an amorphous, heterogeneous, polyphenolic polymer that is synthesized in nature by enzymatic dehydrogenative polymerization of three types of alcohols, namely, *p*-coumaryl, coniferyl, and sinapyl alcohols.[7, 8] Upon polymerization, these alcohols are transformed to the three phenylpropanoid monomers 4-hydroxyphenyl (*p*-hydroxyphenyl), guaiacyl and syringyl, respectively.[7, 8] The relative abundance of each of the three lignin monomers (*p*-hydroxyphenyl, guaiacyl and syringyl) varies depending on the plant species.[4, 6]

The structure of each monomeric unit may further be divided into two major segments: the aromatic segment and the C3 chain.[8] The most commonly found linkage between the phenylpropane monomers in lignin is the aromatic arylglycerol-β-aryl ether unit (30 to 60%).[1, 13] Studies have shown that microorganisms preferentially degrade these arylglycerol-β-aryl ether linkages in lignin.[1, 14] After the arylglycerol-β-aryl ether linkage, the second most abundant linkage in lignin, specifically softwood lignin, is the biphenyl linkage (20 to 25%).[13] These linkages are important targets for biodegradation by microbial enzymes.

6.3. Commercial Applications of Lignin

As a by-product in the paper and pulp industry, lignin is commonly discarded as waste material.[6, 8] Only about 2% of the lignin by-product is utilized for commercial purposes such as the production of vanillin and other aromatic compounds.[6] The remaining 98% is incinerated as a fuel for energy production within the paper mills.[6, 8] Therefore, new industrial applications of lignin are being developed to fully realize the commercial value of this abundant by-product. One of the applications is in the development of lignin-based sorbents to decontaminate industrial wastewater and oil spills.[15] More recently, studies have described the application of lignin in adsorbing and segregating heavy metal ions.[15, 16] Lignin also finds application in the construction industry as a replacement for wood flour and sawdust in the production of porcelain and bricks.[8, 15] Furthermore, lignin is used as a concrete filler in the construction industry.[8]

Lignin is also incorporated in paints, varnishes, and inks as a filler.[8, 17] Belgacem *et al.* demonstrated in 2003 that adding organosolv lignin to paints, inks, and varnishes can reduce misting of these viscous fluids by as much as 87%, thereby improving their commercial application.[17] Other applications of lignin include its use as an environment-friendly soil stabilizer.[8, 18-20] Zhang *et al.* reported that lignin-stabilized silty soil has better mechanical properties than soil stabilized with the conventional stabilizer (quicklime).[20] Similar results were reported by Liu *et al.*, who used sulfur-free lignin, a by-product of the bioethanol industry, as a soil stabilizer.[18] They found that lignin provided a stringer inter-grain bonding in the soil and resulted in an overall reduction in pore size and pore volume, which ultimately led to higher mechanical strength.[18]

Lignin is also a rich precursor for the production of a host of other compounds. It can be used to produce aromatic compounds such as vanillin, quinones, and aromatic aldehydes and acids via oxidation reactions.[9] Other chemicals such as benzene, cresols, phenols, methanol, etc., can also be produced from lignin through degradative pathways.[9] Lignin can also be used as a macromolecular monomer to synthesize polymers such as polyurethanes, which are widely used for commercial purposes such as adhesives, fibers, coatings, electronics as well as construction materials.[9, 10, 12] In a related application, owing to its mechanical strength, thermal stability, and biodegradability, lignin is also used as a filler to reinforce polymeric composites.[9] Furthermore, lignin can be used to synthesize different types of thermoplastics for commercial applications.[11]

Figure 6.1 summarises some of the most common applications of lignin and lignin-derived materials.[8]

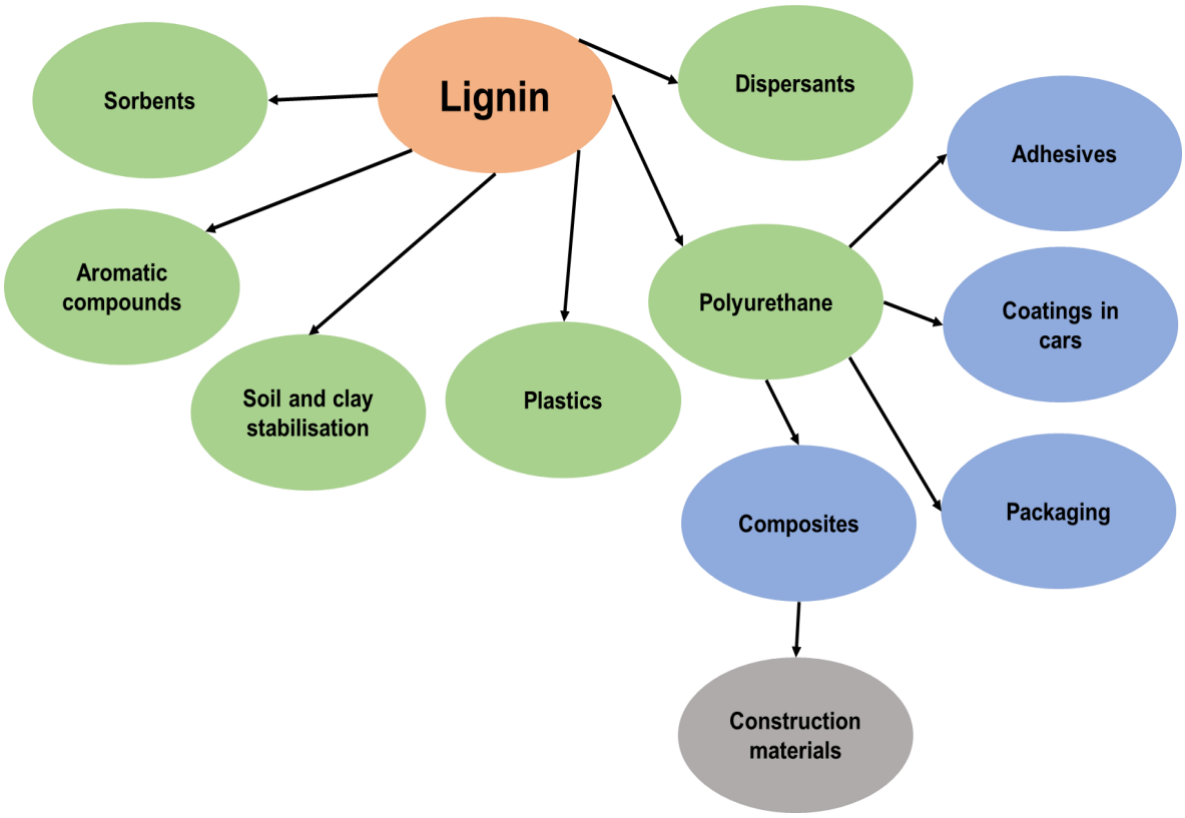

Figure 6.1. Common commercial applications of lignin and lignin-derived materials.

6.4. Lignin Biodegradation

Being an aromatic polymer with a highly branched chemical structure, lignin resists degradation and is thus recalcitrant.[5, 6] However, several microorganisms, including bacteria and fungi, are capable of degrading lignin and recycling its carbon content.[2, 5] This microbial degradation is important in nature as, without it, dead vascular plants would make the soil irreversibly lignified, which would affect soil fertility and crop yield.[5] Lignin

biodegradation became the focus of several studies in the 1970s and continues to be a central research topic in many studies today.[1]

6.4.1. Lignin Degradation by Fungi

The wood-decaying white-rot fungi are abundantly found in hardwood forests and to some extent in softwood forests.[21, 22] They are so named because upon wood decay, they lead to a bleached, light appearance of the tree.[22] White-rot fungi are the most prominent ligninolytic microbial organisms capable of both selectively degrading lignin and simultaneously degrading it alongside other polymeric wood components like cellulose.[22] Degradation of lignin by white-rot fungi preferentially targets the non-condensed aromatic unit of the polymer.[1] It is primarily achieved by the activity of fungal enzymes that target various lignin linkages. The most abundant ligninolytic fungal enzymes, produced by white-rot fungi such as basidiomycetes like *Phanerochaete chrysosporium*, are lignin peroxidases, laccases, and manganese-dependent peroxidases (Table 6.1).[21, 23] These enzymes are non-specific in their activity, which further gives them the ability to degrade other compounds that are structurally similar to lignin, such as environment-polluting xenobiotics like endocrine-disrupting drugs, pesticides, dyes, and chlorinated phenols.[21, 23, 24] Moreover, the enzymes are secreted in the extracellular environment, which makes these microbial species more effective at degrading lignin and other compounds.[21, 23, 24] The relative abundance of these three kinds of ligninolytic enzymes in white-rot fungi varies based on the strain.[23] Some strains of white-rot fungi do not express laccase but produce the other two enzymes, while other strains do not express lignin peroxidases but express both laccases and manganese-dependent peroxidases.[23]

Lignin peroxidases are hydrogen peroxide-dependent enzymes with a heme prosthetic group.[24, 25] These enzymes target the non-phenolic units in lignin.[13] Six lignin peroxidase isozymes have been isolated from *P. chrysosporium* (namely, H1, H2, H6, H7, H8, and H10).[24] On the other hand, manganese-dependent peroxidases comprise a group of 4 heme-containing peroxidase isozymes (H3, H4, H5, and H9) whose peroxidase activity depends on Mn^{2+} ions.[24, 25] Mn^{2+} ions are mono-oxidized to Mn^{3+} ions, which then diffuse through the substrate and act on both phenolic and non-phenolic units in lignin by enabling lipid peroxidation.[13, 25, 26]. Both these types of peroxidase enzymes are involved in the initiation of free radical-mediated oxidation of lignin and other compounds.[24]. The third class of enzymes, laccases, are Cu^{2+} - containing phenol oxidases that catalyze oxygen-mediated oxidation of lignin and other phenol-containing compounds.[23, 24] A less extensively investigated class of ligninolytic peroxidase enzymes, called the versatile peroxidases, was also found in studies conducted in the late 1990s.[13] For example, Martínez *et al.* found that *Pleurotus eryngii*, another white-rot fungal species, produces two peroxidase isozymes in the presence of peptone, both of which show Mn^{2+} independent activities despite successfully carrying out mono-oxidation of Mn^{2+} to Mn^{3+}.[25, 26] These 'versatile' peroxidases thus exhibit the properties of both lignin peroxidases and manganese-dependent peroxidases.[13] Therefore, versatile peroxidases possess the ability to attack both non-phenolic and phenolic/aromatic sites in lignin.[27] In all cases, the ultimate product of lignin biodegradation by fungal enzymes is carbon dioxide.[24]

The relative contribution of the different classes of ligninolytic enzymes differs from one fungal species to another.[28] Tanabe *et al.* found that degradation of *Picea jezoensis* wood

lignin by the white-rot fungus *Porodaedalea pini* was a result of high activity of manganese-dependent peroxidase as well as a significant activity of lignin peroxidase.[28] Laccase did not play a significant role in lignin degradation by *P. pini*.[28] On the other hand, Sethuraman *et al.* observed that cultures of the white-rot fungus *Cyathus stercoreus* in both nitrogen- and glucose-rich nutrient media exhibited increased activity of laccase and manganese-dependent peroxidases.[29] However, lignin peroxidase was not produced at all in the experimental conditions explored in this study.[29] Meanwhile, *P. eryngii* is known to secrete laccase and aryl-alcohol oxidase enzymes, both of which mediate a highly preferential degradation of lignin.[26]

Various species of brown-rot fungi, most commonly belonging to the Basidiomycota division, have also been found to have ligninolytic properties, although these properties are inferior to those of white-rot fungi.[5, 13, 30] This is because, unlike white-rot fungi, brown-rot fungi primarily target cellulose for degradation, but at the same time, they also modify the chemical structure of lignin via demethylation.[5]

Soft-rot fungi, such as *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus* sp. *LPB5,* are also capable of performing lignin degradation through enzymatic processes [31-35]. Soft-rot fungi exhibit typically only low lignin degradation capabilities and take longer than other lignin-degrading fungi [31]. However, in one study, *Aspergillus fumigatus* was shown to have nearly 5 times greater lignin-degrading activity than the white-rot fungus *Coriolus versicolor* [35].

Both white-rot and brown-rot fungi are also known to secrete non-enzymatic low molecular weight compounds that can either directly cause lignin degradation or mediate lignin

degradation via enzymatic mechanisms [36, 37]. These compounds play an important role because lignin-degrading enzymes cannot directly access the cell wall structure in wood owing to their larger size [37]. Thus, the low molecular weight compounds initiate the decay so that the enzymes can access and act upon lignin [37, 38]. Some of these compounds, such as phenolates, specific peptides, veratryl alcohol, linoleic acid, oxalic acid, 3-hydroxyanthranilic acid, have been listed in Table 6.2. Of these, Fe^{3+}-reducing compounds have found particular attention in scientific research [36, 38-41]. Fe^{3+}-reducing compounds reduce the Fe^{3+} (naturally found in wood, soil, as well as water systems) to Fe^{2+} after which an OH radical is generated through the Fenton reaction (Equation 1) [36, 37, 42].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + {}^{-}OH \qquad \text{(Equation 1)}$$

The OH radical then depolymerizes polysaccharides including lignin [38, 43, 44].

6.4.2. Lignin Degradation by Bacteria

Many bacterial species, primarily sourced from soil and wood-eating insects, are also capable of degrading lignin [13, 45]. Such lignin-degrading bacteria come from three classes: α-proteobacteria, γ-proteobacteria and actinomycetes [13, 45]. *Streptomyces viridosporus* is known to produce an extracellular lignin peroxidase [46]. The activity of this peroxidase has been shown to be hydrogen peroxide dependent [13]. *Sphingomonas paucimobilis* also shows lignin peroxidase activity that targets the arylglycerol-β-aryl ether linkage in lignin [13, 47].

Similarly, Ahmad *et al.* identified hydrogen peroxide-dependent lignin-degrading enzymes in the bacterial species *Rhodococcus jostii* RHA1 [48]. They found that *R. jostii* RHA1 produces an extracellular manganese-dependent lignin peroxidase, which they annotated as

DypB [48]. On the other hand, *Pseudomonas putida* shows lignin-degrading activity when hydrogen peroxide is absent [13, 48].

In another study, Huang *et al.* isolated 140 bacterial strains from the soil of a Peruvian rainforest, of which 2 (namely, *Bacillus pumilus* and *Bacillus atrophaeus*) showed significant laccase activity [45]. Interestingly, unlike fungi and most other lignin-degrading bacteria, these rainforest bacterial strains exhibited both intracellular and extracellular laccase activity, targeting the arylglycerol-β-aryl ether linkage in lignin [45].

Recently, Zhang et al. (2019) reported for the first time the lignin-degrading ability of *Mycobacterium smegmatis* [49]. They found that *M. smegmatis* showed lignin degradation comparable to other bacterial strains, and preferentially degraded lignin over cellulose. The mechanism of lignin degradation was found to be similar to fungi, involving the Fenton reaction [49].

A recent study demonstrated that catabolic enzymes in lignin-degrading bacteria (such as *Pseudomonas putida*, *Rhodococcus jostii* and *Amycolatopsis*) are sorted into outer membrane vesicles (OMVs) which are secreted into the extracellular environment [50]. The OMVs can then either be endocytosed by the target cells, or lyse in the extracellular environment, allowing the released catabolic enzymes to directly access their substrates [50].

Table 6.1. Overview of common lignin-degrading microorganisms and their ligninolysis mechanisms in literature.

Broad classification of lignin-degrading microorganisms	**Lignin-degrading microbial species**	**Mechanism of lignin degradation**	**Reference(s)**

White-rot fungi	*Phanerochaete chrysosporium*	Free radical mechanism via enzymes such as lignin peroxidases and manganese-dependent peroxidases. Laccases also found.	Cameron *et al.* (2000), Su *et al.* (2016) [24, 51]
	Bjerkandera	Free radical mechanism via lignin peroxidases and manganese-dependent peroxidases	Cameron *et al.* (2000) [24]
	Trametes versicolor	Free radical mechanism via lignin peroxidases and manganese-dependent peroxidases	Cameron *et al.* (2000), Rodríguez-Couto (2017), Su *et al.* (2016) [21, 24, 51]
	Tramtes hirsute	Lignin peroxidase, manganese-dependent peroxidase and laccase	Su *et al.* (2016) [51]
	Pleurotus ostreatus	Only manganese-dependent peroxidases found	Cameron *et al.* (2000), Rodríguez-Couto (2017) [21, 24]
	Pleurotus eryngii	Laccase, aryl-alcohol oxidase, versatile peroxidase	Martínez *et al.* (1996) [26]
	Phlebia radiata	Only manganese-dependent peroxidases found	Cameron *et al.* (2000), Martínez *et al.* (1996) [24, 26]
	Ceriporiopsis subvermispora	Only manganese-dependent peroxidases found	Cameron *et al.* (2000), Rodríguez-Couto (2017) [21, 24]
	Pycnoporus cinnabarinus	Produce high concentrations of laccases	Cameron *et al.* (2000) [24]
	Cyathus stercoreus	High concentrations of laccases and manganese-dependent peroxidases	Rodríguez-Couto (2017), Sethuraman *et al.* (1999) [21, 29]

	Pycnoporus cinnabarinus	Laccase pathway	Li *et al.* (2001) [52]
	Perenniporia medulla-panis	Fenton reaction mediated by Fe^{3+}-reducing compounds (hydroxybenzenes, cinnamic acid, veratryl alcohol, peptides and long chain saturated fatty acids)	Arantes *et al.* (2011) [36]
	Porodaedalea pini	Highly active manganese-dependent peroxidases followed by lignin peroxidase; low laccase activity	Tanabe *et al.* (2016) [28]
Brown-rot fungi	*Wolfiporia cocos*	Fenton reaction mediated by Fe^{3+}-reducing compounds including hydroxybenzenes, benzoic acid, cinnamic acid and phenyl acetic acid, hydroxyphenone, peptides and long chain saturated fatty acids.	Arantes *et al.* (2011) [36]
	Serpula lacrymans	Reduction of Fe^{3+} by iron reductase enzymes, followed by non-enzymatic Fenton reaction	Nurika *et al.* (2020) [53]
	Gloeophyllum trabeum	Fenton reaction mediated by the Fe^{3+}-reducing peptide Gt factor	Wang *et al.* (2006) [54]
Soft-rot fungi	*Aspergillus flavus*	Laccase-mediated degradation	Ghosh & Ghosh (2017) [32]
Bacteria	*Streptomyces viridosporus*	Lignin peroxidase activity	Ramachandra *et al.* (1988) [46]
	Bacillus pumilus	High laccase activity	Huang *et al.* (2013) [45]

	Bacillus atrophaeus	High laccase activity	Huang *et al.* (2013) [45]
	Sphingomonas paucimobilis	Lignin peroxidase activity	Masai *et al.* (2007) [47]
	Rhodococcus jostii RHA1	Manganese-dependent lignin peroxidase DypB	Ahmad *et al.* (2011) [48]
	Mycobacterium smegmatis	Glucose-methanol-choline (GMC) oxidoreductases, catalase-peroxidase	Zhang et al. (2019) [49]
	Pantoea ananatis	Lignin peroxidase, laccase, as well as Fenton reaction mediated by GMC oxidoreductases	Ma *et al.* (2016) [55]
	Pseudomonas putida	Laccase-like hydrogen peroxide-independent activity	Bugg *et al.* (2011), Ahmad *et al.* (2011) [13, 48]

Table 6.2: Non-enzymatic low molecular weight compounds involved in lignin degradation by fungi (adapted from [36]).

Low molecular weight compound	**Mechanism of lignin degradation**	**Reference(s)**
Fe^{3+}-reducing compounds (phenolate derivatives and peptides)	Enable the Fenton reaction	Arantes *et al.* (2011) [36]
Linoleic acid	Fatty acid peroxidation: Manganese-dependent peroxidases can lead to the formation of acyl radicals	Kapich *et al.* (2007) [56]

	from the fatty acid, leading to peroxidation and formation of radicals such as lipid peroxyl which can oxidize lignin	
Oxalic acid	Direct acid hydrolysis as well as mediating effect through m anganese-dependent peroxidases	Shimada *et al.* (1997) [57]
Veratryl alcohol	Interacts with lignin peroxidase to release cation radical which may mediate lignin depolymerisation	Evans *et al.* (1994) [44]
3-Hydroxyanthranilic acid	Mediating effect through laccases to oxidise non-phenolic lignin subunits	Feng *et al.* (2019) [58]
2-Chloro-1,4-dimethoxybenzene	Acts as a redox mediator in the lignin peroxidase-based degradation pathway	Teunissen *et al.* (1998) [59]

6.5. Growing Industrial Applications of Microbial Biodegradation of Lignin

In the paper and pulp industry, cellulose and lignin need to be segregated mechanically or chemically to produce high-quality paper [21]. Chemicals harmful to the environment are typically used to solubilise lignin after which chlorine, hydrosulfites, oxygen or hydrogen peroxide are used to remove the brown lignin residue [21, 60]. A recent approach involves pre-exposing the wood used to produce paper with white-rot fungi in a process that is known as biopulping [21, 61, 62]. This process involves allowing white-rot fungi to grow on lignocellulosic raw materials with the help of nutrient supplementation [62]. Biopulping with white-rot fungi is a more energy-efficient, eco-friendly and cost-effective approach to eliminating lignin contaminants from wood before it is pulped mechanically or chemically for paper production [21, 62]. Similarly, in the recently booming bioethanol production industry, lignocellulose biomatter is pre-treated with white-rot fungi to enrich the fermentable carbohydrates in the biomass [21]. To repurpose lignocellulosic biowaste for the purpose of ruminant feeding, lignin-selective white-rot fungi are being explored so that the energy-producing cellulosic content remains intact while lignin is selectively removed [21].

Microbial biodegradation of lignin has also been investigated as an important pre-treatment step in the production of biodiesel from cellulose. At the same time, lignin degradation can also directly result in the formation of lipids (such as palmitic acid, oleic acid, tetracosanoic acid and several others) which can act as feedstock for the production of biodiesel, as

demonstrated by Zhang et al. (2019) in *Mycobacterium smegmatis* [49] and Wang et al. (2019) in *Rhodococcus opacus* [63].

Such industrial applications often require large amounts of lignin-degrading enzymes to be viable. Various recent studies have thus focused on optimizing the growth conditions that enhance the production of lignin-degrading enzymes from fungi. Ghosh and Ghosh (2017) found that adding sodium chloride to a liquid growth medium with agro-waste growth substrate improved laccase production by *Aspergillus flavus* [32]. Further, supplementing the growth medium with soluble starch led to a 1.8-fold increase in laccase production compared to when glucose was used [32]. Importantly, the type of lignocellulosic agro-waste used as substrate also affected the production of laccase by *Aspergillus flavus*, with ribbed gourd peel inducing the highest laccase production [32].

On the other hand, Wu et al. (2018) recently demonstrated a method that can help expand the industrial applications of lignin [64]. They genetically engineered *Escherichia coli* as a means to convert aromatic lignin degradation products such as vanillin to more industry-useful products like catechol which may then be used as precursors for other polymers [64]. Such strategies can prove to be useful in improving the efficiency of the existing industrial applications of lignin as well as in developing new potential applications.

6.6. Fungal *vs* Bacterial Degradation of Lignin

Fungal degradation of lignin has been found to be more efficient, particularly in the context of the use of white-rot fungi in industrial lignin removal applications [21, 65, 66]. White-rot fungi are able to tolerate and even degrade a variety of xenobiotics that are toxic to other

bacteria [21, 67-70]. This is primarily because white-rot fungi can survive in a range of environmental conditions and employ an extracellular lignin-degrading mechanism [21, 22, 71]. Moreover, these fungi can utilise their lignocellulosic substrates to obtain nutrition, obviating any expensive nutrient media for their growth [21, 63].

At the same time, fungal enzymes have been found to be less economically feasible for commercial production [45]. Moreover, these enzymes are more susceptible to degradation in extreme temperature and pH conditions than their bacterial counterparts [45]. Furthermore, maintenance of pH has been found to be difficult in white-rot fungal cultures [29]. Therefore, commercialisation of fungal biodegradation of lignin has still not succeeded beyond a few applications [45].

6.7. Summary and Future Directions

Lignin waste is generated in large amounts in a number of industries, including paper and pulp and bioethanol industries. Chemical means to treat and eliminate lignin waste products are harmful to the environment. Natural degradation by a variety of fungal and bacterial species provides an environment-friendly alternative to chemical degradation of lignin. The use of microbial degradation to repurpose lignin and lignin-containing by-products in various industries could be an important commercial avenue as well as a scientific one. However, as discussed in the present review, research on lignin-degrading microorganisms has revealed significant differences in the ability of different species to degrade lignin, most commonly owing to the variations in expression and/or activity of the various isozymes of lignin peroxidases, manganese-dependent peroxidases, versatile peroxidases and laccases.

Furthermore, microbial growth conditions also affect their ability to produce lignin-degrading enzymes. For example, Martínez *et al.* observed that the *in vitro* production of manganese-dependent peroxidases by four *Pleurotus* species (*P. eryngii*, *P. sajor-caju*, *P. ostreatus* and *P. pulmonarius*) was dependent on the type of nutrient media used to culture the fungi [26]. Indeed, the use of peptone as the culture medium elicited a production of these lignin-degrading enzymes, while a mixture of glucose and ammonium tartrate did not [26].

Overall, the literature reviewed in the present article suggests that one area where further biological research could significantly improve our knowledge as well as commercial applications of microbial degradation is the optimisation of *in vitro* lignin degradation conditions. Current literature about lignin-degrading microorganisms is scattered in its focus and scope. Systematic and comprehensive studies need to be undertaken to determine the degree of impact that the following factors have on lignin-degrading capabilities of microorganisms:

(1) Taxonomical differences among lignin-degrading microbes, at kingdom level (fungi vs. bacteria) or lower levels (white-rot fungi vs. brown-rot fungi, and inter-species differences).

(2) Taxonomical differences among the wood or lignin source (such as different species of trees or grasses, etc.) as the fraction of lignin in the lignocellulosic wood content varies from one species to the other.

(3) Controllable environmental conditions for laboratory- or industry-based degradation. The conditions evaluated must include temperature, humidity and the composition of the

microbial growth media. Such investigations could help determine the optimal growth conditions for lignin-degrading microbes to degrade lignin-containing biowaste.

(4) The types, relative abundance, number of isozymes and activities of various lignin-degrading enzymes produced by microorganisms.

Studies that investigate two or more of the above-mentioned factors in an integrated manner may be able to provide useful and novel insights into lignin degradation mechanisms in nature and also help further commercial uses of microbial lignin degradation.

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Chapter 7: CONCLUSIONS AND RECOMMENDATIONS

Lignin is a complex biopolymer that is abundantly found in all vascular plants. It plays a key role in building connective tissues, giving them strength, rigidity, and resistance to environmental factors such as pathogens. Lignin also finds diverse applications in the commercial sector. In fact, extracted lignin obtained from the forestry and agriculture sectors are important raw materials that show immense potential in novel value-added applications that are becoming the trend of the future. Therefore, it is important to explore and develop optimum and sustainable processes for lignin extraction. This study aimed to examine the different lignin extraction methods for various wood species present in Newfoundland: balsam fir, pine and spruce (softwood), and birch, maple and oak (hardwood). Two different methods of lignin extraction were studied: (1) the Formacell method, that uses a mixture of acetic acid/formic acid/water; and (2) the BioEB method, which uses only formic acid/water. Various parameters were tested, including solvent concentration, temperature, cooking time and wood species (hardwoods and softwoods). Among all conditions tested for Formacell extraction, lignin yield was the highest with 55/30/15 (v/v/v) formic acid/acetic acid/water ratio, a temperature of 80°C and a cooking time of 3 h. Meanwhile, among all treatment conditions tested for the BioEB method, the lignin yield was the highest after treatment at 90°C for 3 h. Overall, the lignin yield of the BioEB method was higher than that of Formacell method (for both hardwoods and softwoods). However, in both the methods, the extraction of lignin from hardwood was

much easier than from softwood. Overall, the results of this study could be applied to inform and improve industrial lignin extraction processes to obtain better yields in the most optimal manner. Future studies should focus more on understanding the differences in lignin composition and structure as a function of the extraction method and parameters used.

The thesis further discussed the latest developments related to value-added uses of extracted lignin oligomers for the preparation of novel biochemicals and various bio-based materials, with the added advantage of eco-friendliness, sustainability, as well as promoting the low-carbon economy. Additionally, microbial biodegradation of lignin provides a host of possibilities to overcome the challenges of using harmful chemicals to degrade lignin biowaste in many industries. This thesis placed emphasis on the degradation of lignin by natural means through the ligninolytic activities of various fungal (white-rot and brown-rot fungi) and bacterial species. It became clear from this review that further biological research can significantly improve our knowledge as well as commercial applications of microbial degradation. To achieve this, it is important to optimise *in vitro* ligninolysis conditions. Particularly, systematic and comprehensive studies need to be undertaken to determine the degree of impact that the following factors have on ligninolytic capabilities of microorganisms: (1) taxonomical differences among lignin-degrading microbes, at kingdom or lower levels; (2) taxonomical differences among the wood or lignin source; (3) controllable environmental conditions for laboratory- or industry-based degradation, such as temperature, humidity and the composition of the microbial growth media; and (4) the

types, relative abundance, number of isozymes and activities of various lignin-degrading enzymes produced by microorganisms.

Overall, the present thesis was able to address the research gaps identified in Section 1.1. It provided a comprehensive review that compiled all the different industrial applications of lignin. It identified an extraction method (the newly developed, relatively unexplored BioEB method) that not only operates at feasible ranges of parameters such as temperature yet yields high lignin concentrations, but is also cost-effective and eco-friendly and works on multiple wood species. This method can help meet the increasing industrial demands of lignin. The thesis also elucidated the synthesis and characterization of industrially relevant lignin-epoxy resins in the context of the type of epoxy source used. At the same time, the thesis addressed the evolving field of lignin degradation by microbial organisms in a unique manner by discussing microbial biodegradation of lignin in the context of its growing industrial applications.

APPENDIX A: ENVIRONMENTAL IMPACT OF BIOPLASTIC USE: A REVIEW

A.1. Introduction

Plastics have become commonplace manufacturing materials that find applications in a variety of industries, from packaging to the production of toys, from grocery bags to plastic cutlery, from straws to 3D printed rocket nozzles.[1-5] Chemically, plastics are high molecular weight polymers typically comprising between 1000 to 10000 monomeric repeating units.[1,6,7] Conventional petroleum-based synthetic plastics are produced in a series of steps, the first of which is the distillation of crude oil in an oil refinery. This process separates and fractionates the heavy crude oil into groups of lighter components, called segments. Each segment is a mixture of polymeric hydrocarbon chains, which differ in terms of size and structure. One of these fractions, naphtha, is the crucial component needed to generate monomers such as ethylene, propylene, and styrene to produce plastics. These monomers form plastics through polyaddition and/or polycondensation aided by specific catalysts.[8,9] However, this conversion produces pollutants and greenhouse gases such as carbon dioxide (CO_2), thus contributing to environmental pollution and global warming.[3] Moreover, several petroleum-based plastics are nonbiodegradable, which leads to their persistence at the site of disposal and harms the environment.[10] Over the past two decades, several studies have suggested alternatives to the conventional petroleum-based plastics. One such alternative is bioplastics, which are polymeric compounds that are both

functionally similar to synthetic plastics and largely environmentally sustainable. However, bioplastics are surrounded by myths that claim that all bioplastics are biodegradable and good for the environment. The truth is that some bioplastics may contribute significantly to global warming, pollution and drastic land use change. Still, while many reviews discuss bioplastics, few comprehensively and simultaneously address the positives and negatives of bioplastic use for the environment. The primary focus of the present review article is to address this gap in present research. To this end, this review addresses the following questions:

(1) What are the different types of bioplastics that are currently in commercial use or under development in the industry?

(2) Are these bioplastics truly good for the environment?

(3) How can we better resolve the controversial impact of bioplastics on the environment?

A.2. Methodology

This review article collates and summarises primary data produced and presented by other academic and industrial scholars through their research on bioplastics and their impact on the environment. The following search terms were used on Google Scholar to identify relevant studies to discuss in this review: plastics, petroleum-based plastics, bioplastics, bio-based plastics, biodegradable plastics, plastic waste disposal, bioplastic waste disposal, plastic recycling, bioplastic recycling, life cycle analysis. Industrial research data, such as primary data available on company websites, was not excluded from this review. To

specifically meet the objectives of the present review, only those studies that discussed existing or new classes of bioplastics, and/or their impact on the environment (positive or negative) were included.

The results of this literature review are presented in four sections. The first of these sections, titled 'Plastics and the environment', discusses conventional plastics, their degradability and their impact on the environment. The second section introduces bioplastics as a way to replace conventional plastics and discusses some of the most important as well as recently developed bioplastics currently in commercial use or industrial testing. The third section elucidates the debate about whether or not bioplastics are good for the environment, presenting both the positive and negative effects of these materials on the environment. The last of the four sections introduces life cycle assessment as a means to address the debate around the eco-friendliness of bioplastics, referencing some preliminary analyses published by other researchers.

A.3. Plastics and the Environment

The global consumption of plastics has increased over the years, particularly because they are lightweight, resilient, relatively low-priced and long-lasting. The plastic industry generates approximately 300 million tons of plastics annually, which are used once and discarded after use.[11] Discarded plastic waste, owing to the durability and low degradability of these polymers, may take hundreds to thousands of years to decompose.[11] Moreover, of the total produced quantity of plastics, only 7% is recycled, while about 8% is incinerated and the residual landfilled.[12] The National Academy of Sciences in 1975

assessed that 14 billion pounds of garbage was dumped every year, either buried underground or buried in the oceans. Consequently, oceans and landmass are infested with plastics. In fact, more than 10 million tons of plastic waste is dumped in the oceans alone, due to which the majority of anthropogenic debris littering the oceans is composed of human-made plastics. Reports suggest that plastics can now be used as a geological stratigraphic indicator of the Anthropocene era.[13-16] This anthropogenic debris threatens ocean safety, integrity, and sustainability.[17] Overall, plastic waste contributes to a pertinent environmental problem that is as yet unsolved.

A.3.1. Why Plastics Are Nondegradable

The production of synthetic plastics, particularly nondegradable ones, is an environmental burden. This is because 'nondegradable' plastics take decades or centuries to break down.[18] Nonbiodegradability of certain plastics suggests that their chemical structure cannot be adequately modified by naturally-occurring microorganisms, water, carbon dioxide or methane to degrade them.[10,19] Meanwhile, 'biodegradable' plastics are truly compostable materials that can almost entirely be converted into benign trash after a matter of months in a composter.[18]

Studies on biological decomposition of plastics by various microorganisms under different environmental conditions have revealed that these decomposition conditions are governed by the physical and chemical characteristics of the type of plastic discarded, such as mobility, crystal structure, molecular weight, functional groups etc.[20] High molecular weight, high degree of crystallinity, high hydrophobicity as a result of linearity of the

polymeric carbon chain backbone, and general insolubility in water are some of the factors that typically reduce the degradability of plastics.[20-22] Indeed, these are the properties that make the petroleum-based plastics polyethylene and polypropylene nonbiodegradable.[10,22]

Notably, not all petroleum-based plastics are nonbiodegradable. For example, polycaprolactone (PCL) and poly(butylene succinate) (PBS) are both petroleum-based plastics which can undergo microbial degradation.[10] However, the biodegradability of these polymers is affected by their physicochemical properties such as degree of crosslinking, degree of crystallinity, molecular weight and the species of microorganisms used.[23] Indeed, studies have revealed that crosslinked polymers have the lowest rate of degradation, followed by crystalline and then amorphous polymers.[23]

A.3.2. How to Eliminate Plastics

There are many alternatives currently available for reusing and recycling existing plastics, and a significant amount of ongoing research seeks to completely replace plastics with more sustainable alternatives in the future. At the same time, a large amount of plastic waste is already present in the environment and needs to be disposed. Moreover, recycling of plastics has not been effectively adopted. Also, plastics can only be recycled a limited number of times before they become contaminated to the point when they can no longer be used.[17]

The challenge of plastic disposal can be addressed in various ways. One way is to convert the plastic discards into energy by incineration.[24] However, this will give rise to large amounts of carbon dioxide and contribute to global warming. A more sustainable means of

disposing old plastics is to develop the capability to recycle old plastic materials into new ones. An example is the production of recycled oxy-degradable plastics (synthetic wood) from high-molecular polyethylene to replace wood for discarded garden furniture.[25] Other alternative approaches to plastic recycling include mechanical and chemical recycling. Mechanical recycling permits plastic discards to be used as raw material for other new types of plastic products.[26] When mechanical recycling is not possible, chemical recycling technologies can be used to convert plastic waste into different products through breakdown chemical processes.[26] Chemical recycling of plastic waste involves depolymerization to the constituent monomers achieved through hydrolysis, alcoholysis, glycolysis, ammonolysis, pyrolysis, hydrogenation, and gasification.[26] However, whether recycled plastics are better for the environment can only be determined after knowing if the production of new plastic materials will allow overall reductions in energy expenditure, water use and greenhouse gas emissions.[27,28]

Lastly, another method of eliminating plastic waste is to use it to generate gaseous matter with high hydrogen content or synthesis gas.[7] This is a promising alternative to waste treatment because not only is waste eliminated, but it is also used as fuel.

A.4. Bioplastics

The environmental problems caused by discarded synthetic plastics have paved the way for the search for substitutes. Bioplastics, which are both functionally similar to synthetic plastics and environmentally sustainable, are touted as promising new materials to address these problems. Bioplastics is a term used to refer to plastics that (1) are biodegradable,

such as PCL or PBS; or (2) may or may not be degradable but are produced from biological materials or renewable feedstock, such as starch, cellulose, vegetable oils, and vegetable fats.[10,19] Like any other polymeric material, the degradability of bioplastics is also a factor of their composition, degree of crystallinity and environmental factors, leading to degradation times ranging from several days to several years. For these reasons, the development of biodegradable bioplastics has gained attention in recent years.[24,26,28,29]

Based on degradation mechanisms, there are two main categories of biodegradable bioplastics, namely oxo-biodegradable and hydro-biodegradable.[30] Oxo-biodegradable plastics are made of petroleum-based polymers mixed with a pro-degradant additive that catalyzes the plastic's degradation process.[31] The additive is a metal salt (manganese or iron salts), which enhances the abiotic degradation process of the oxo-biodegradable plastic in the presence of oxygen.[32,33] Presently, oxo-biodegradable plastics are mainly produced from naphtha, a by-product of oil or natural gas.[34] Interestingly, the time taken by biodegradable oxo products to degrade can be 'programmed' at manufacture, like the methane or nitrous oxide industrial processes.[31] The degradation of oxo-biodegradable plastics usually takes months to years.[32] On the other hand, hydro-biodegradable plastics decompose hydrolytically at a rate faster than oxo-degradable plastics. These plastics can be converted to synthetic fertilizers. Examples include bioplastics produced from plant sources (such as starch), and polylactic acid (PLA). Forthcoming paragraphs summarize the most recent literature on different types of bioplastics that have been or are currently being developed.

A.4.1. Thermoplastic Starch

Starch is a biodegradable, cheap, renewable, easily modifiable biopolymer acquired from renewable plant resources.[35,36] It consists of two main constituent polymers, amylose and amylopectin. Amylose is a linear polysaccharide composed of α-D-glucose monomers linked by α-1,4-glycosidic linkages, whereas amylopectin has the same composition but is highly branched through another type of linkage, the α-1,6-glycosidic linkage.[37] It should be noted that starch chains bind together via strong hydrogen bonding, which results in a rigid structure composed of highly ordered crystalline regions.[37-40]

Starch can be formulated into suitable thermoplastic material that can be readily processed into usable forms.[40,41] Starch's thermal processing involves a change in its microstructure, phase transitions and rheology. Furthermore, starch can be chemically modified and blended with other biopolymers to reduce its brittleness. Starch-based bioplastics are used for packaging materials and for producing food utensils such as cups, bowls, bottles, cutlery, egg cartons, and straws.

A.4.2. Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are a class of bio-based bioplastics belonging to the polyhydroxyester family of 3-, 4-, 5- and 6-hydroxy alkanoic acids.[42] The general chemical structure of PHA is shown in Figure A.1. PHAs are biocompatible, biodegradable, and non-toxic polyesters synthesized by certain bacteria and plants from renewable sources.[42] In particular, PHA can be produced from methane released from feedstock in wastewater treatment facilities, landfills, compost facilities, farms and food processors,

waste haulers, bio-refinery operators, and plastic compounders can be used as feedstock for successful, low-cost commercial production of PHA.[43,44] PHA can also be produced from wood biomass, grass, energy, and crop residues instead of more expensive biomass obtained from edible crops (Renmatix, Pennsylvania, USA).[45] Renmatix's technology separates biomass from water and uses heat instead of acids, solvents, or enzymes to produce PHA bioplastics in a clean, fast and relatively inexpensive process.[43] The PHA thus produced can be used for commercial purposes, such as bioplastic wraps, shampoo bottles, or polyester fibers that can be combined with natural materials for clothing. PHA bioplastics can be digested naturally by marine microorganisms when they are decomposed into methane and reach the ocean.[43] At the end of its life cycle, the developed bioplastic can be broken down into virgin plastic since it is compostable and marine-degradable.[43,46]

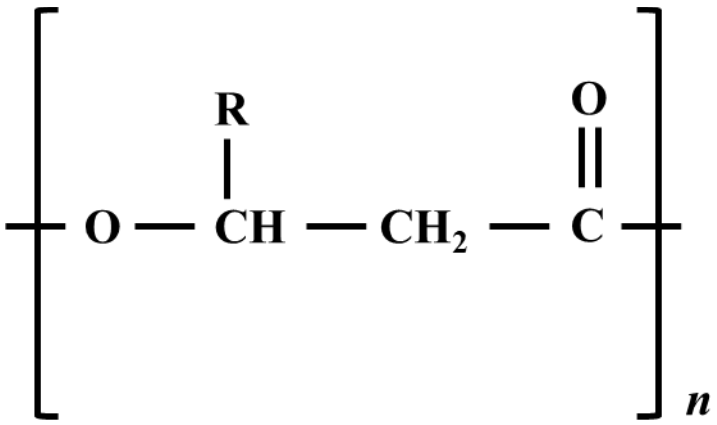

Figure A.1. Chemical structure of PHA.

Polyhydroxybutyrate (PHB) is a widely-used PHA (Figure A.2) produced by a variety of microorganisms (such as *Cupriavidus nectar*, *Methylobacterium rhodesianum* or *Bacillus megaterium*) from methane.[47-49] Methane is first oxidized to methanol via the methane monooxygenase enzyme catalytic pathway.[50] This is followed by methanol dehydrogenase-dependent conversion of methanol to formaldehyde.[50] Methanotrophic bacteria, such as γ-proteobacteria and α-proteobacteria, can further convert formaldehyde to acetyl coenzyme A (Acetyl-CoA).[50,51] Acetyl CoA is condensed into the dimer acetoacetyl-CoA, which is then reduced by acetoacetyl-CoA reductase enzyme to form PHB monomer β-hydroxybutyrl-CoA.[50] Finally, β-hydroxybutyrl-CoA is polymerized to PHB via the PHB synthase enzyme.[50]

PHB bioplastics are biodegradable, making them an attractive environment-friendly alternative to fossil-based thermoplastics.[52,53] Melt-processable PHB can be formed by using semi-crystalline thermoplastics produced from the fermentation of renewable carbohydrate feedstock.[54] Moreover, commercial grades of PHB possess properties very similar to fossil fuel produced polypropylene (PP).[55,56]

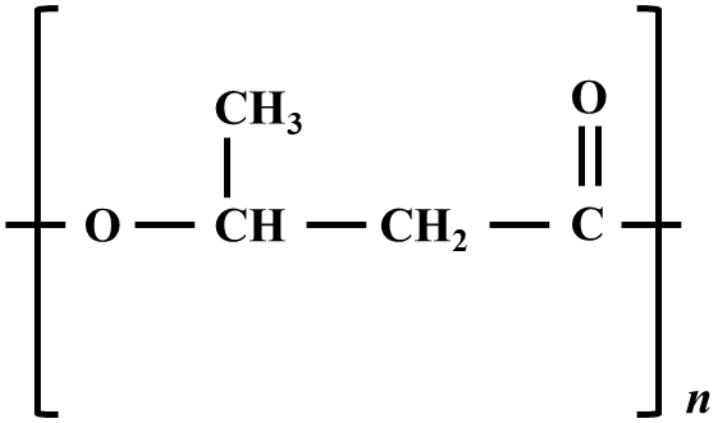

Figure A.2. The structure of PHB plastic.

Common applications of PHB include disposable tableware articles, soil retention sheathing, waste wrapping, and packaging material. PHB also finds applications in the field of biomedical engineering where it can be spun into surgical sutures and used as drug delivery systems.[56]

A.4.3. Polylactic Acid

Polylactic acid (PLA) is a thermoplastic aliphatic polyester obtained by polymerizing lactic acid from renewable resources, such as corn starch, tapioca roots, chips or starch, and sugarcane.[57] PLA is used mainly in the food industry to prepare disposable tableware articles like drinking cups, cutlery, trays, food plates, food containers and packaging for sensitive food products. However, PLA bioplastics are too fragile and cannot be used for other packaging manufacturing processes. For this reason, PLA needs additives to make it

more durable.[58] Notably, PLA is the most biodegradable thermoplastic, typically degrading via hydrolysis (Figure A.3).[59]

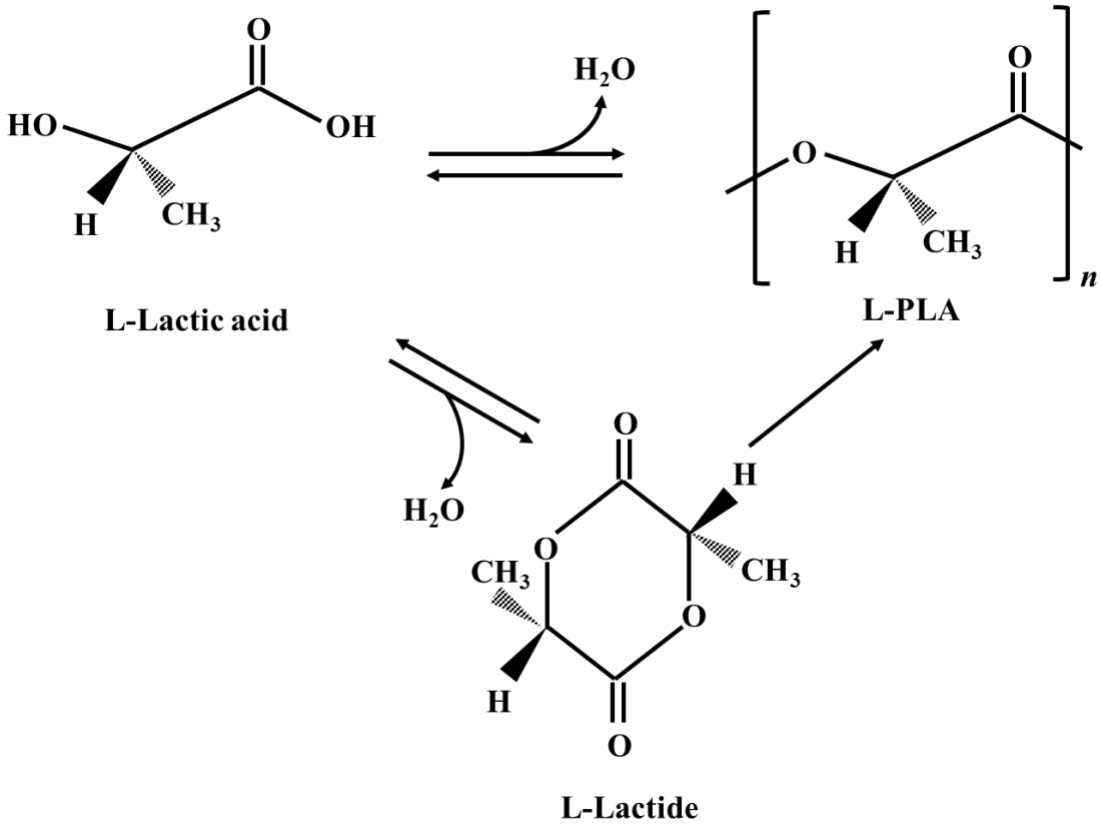

Figure A.3. Polylactic acid hydrolysis.

Several commercial grades of PLA are specifically designed for processes such as thermoforming and extrusion/injection moulding.[60] It can also be used for soil retention sheathings, agriculture films, waste shopping bags, and the use of packaging material.[59]

Furthermore, PLA can be converted into fibers by spinning and used to manufacture woven, disposable and biodegradable fabric articles such as disposable garments, feminine hygiene products, and diapers.[44,59]

A.4.4. Bioplastics Produced by Blue-Green Algae and Cyanobacteria Through Photosynthesis

Recent studies have described the production of bioplastics by using blue-green algae and cyanobacteria blooms that use sunlight to produce chemicals through photosynthesis.[61] Instead of feeding sugar from corn or sugarcane to plastic-producing bacteria, advances have been made to improve the cyanobacteria to produce plastics naturally by using their self-synthesized glucose. Cyanobacteria can convert glucose to acetyl-CoA, which, as explained earlier, is then converted to acetoacetyl-CoA, followed by β-hydroxybutyryl-CoA and finally, PHB.[61] Moreover, it has been shown that it is also possible to produce polymers from genetically engineered cyanobacteria that feed on sugars, a method that could replace fossil-fuel-based processes.[62-64] Overall, cyanobacterial species such as *Scytonema geitleri Bharadwaja*, when stressed, store the intracellular poly-β-hydroxybuyrate granules for energy and carbon reserves inside their cells.[65] The biodegradable and eco-friendly PHB can then be gathered and used to form biocompatible thermoplastics.[64]

However, researchers have pointed out a possible issue with bioplastic production that relies on feeding plastic-producing bacteria with large quantities of sugars obtained from natural crops. Since the natural crops are used as food to sustain people and animals, we

risk compromising the competing balance for the limited agricultural resources.[66] As a potential solution for this issue, a recent study has demonstrated the development of finely tuned cyanobacteria of the *Spirulina* strain, which can constantly produce sugar and leak it into the surrounding saltwater, which contains natural bacteria.[67] These bacteria usually feed off the leaked sugar and convert it to produce bioplastic. This means that the cyanobacteria create sugar during photosynthesis, which is food for the natural bacteria that converted it into bioplastics.[67]

Promising new strategies involving genetic engineering of cyanobacteria have also been reported to produce small substrate chains like poly (3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV and poly (3-hydroxybutyrate-co-4-hydroxybutyrate) PHB4B, and PHBHx copolymers containing 3-hydroxyl hexanoate units.[61] This involves the use of a mixture of substrates, such as glucose and valerate, to cause the formation of random copolymers.[61] Hence, when these substrates are alternately bonded during copolymerization, it is possible to obtain PHA block copolymers synthesized by bacteria.[68] The chemical structures of these copolymers are shown in Figure A.4.

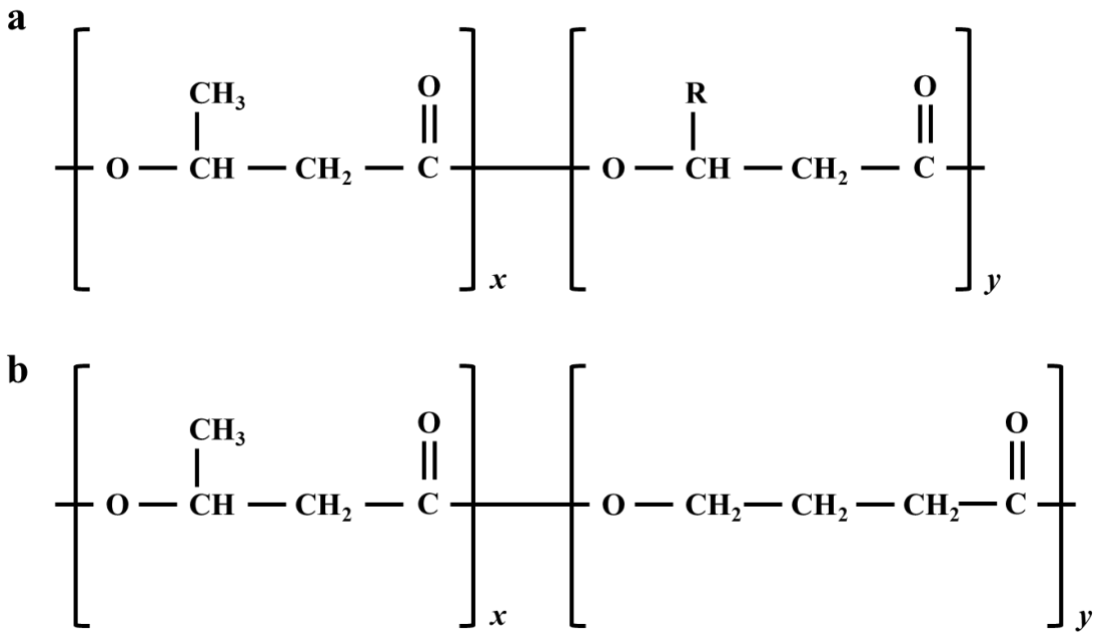

Figure A.4. (a) Poly-hydroxybutyrate copolymers. (b) Poly (3-hydroxybutyrate-co-4hydroxybutyrate) (PHB4B).

A.4.5. 1,2-, 1,4- and 2,3-Butanediol Bioplastics

Butanediol (BDO) is an industrial chemical used as a solvent and building block in bioplastics, elastic fibers, and polyurethanes.[69] BDO contains terminal, primary hydroxyl groups which allow it to be used as a cross-linking agent for the synthesis of thermoplastic urethanes, polyester plasticizers, paints and coatings, copolyester hot melt and solvent-borne adhesives.[70] In polyurethane applications, 1,4-BDO is primarily used as a component of polyesters or as a chain extender. Bioplastics formed from BDO are completely biodegradable. An example is poly (1,4-butylene succinate) (PBS). PBS, which

typically exists behaves as a semi-crystalline thermoplastic, is chemically synthesized from succinic acid and 1,4-BDO (Figure A.5).

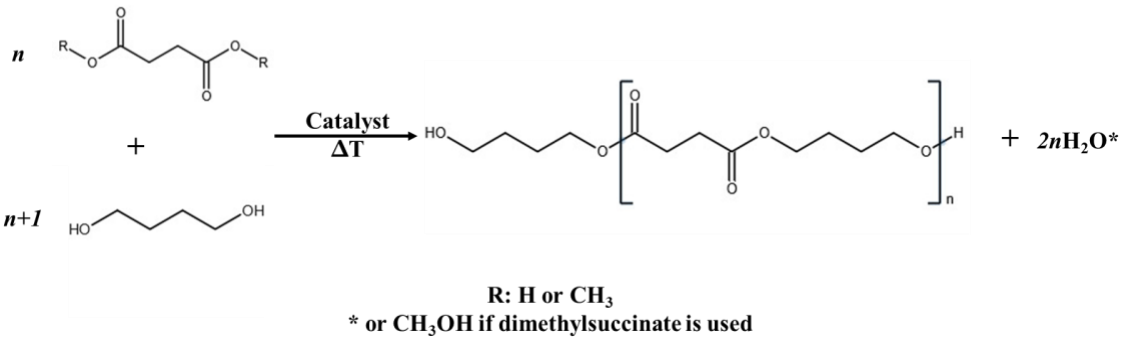

Figure A.5. Poly (1,4-butylene succinate).

The mechanical properties of PBS are comparable to that of widely used high-density polyethylene and isotactic polypropylene.[71-73] Moreover, it is relatively more cost-effective compared to other biopolymers such as PLA, PBAT, and PHB.[71-73] As such, it is used for a variety of applications such as disposable food packaging, mulch film, plant pots, hygiene products, fishing nets, and fishing lines.[71-73] It can also be utilized as a 'matrix polymer' or in combination with other biopolymers such as PLA.[71-73]

The key monomer for PBS, namely, 1,4-BDO, is currently produced through feedstocks derived from oil and natural gas.[74] Furthermore, it is also possible to synthesize 1,4-BDO via direct biocatalytic routes from renewable carbohydrate feedstocks (glucose and sucrose).[74] It has also been found that an engineered *Escherichia coli* host enhances the anaerobic operation of the oxidative tricarboxylic acid cycle, thereby generating reducing power to drive the BDO pathway.[75] *E. coli* produce BDO from glucose, xylose, sucrose,

and biomass-derived mixed sugar streams. The creation of such engineered bacteria has allowed for a systems-based metabolic engineering approach to strain design and development that can enable new bioprocesses for commodity chemicals that are not naturally produced by living cells.

In addition to 1,4-BDO, it has been established that 2,3-butanediol (2,3-BDO) is an excellent bio-based chemical possessing important industrial applications. 2,3-BDO has been used extensively for synthetic rubber precursor, food additives, and cosmetics. As in the case of 1,4-BDO, *E. coli* has been metabolically engineered to promote the production of 2,3-BDO by expressing the *Bacillus subtilis alsS*, *alsD*, and *ydjL* genes encoding α-acetolactate synthase, α-acetolactate decarboxylase, and acetoin reductase/2,3-butanediol dehydrogenase, respectively, along with *Deinococcus radiodurans dr1558* gene encoding a response regulator.[76,77] In another study, USA-based Genomatica, Inc. developed a commercial, bio-based processes to manipulate *E. coli* to produce bio-butanediol (Bio-BDO) directly.[78] This bio-butanediol (Bio-BDO) chemical can be used to create a wide range of products: from spandex to car bumpers, in a more energy-efficient way and without oil or natural gas.[78]

A.4.6. Seaweed Polysaccharide Bioplastics

Seaweeds are excellent candidates for the production of bioplastics.[79] Seaweeds possess the ability to grow in a wide range of environments, which simplifies their cultivation in the natural environment.[80] Using seaweeds for bioplastics production can minimize the

impact on the food chain.[79,81] Furthermore, seaweed-based bioplastics are chemical-independent.[79,81]

The most commonly used seaweed types in the industry contain polysaccharides such as agar, alginate, carrageenan, galactans and starch.[79] These polysaccharides consist of mannuronic and guluronic acid residues.[44,82] The seaweed polysaccharide backbones are frequently functionalized with various substituent sulphate and methoxyl groups, which impart negative charge to them.[83] This allows them to interact to variable extent with cations, resulting in the formation of gels.[83] These gels have properties that cover a wide range of industrial applications required by all thermo-mechanical bioplastics.[83]

Seaweed polysaccharides are extracted from dried and ground seaweeds by following a hot extraction method.[79] This is followed by a two-step purification process, the first of which involves the removal of dense cellulosic contaminants by centrifugation and subsequent filtration, and the second one involves the concentration of the purified mixture by allowing the water to evaporate.[79] From the enriched mixture, potassium chloride can be added to cause gelation of seaweed polysaccharides.[79] Alternatively, isopropyl alcohol can be used to cause precipitation of the polysaccharides.[79] The concentrated mass of polysaccharides can be frozen and freeze-dried to be used in the manufacturing of bioplastics.[79] An example is the production of thermoplastic starch from seaweed starch, as discussed previously in section A.4.1.

Seaweed polysaccharides can be useful in various food industry applications such as texture modification, colloidal stabilization, fat reduction and shelf-life extension.[83] It is also

possible to produce biodegradable water bottles made from seaweed.[77,79] Other applications include lenses, coatings for telephones and DVDs and packaging materials.[84]

A.4.7. Fungal Mycelium-Based Bioplastics

Evocative, a New York-based company, has used mycelium – vegetative fungal extensions that give rise to mushrooms – to make plastic-like materials for biodegradable packaging and tiling.[85,86] Mycelium is composed of polysaccharides, chitin, proteins and lipids, which together result in adequate mechanical properties for this biomaterial to be used in a range of industrial applications.[87] The mushroom-producing mycelium provides for a fibrous biomaterial that can be combined with agricultural by-products (such as the peel of the seeds and the corn stalk) to make composite materials for industrial use.[85,88] This new material is being used by IKEA company which, to fulfill its commitment to sustainable innovation, has decided to use mushroom-based packing that eliminates the need for other wasteful materials.[89]

A.4.8. Bioplastics from Crab Shells and Tree Discards

Jie Wu (2014) created a novel bioplastic derived from crab shells and tree fibers that can be used as an alternative for the flexible plastic packaging used to keep food fresh.[90] Multiple layers of chitin from crab shells and cellulose from trees were sprayed to form a flexible film similar to plastic packaging film. This new bioplastic was compared to polyethylene terephthalate (PET), the most common petroleum-based plastic used as transparent packaging. The study revealed that this new packaging could be more effective and safer to contain liquids and foods.[91,92] In comparison to fossil fuel-based PET plastics,

the novel bioplastic material showed a 73% reduction in oxygen permeability, thereby enabling food to stay fresh for longer.[93]

A.4.9. Lignin-based Bioplastics

As discussed previously in Chapter 2, the high abundance of lignin and lignocellulosic material as by-products of several wood-based industries makes them useful resources for other applications, such as bioplastics. A considerable number of lignin-based bioplastics have been developed and characterized over the past decade.[134-141] In the realm of bioplastics, lignin is most commonly used as a component in copolymers or composites.[134] In general, lignin is easily incorporated in polymers with a large number of polar groups, for example, PHB and PET.[135] Moreover, the addition of lignin to other polymeric materials generally results in improved mechanical properties, and higher stability and biodegradability.

For example, lignin has been utilized in the synthesis of environment-friendly biodegradable polyurethane by synthesising polyurethane-lignin copolymers. Conventional non-biodegradable polyurethane is synthesized through the reaction between a diisocyanate and a diol. However, the polymerization of natural lignin with the resulting polyurethane can impart biodegradability to it, thereby making it more environment-friendly.[136] Introducing lignin in polyurethanes has also been shown to provide better foam properties to the resulting composite as compared to conventional polyurethanes.[136-139] Lignin-based polyurethanes can either be produced via a one-step method that involves the copolymerization of lignin with a diisocyanate or a two-step method that involves the

formation of a conventional polyurethane polymer followed by its polymerization with lignin.[137]

In another study, Spiridon et al. utilized both softwood and hardwood lignin to form composite bioplastics with PLA.[140] They found that the presence of lignin in the composite bioplastics provided them with improved thermal and mechanical properties. For example, the tensile strength of lignin-containing PLA bioplastics was more than two-fold greater than PLA alone. Similar trends were observed for impact strength. Moreover, thermal degradation of lignin-containing bioplastics was initiated at higher temperatures than PLA alone.

In a more recent study, Tedeschi et al. performed an amorphous reassembly of hydrolysed lignin in combination with other wood-derived polymers including cellulose and hemicellulose to create bioplastics.[141] By changing the formulation of the amorphous mixture, lignin-based bioplastics could be developed to exhibit a wide range of properties, including optical, mechanical, antimicrobial and antioxidant properties. This study revealed that the use of hydrolysed lignin in bioplastics acts as a barrier to oxygen, water vapour and grease. Other recently explored lignin-based bioplastics include starch-lignin bioplastics, protein-lignin bioplastics, and cellulose-lignin bioplastics.[134]

A.5. Are Bioplastics Good or Bad for the Environment?

Bioplastics are emerging to be highly controversial when it comes to determining their impact on the environment. While bioplastics are often hailed as excellent alternatives to conventional plastics, they are also associated with some shortcomings.[94] Let us consider

the case of biodegradable bioplastics. Biodegradable bioplastics can decompose into natural materials through microbial mechanisms and blend harmlessly into the soil.[95,96] This decomposition process is aided by water and/or oxygen. For example, when a cornstarch-derived bioplastic is composted, the cornstarch molecules slowly absorb water and swell up when buried. This causes the starch bioplastic to break apart into small fragments that can then be easily digested by bacteria.[95,97-100]However, some low-degrading or nondegradable bioplastics only break-down at high temperatures or when treated in municipal composters or digesters.[101-103] Moreover, some biodegradable plastics can only degrade in specific active landfill sites under certain definite and tried conditions.[104] Decomposition during composting produces methane gas, a greenhouse gas many times more potent than carbon dioxide.[105,106]This greenhouse gas contributes to the problem of global warming.[107]

Furthermore, producing bioplastics from plants such as corn and maize requires repurposing of land for producing plastic instead of fulfilling food requirements.[108] A recent statistical study revealed that almost a quarter of the agricultural land producing grains is used for the production of biofuels and bioplastics. As more agricultural land gets used for the production of biofuels and bioplastics, there may be a significant rise in food prices, affecting the economically weaker sections of the society.[109]

Moreover, a recent study, which compared seven traditional plastics, four bioplastics, and one made from both fossil fuel and renewable sources, determined that bioplastic production resulted in greater amounts of pollutants, owing to the fertilizers and pesticides employed in cultivating the crops, in addition to the chemical processing needed to turn

organic material into the plastic.[110] It was also found that bioplastics contribute more to ozone depletion than traditional fossil fuel-derived plastics.[111] Furthermore, it has been found that B-PET hybrid bioplastic is a potential carcinogen and also has pernicious toxic effects on earth ecosystems.[112,113]

At the same time, bioplastics also have some eco-friendly characteristics. For example, production of PLA saves two-thirds of the energy needed to make traditional plastics.[52] Moreover, it has been scientifically established that during the biodegradation of PLA bioplastics, there is no net increase in carbon dioxide gas.[59] This was evidenced by the fact that the plants from which they were produced absorbed the same amount of carbon dioxide when they were cultivated as was released during their biodegradation.[59,114] Notably, PLA emits 70% less greenhouse gases when it degrades in landfills.[30] Other studies have also found that substituting traditional plastic with corn-based PLA bioplastics can reduce greenhouse gas emissions by 25%.[111,113] Such examples provide assurance that the future production of new bioplastics can be accomplished by using renewable energy while substantially reducing greenhouse gas emissions.

A.6. Life Cycle Analysis – A Way to Address the Controversy around the Eco-friendliness of Bioplastics

To comprehensively compare bioplastics with conventional plastics, it is crucial to evaluate bioplastics' environmental impact from the initial production, utilization, and finally to disposal.[115,116] The most important tool to evaluate the environmental impact of bioplastics and/or conventional plastics is life cycle assessment (LCA) or cradle-to-grave analysis, a

process that can help determine the overall impact of a bioplastic on the environment at each stage in its life cycle.[116, 117] This signifies that the whole life of this industrial product is evaluated, starting from the raw material extraction to the various stages of materials processing, manufacture, distribution, and use.[117] An LCA impact study involves the assessment of global warming, human toxicity, abiotic depletion, eutrophication and acidification.[118,119] In addition, when conducting the LCA, it is essential to consider Land Use Change (LUC)-related emissions and the cost and benefits of bioplastic disposal.[120] LUC is a guide to consider when land is converted to spaces for composting, biofuel feedstock production or other uses.[121]

It is essential to understand the LCA of different bioplastic composting, recycling and disposal scenarios of . Indeed, a meticulously performed LCAs can serve as an important reference material for policymakers.[122] For example, numerous protocols have been established to conduct LCA/cradle-to-grave studies on PLA bioplastics currently in the market.[123] These studies involve comparisons of their LCA with that of fossil-fuel plastics such as polyethylene and PET.[124] For instance, a recent study revealed that there was a significant reduction in greenhouse gases when manufactured bottles were made by subsisting 20% of the PET bottles with PLA bottles.[125] This study was carried out by using the Intergovernmental Panel on Climate Change (IPCC) method and a LCA cradle-to-grave study.[125-127] Another study, using the Global Warming Potential (GWP) guide in which the greenhouse gas emission was measured in kg of CO_2 equivalents, showed that it was possible to reduce greenhouse gas emissions by substituting petroleum-based plastics with

bioplastics.[128,129] Additional, separate LCAs for other bioplastics can also provide such valuable data.

LCA also provides an important means of identifying the best method of bioplastic waste management and disposal. For example, LCA has revealed that incineration or landfilling of bioplastic products is not a useful alternative.[95,130] A plausible solution to bioplastic waste management problem was confirmed by adhering to the LUC emissions principle, which established the reliability of bioplastics as an excellent replacement for petroleum-based plastics.[131,132] Compared to conventional petroleum-derived plastics, the use of PLA and thermoplastic starch significantly reduces carbon dioxide emissions, in the case of the former, by 50-70%.[133] Similarly, bio-urethanes and poly(trimethyleneterephthalate) (PTT) have respectively 36% and 44% lower greenhouse gas emissions than their petroleum-derived counterparts.[133] However, to continue the smart management of bioplastic wastes, it has been proposed that the reduction of greenhouse gas emissions must reach zero LUC emissions.[120,131] Future studies should focus on conducting individual LCAs for the ever-growing range of bioplastics, many of which have been discussed earlier in this review.

A.7. Conclusion

A variety of bioplastics have been developed to address the environmental issues associated with conventional petroleum-derived plastics – from well-known and well-studied biodegradable and/or bio-based bioplastics like PHB, PCL and PLA to recent additions such as mycelium-based and chitin-based biopolymers. Importantly, however, bioplastics are associated with some shortcomings. It should be understood that similar to petroleum-

based plastics, some bio-based bioplastics cannot be recycled. Consequently, many biodegradable bioplastics end up in landfills, which decompose gradually and produce methane gas. For these reasons, people are starting to believe that bioplastics should be used only when needed, with tailor-made properties. However, it is important that we weigh these environment-related shortcomings of bioplastics against the harms caused by conventional plastics. Studies, including several discussed in the present review article, show that the harms associated with bioplastics are still less severe when compared to conventional plastics. Moreover, as new types of bioplastics such as those discussed in this article keep getting developed by academic and industry-oriented researchers, it is possible that the drawbacks of currently used bioplastics can be addressed adequately. In order to confirm the eco-friendliness of these new bioplastics, future studies should conduct thorough LCAs and LUC analyses. Such studies will help policymakers to determine whether the use of new-generation bioplastics is indeed beneficial to the environment.

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APPENDIX B: MASS SPECTROMETRY AND TANDEM MASS SPECTROMETRY ANALYSES OF OAK LIGNIN OLIGOMERS

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1) Mikhael, A., Jurcic, K., Fridgen, T. D., Delmas, M., & Banoub, J. (2020). *Rapid Commun. Mass Spectrom. 34*(18), e8841

2) Mikhael, A., Fridgen, T. D., Delmas, M., & Banoub, J. (2020). *J. of Mass Spectrom.* e4676.

B.1. APPI-QqTOF-MS, ESI-QqTOF-MS, and low energy CID-MS/MS

Mass spectrometry was performed using an Applied Biosystems (Foster City, CA, USA) API-QSTAR-XL MS/MS QqToF-MS/MS hybrid instrument. APPI was performed with a PhotoSpray ion source (Applied Biosystems) operated at 1300 V at a temperature of 400°C, with all acquisitions completed in the positive ion mode. Samples were infused into the mass spectrometer with an integrated Harvard syringe pump at a rate of 0.1 ml/min. The auxiliary nebulizer gas pressure setting was fixed at 25 psi and the nebulizer gas pressure at 74 psi. The curtain gas pressure was set at 30 psi. The declustering potential (DP) was established at +100 eV. The focus potential (FP) was adjusted to +100 V. Toluene was

selected as the dopant for its ability to undergo trouble-free photoionization at 8.83 eV. The eluent was composed of dioxane/methanol (2:1). No modifier was used to enhance ion production. ESI-QqTOF-MS (positive ion mode) analysis was performed with the same MS instrument equipped with a TurboIonspray source operated at 4.5 kV at a temperature of 200°C. The mass calibration of the TOF analyzer in the positive ion mode was performed with the PhotoSpray ion source, using 1,2,3,5-tetra-Oacetyl-β-D-ribofurnanose and checking for the exact masses of the $[M + H]^+$ ion $[C_{13}H_{18}O_9 + H]^+$ at *m/z* 319.1029 and the $[M + H–AcOH]^+$ ion $[C_{11}H_{14}O_7 + H]^+$ at *m/z* 259.0817. Calibration for higher masses was performed with octa-O-acetyl-β-D-lactopyranose and checking for the $[M + H]^+$ ion $[C_{28}H_{38}O_{19} + H]^+$ at *m/z* 679.2085. Product ion spectra were obtained on the same instrument, as described above. Nitrogen was used as the collision gas for MS/MS analyses with collision energies varying between 10 and 35 eV. Collision energy (CE) and CID gas conditions were adjusted in each acquisition such that the precursor ion remained abundant in the product ion spectra. In general, we have used a 1 *m/z* unit resolution for the MS/MS selection of the precursor ion for the simplification of the analysis.

B.2. MALDI-TOF-MS/MS (Negative ion mode) and High energy-CID-TOF/TOF-MS/MS Analyses

Mass spectrometric data were obtained using an AB Sciex 5800 MALDI TOF/TOF mass spectrometer (Framingham, MA, USA). Data acquisition and data processing were, respectively, performed using TOF Series Explorer and Data Explorer (both from AB Sciex). The instrument is equipped with a 349 nm Nd:YLF OptiBeam On-Axis laser, and

the laser pulse rate was 400 Hz. Reflectron negative ion mode was used, and MS spectra were acquired in the mass range from *m/z* 100 to 1000. The reflectron and MS/MS modes were externally calibrated at 50 ppm mass tolerance. Each MS spectrum was collected as a sum of 500 laser shots, while MS/MS spectra were obtained as a sum of 900 shots. Raw files were viewed by using the Mmass program. In addition, this latter program was used to find possible formulae for the selected ions. The search for possible formulae was done within +/- 0.2 *m/z* units from the experimental masses. The resulting list of formulae was arranged according to their isotopic distribution fitting percentage to help us in assigning the most likely formulae for the studied ions. All assigned chemical structures are supported by tandem mass spectrometry, especially for those with a low isotopic distribution fitting percentage.

B.3. Negative Ion Mode MALDI-MS/MS

The negative-ion mode MALDI-MS/MS (Figure B.1) of the virgin released lignin (VRL) extracted from the Oak wood showed the complexity and the diversity of the lignin mixture. From this study, several types of compounds were identified: Seven tricin derivatives, five syringyl lignin derivatives, two flavonolignin derivatives, and six miscellaneous compounds such as luteoferol, lariciresinol isomer, 5-hydroxy guaiacyl derivative, syringyl -$C_{10}H_{10}O_2$ dimer, trihydroxy benzaldehyde derivative, and aryl tetralin lignan derivative. Interestingly, most of the detected compounds were carbohydrate and/or shikimic acid complexes. These identified structures were supported by tandem mass spectrometry (MS/MS) and/or their fragmentation mechanism, as shown in some selected schemes from

this work (Figure B.2-B.17 and Schemes B.1-B.16). These identified compounds support the abundance of syringyl lignin units in the oak VRL sample and the association of lignin with carbohydrate in the wood network.

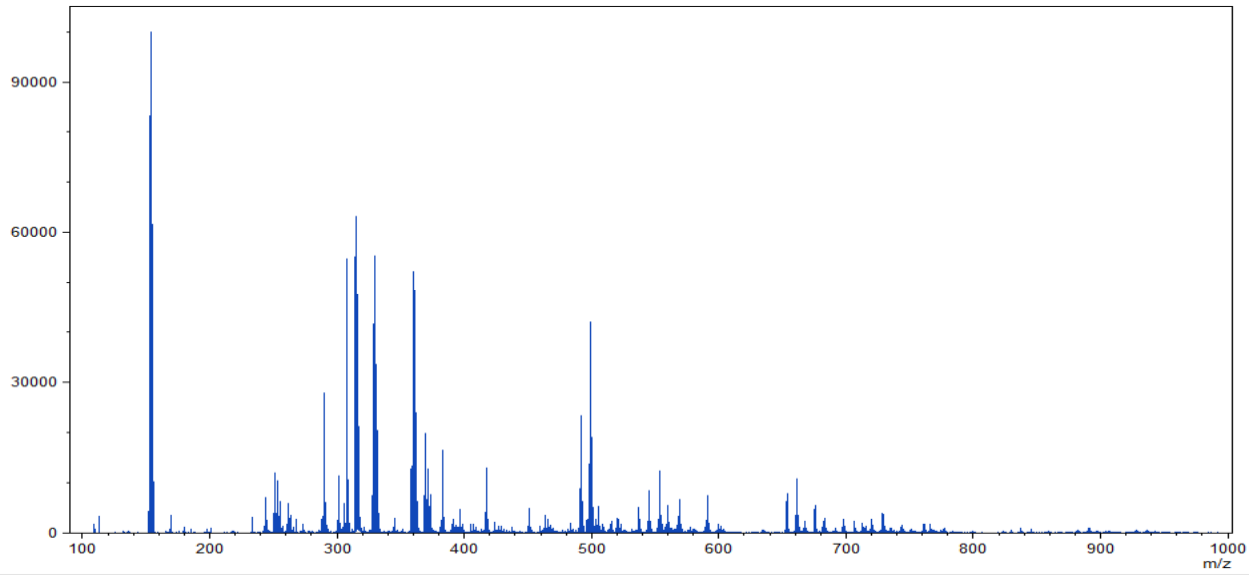


Figure B.1. MALDI-TOF-MS of the VRL lignin oligomers extracted from oak wood.

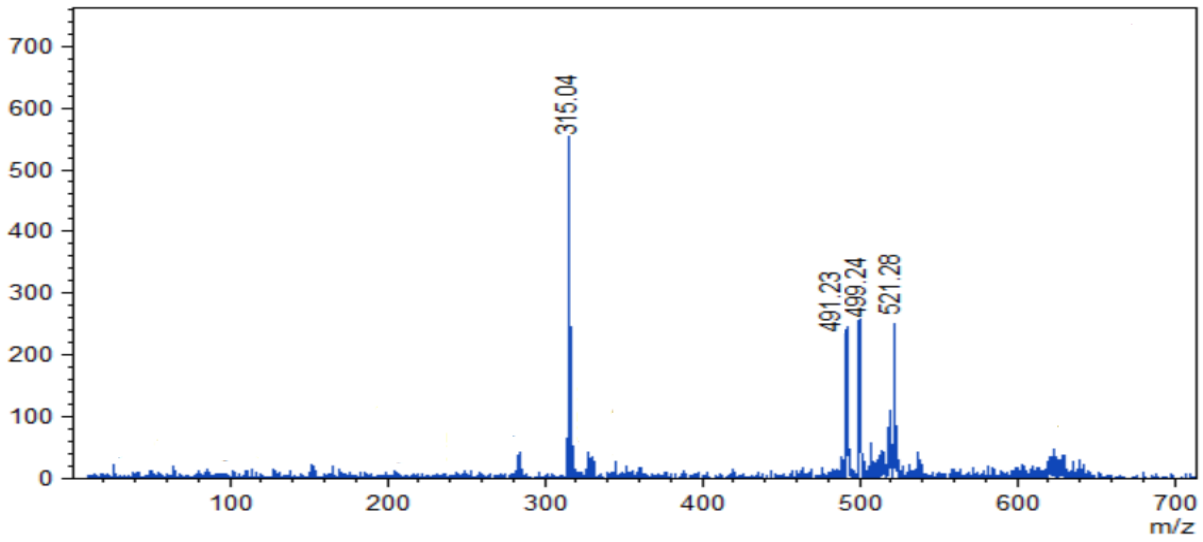
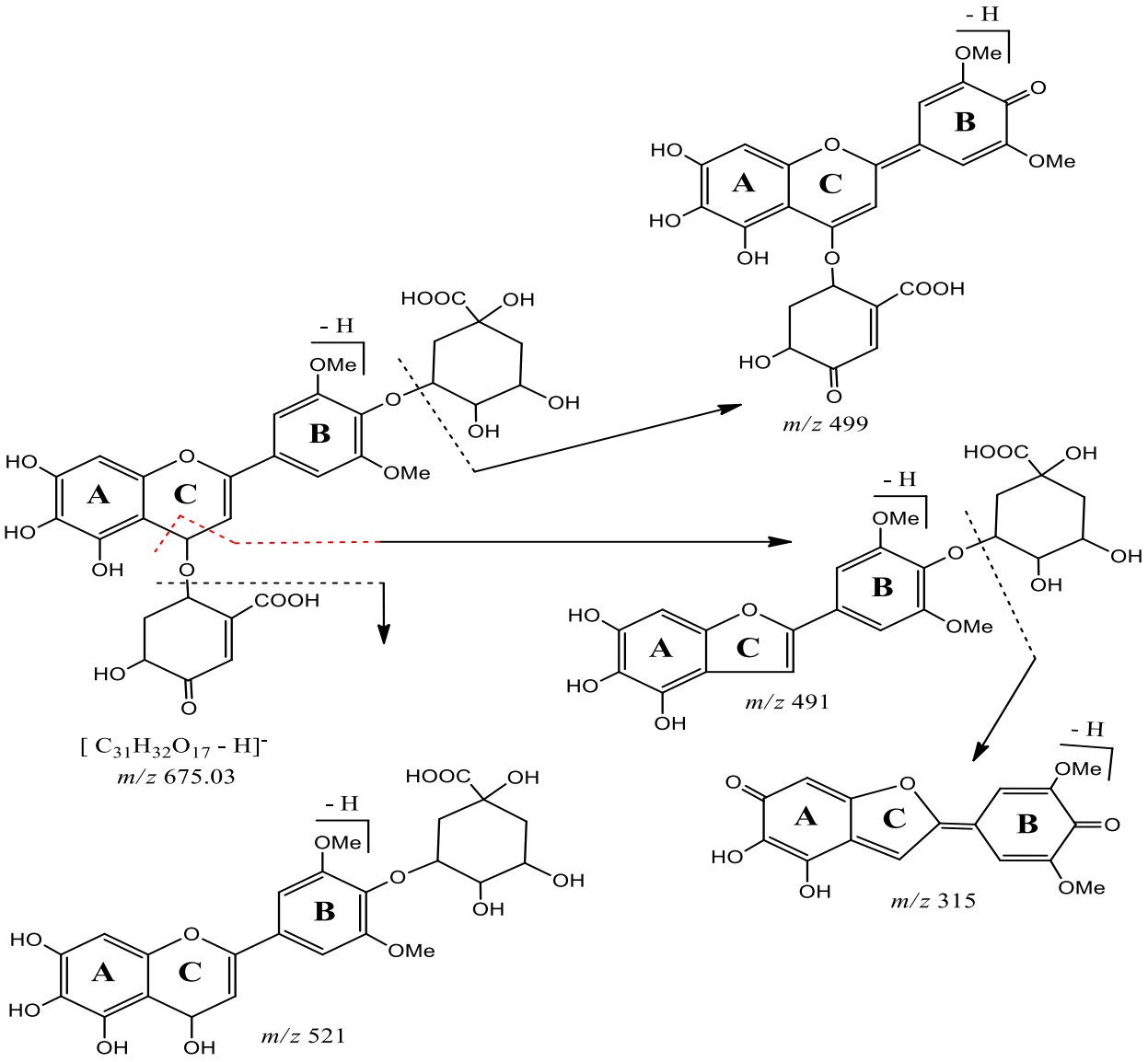


Figure B.2. Product ion scan of the precursor ion at *m/z* 675.03



Scheme B.1. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 675.03

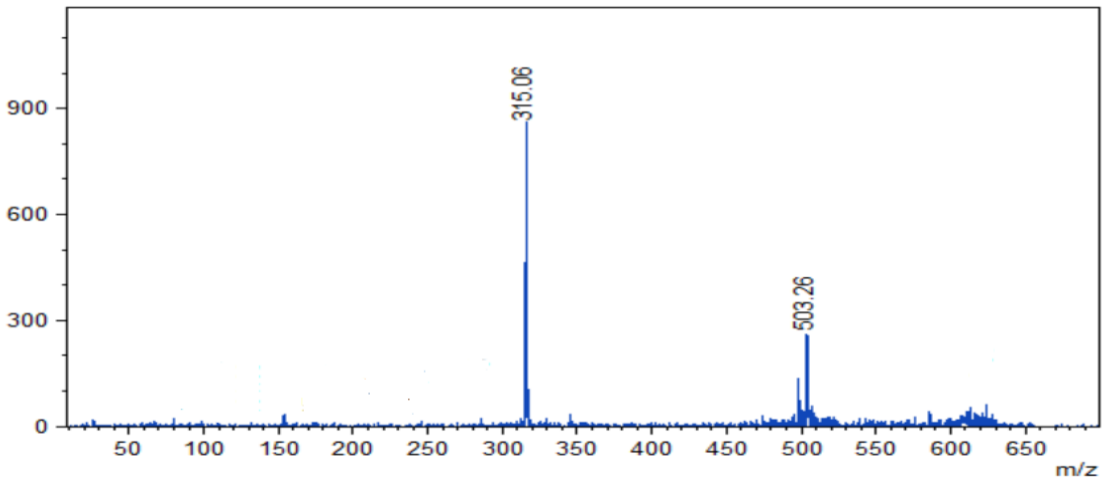
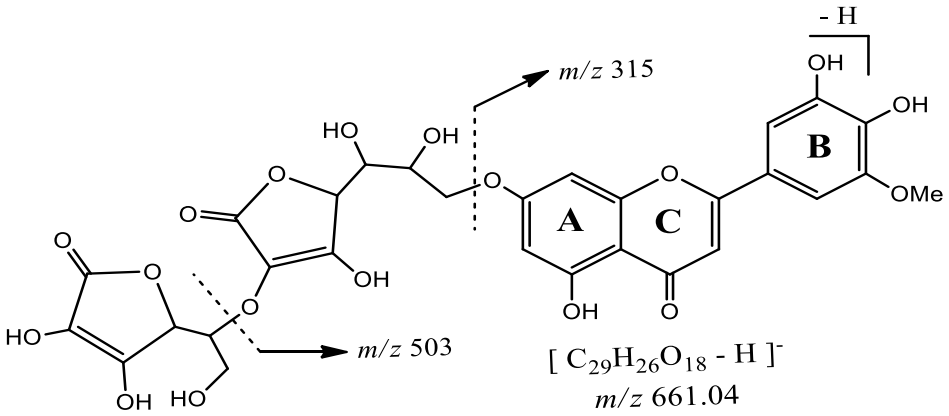


Figure B.3. Product ion scan of the precursor ion at *m/z* 661.04



Scheme B.2. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 661.04

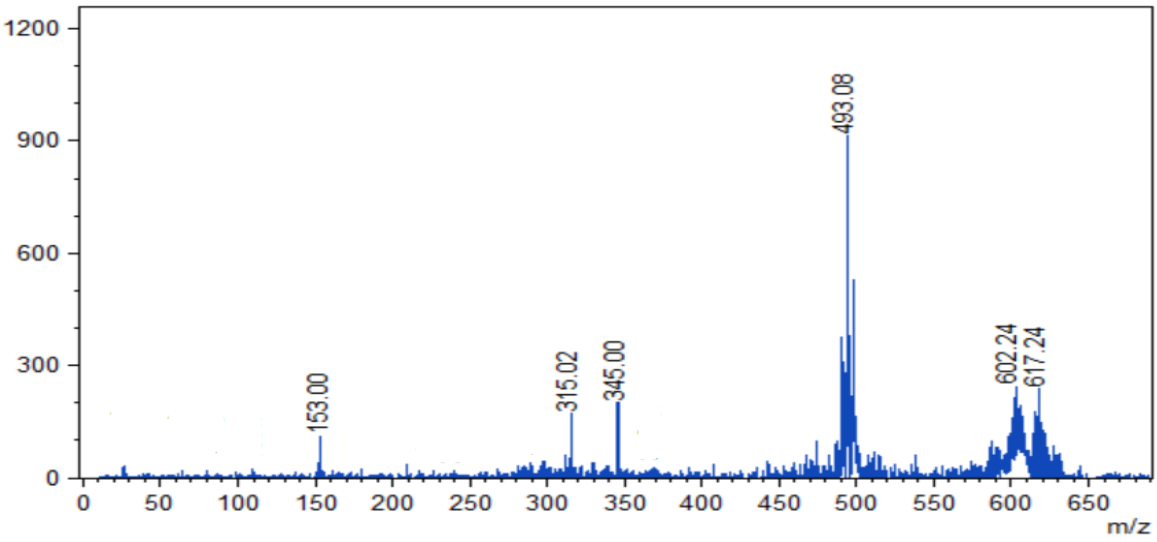
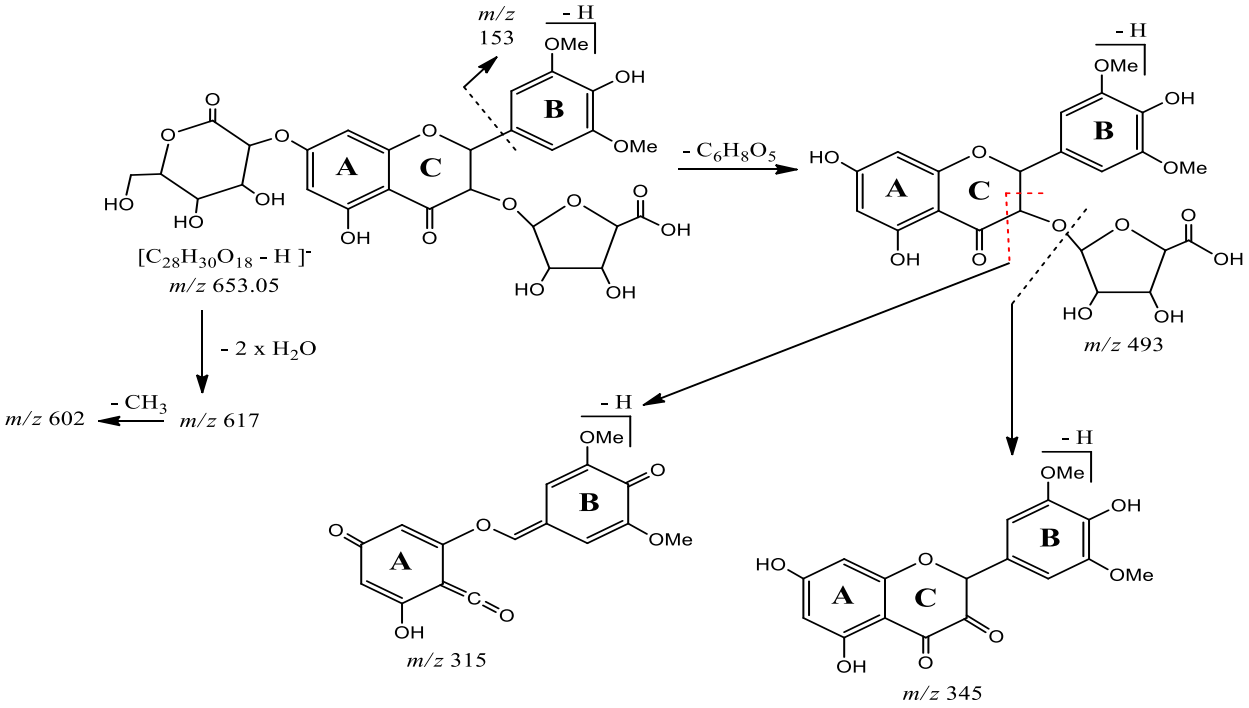


Figure B.4. Product ion scan of the precursor ion at *m/z* 653.05



Scheme B.3. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 653.05

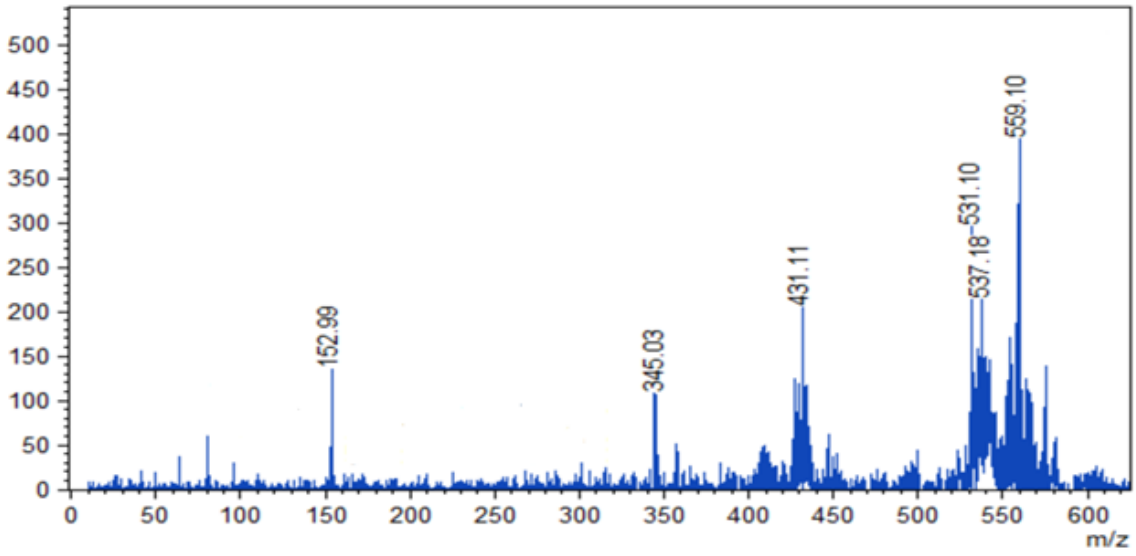
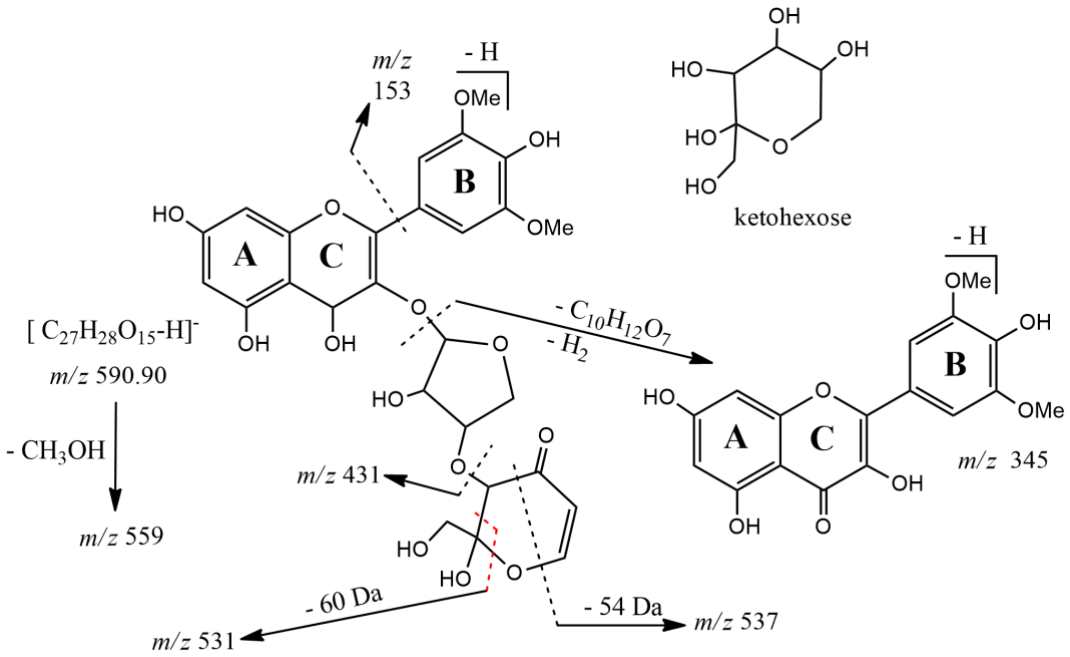


Figure B.5. Product ion scan of the precursor ion at *m/z* 590.90



Scheme B.4. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 590.90

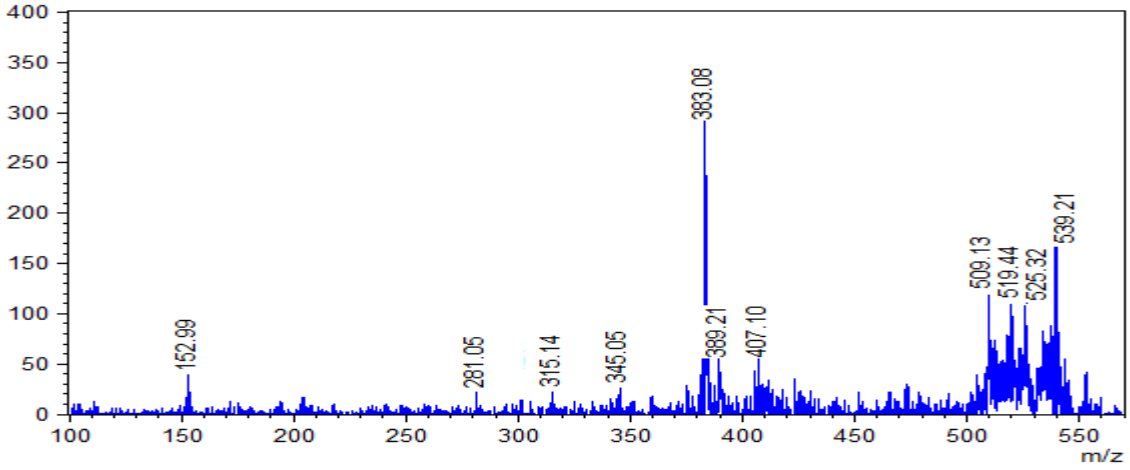
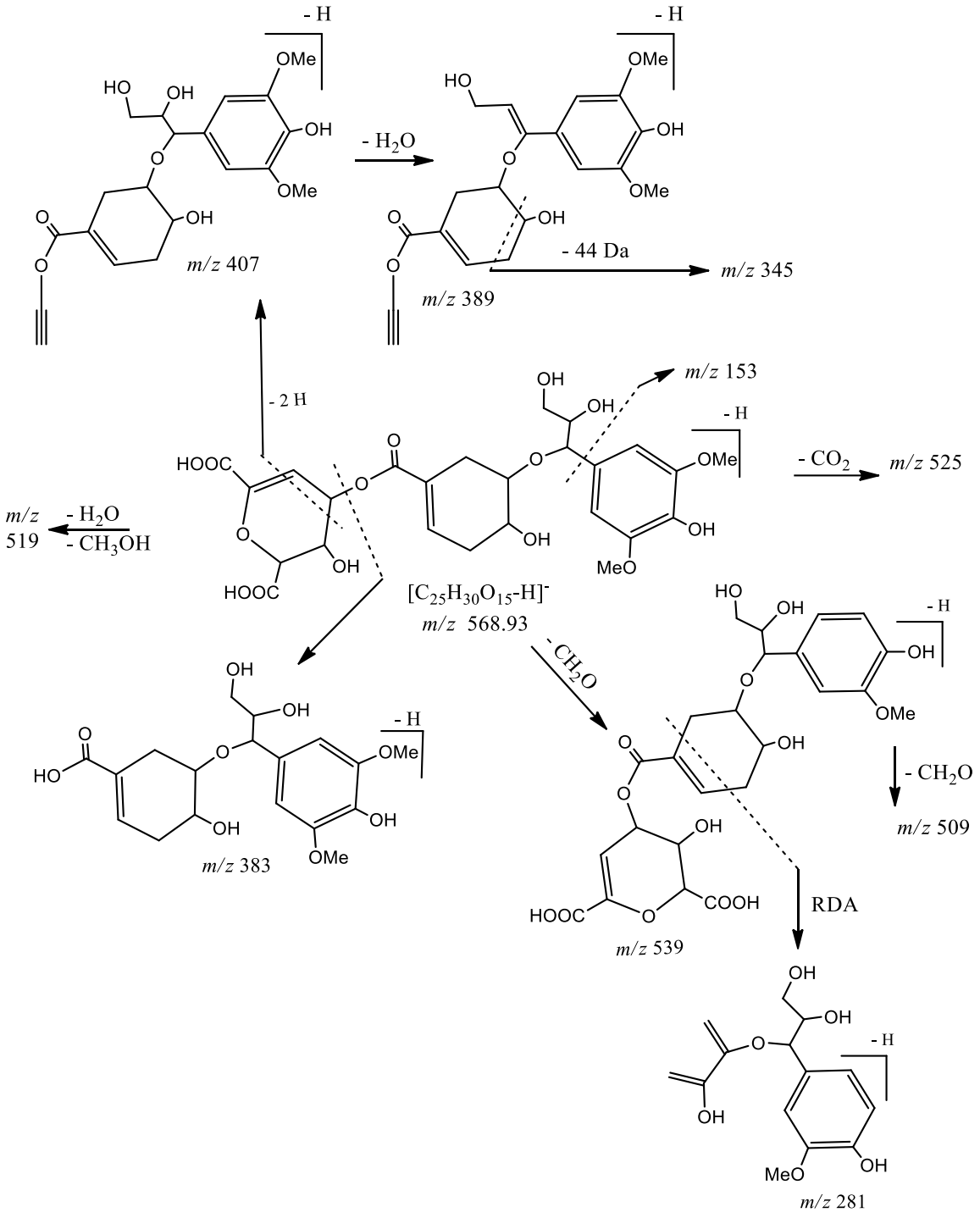


Figure B.6. Product ion scan of the precursor ion at *m/z* 568.93



Scheme B.5. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 568.93

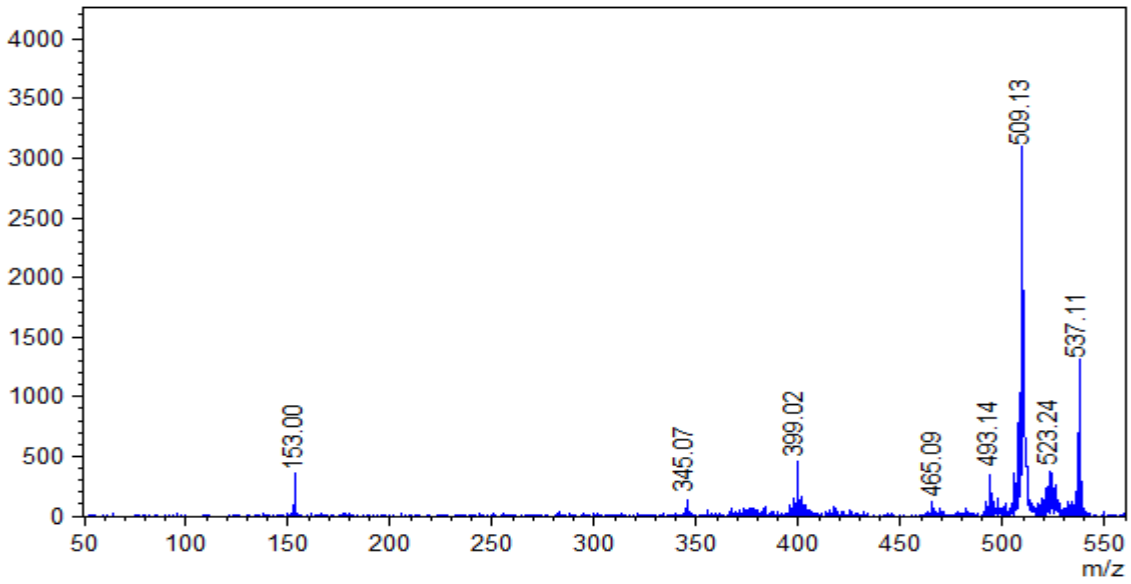
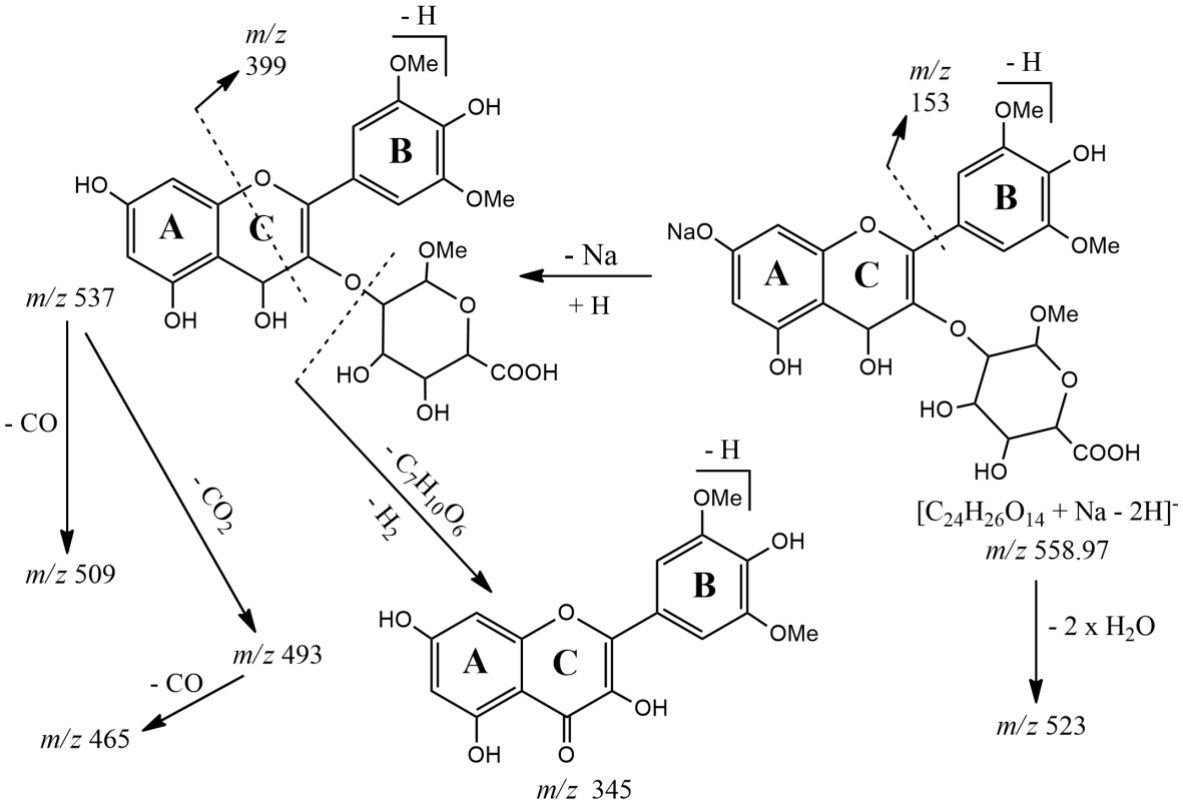


Figure B.7. Product ion scan of the precursor ion at *m/z* 558.97



Scheme B.6. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 558.97

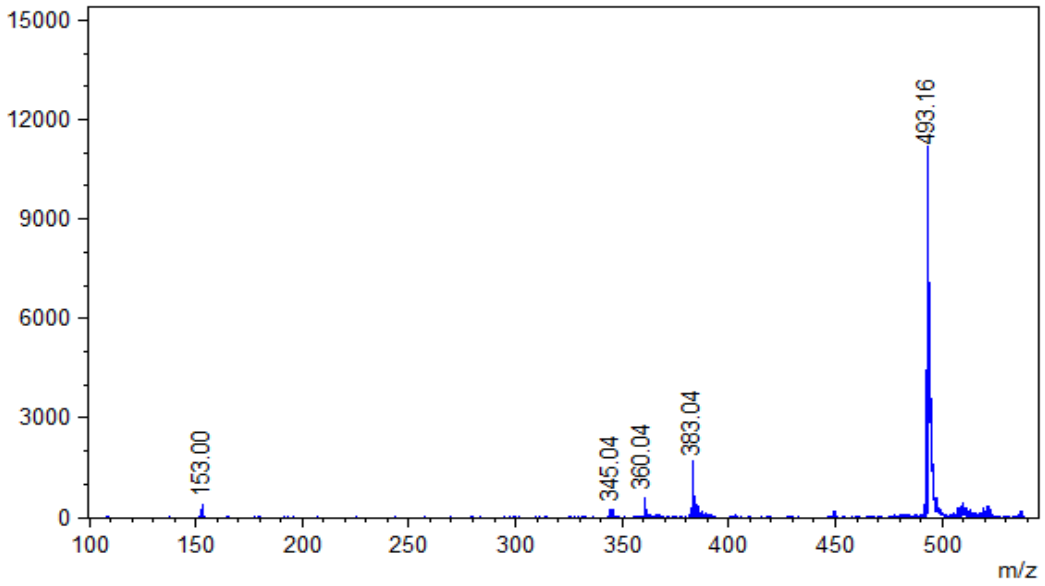
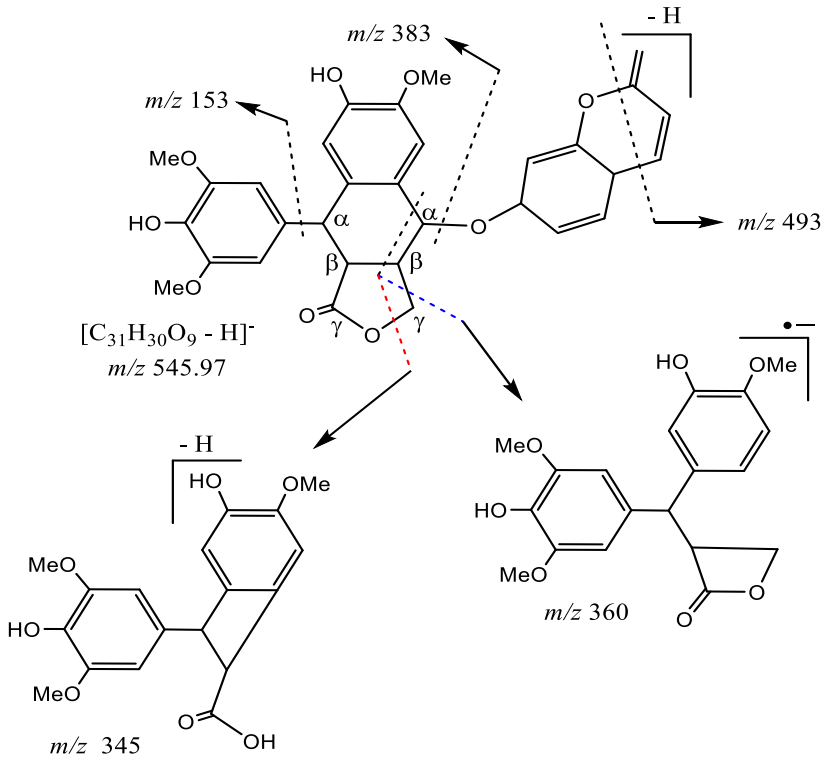


Figure B.8. Product ion scan of the precursor ion *20* at *m/z* 544.97



Scheme B.7. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 544.97

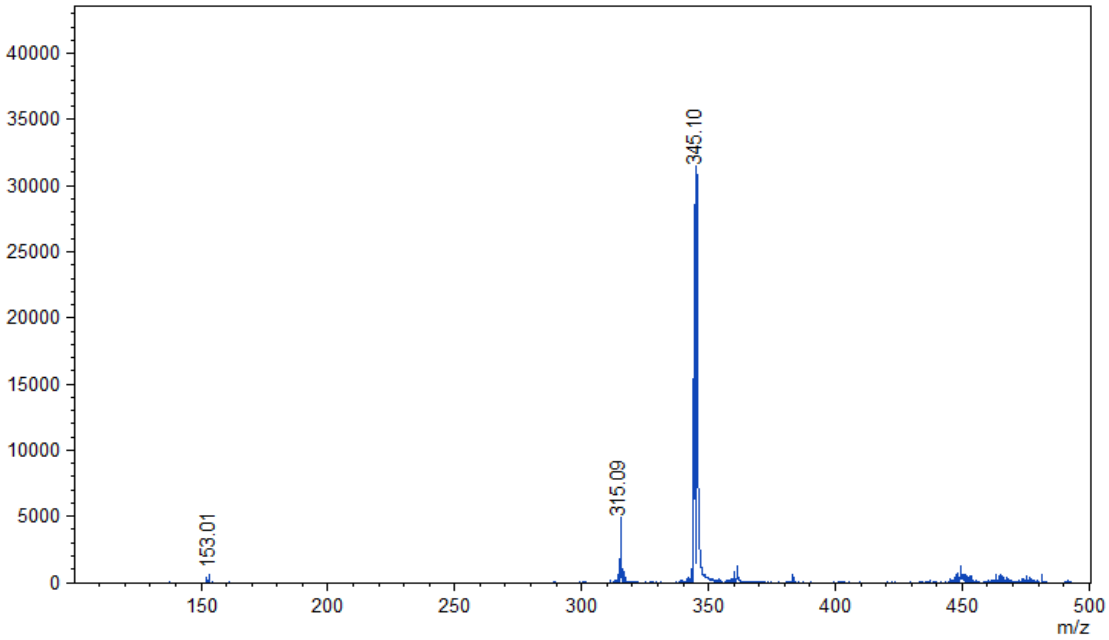
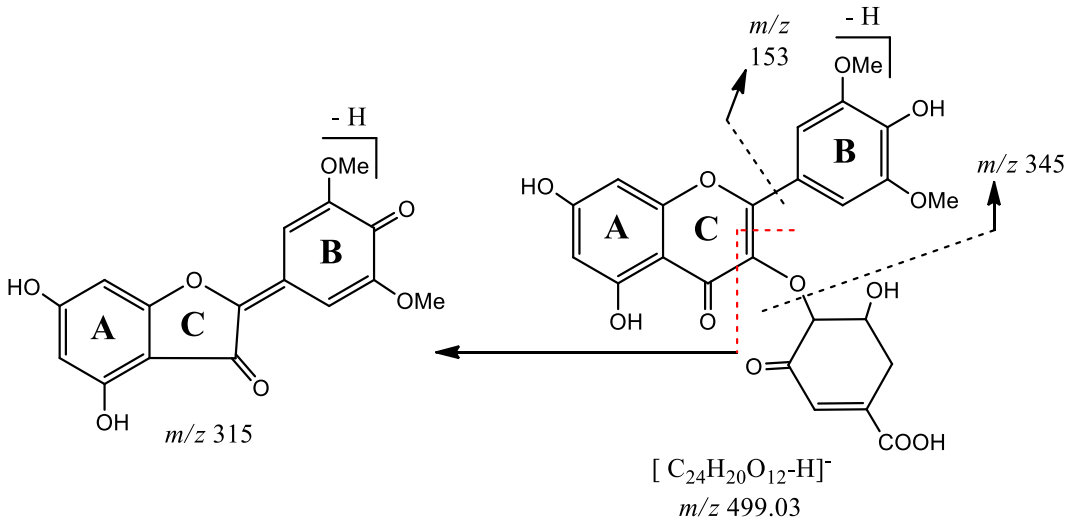


Figure B.9. Product ion scan of the precursor ion at *m/z* 499.03



Scheme B.8. High energy CID-MS/MS fragmentation mechanism of the precursor anion at *m/z* 499.03

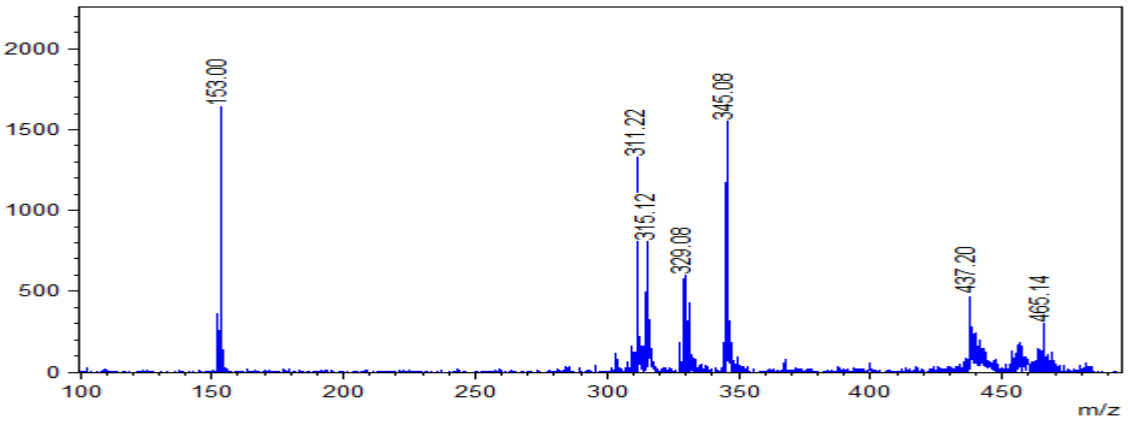
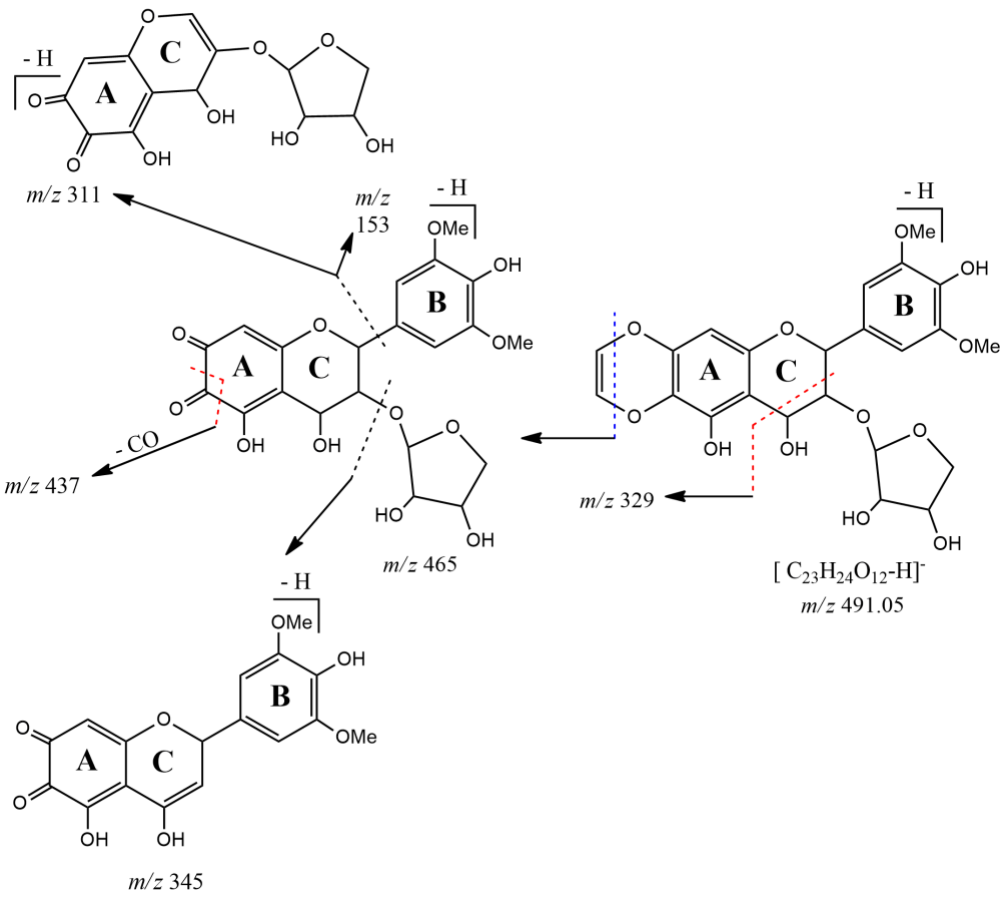


Figure B.10. Product ion scan of the precursor ion at *m/z* 491.05



Scheme B.9. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 491.05.

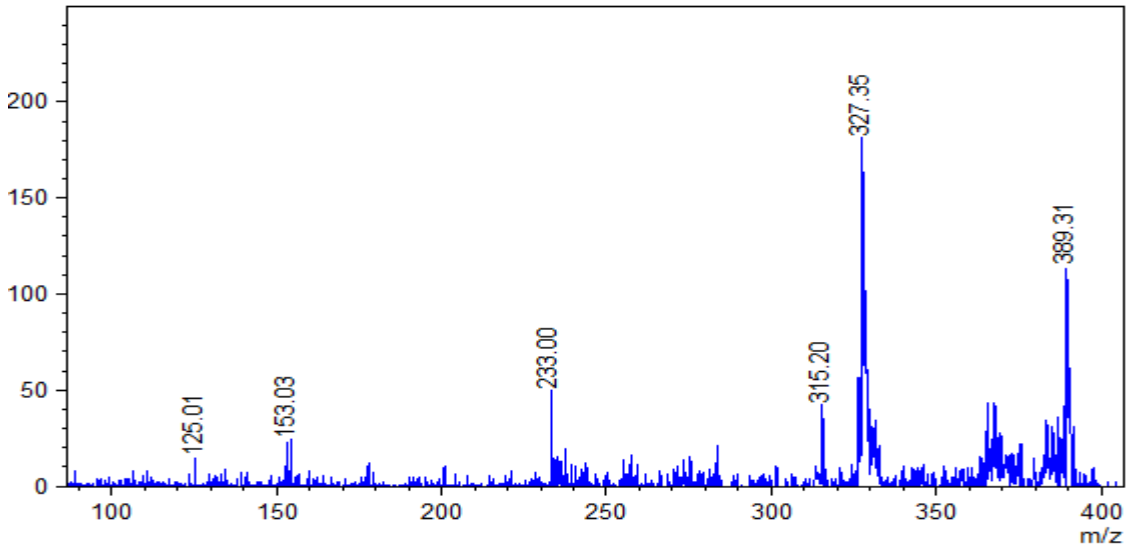
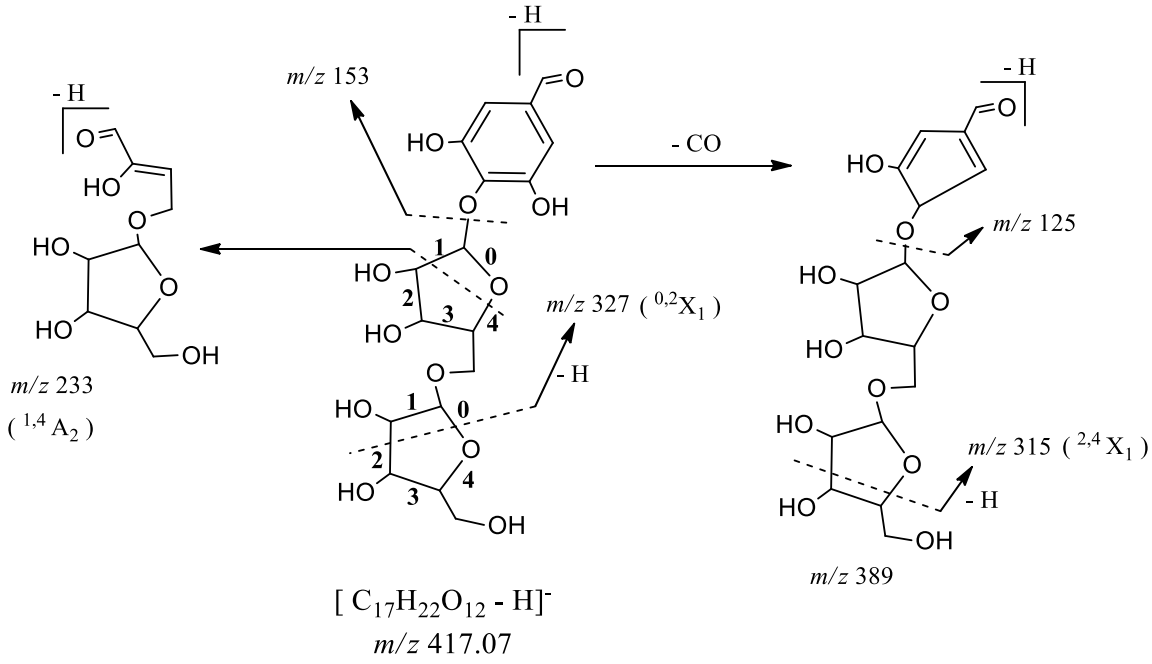


Figure B.11. Product ion scan of the precursor ion at *m/z* 417.07



Scheme B.10. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 417.07

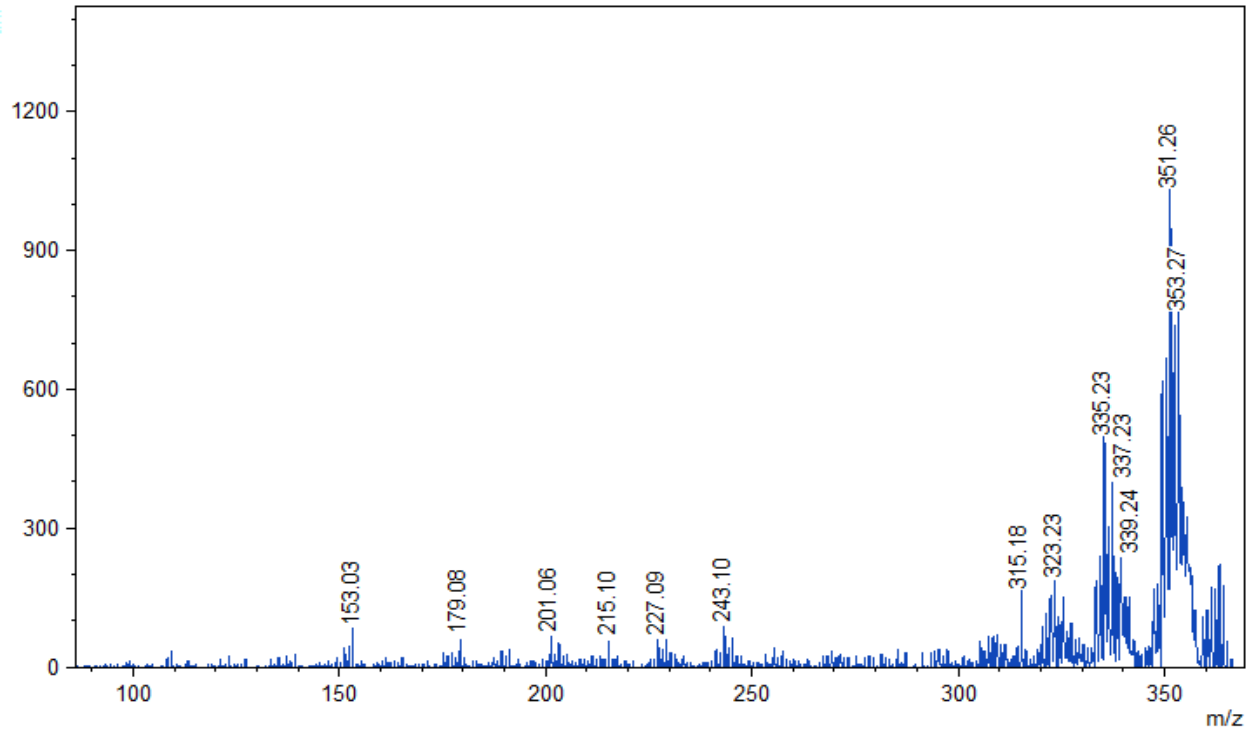
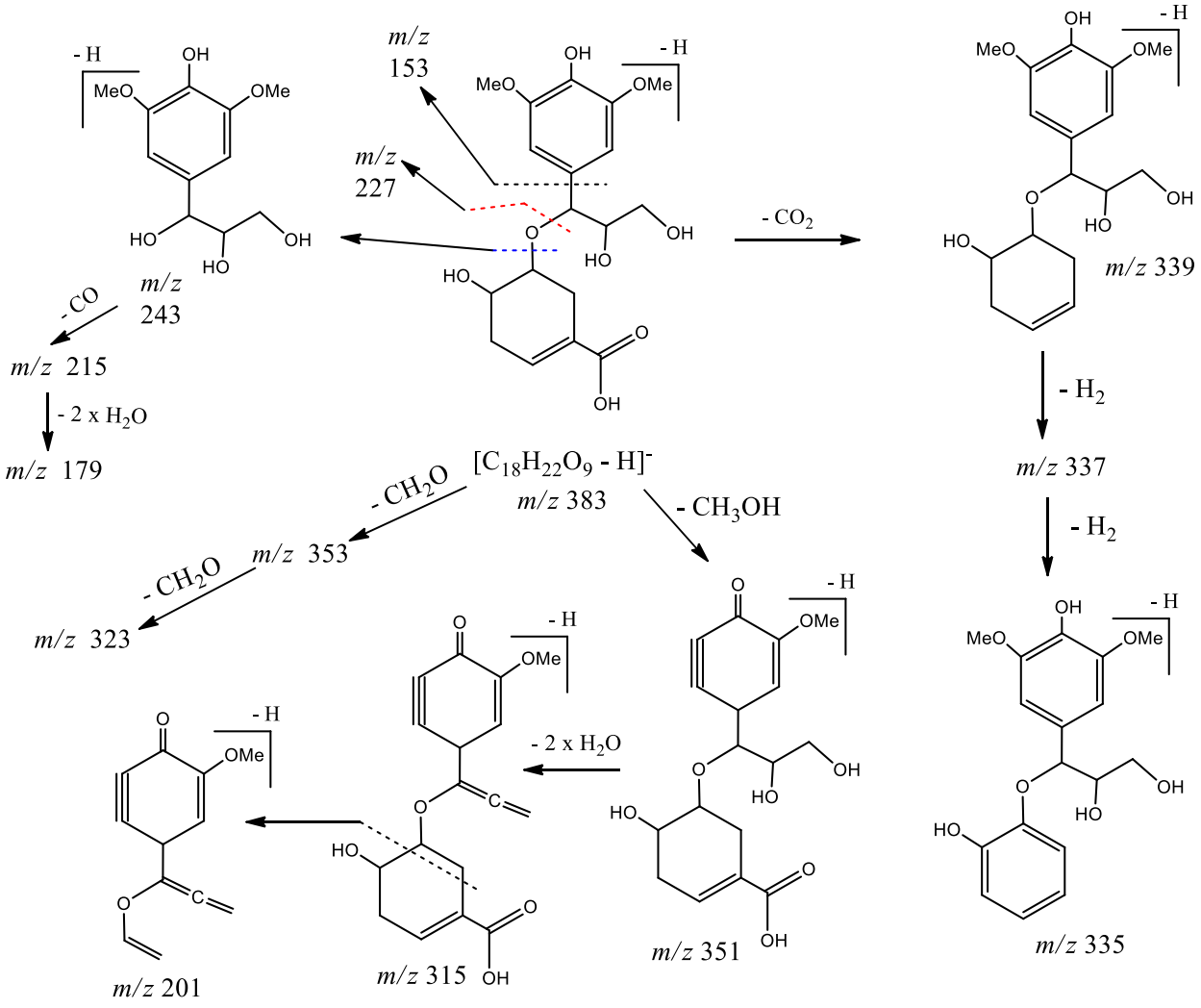


Figure B.12. Product ion scan of the precursor ion at *m/z* 382.96



Scheme B.11. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 382.96

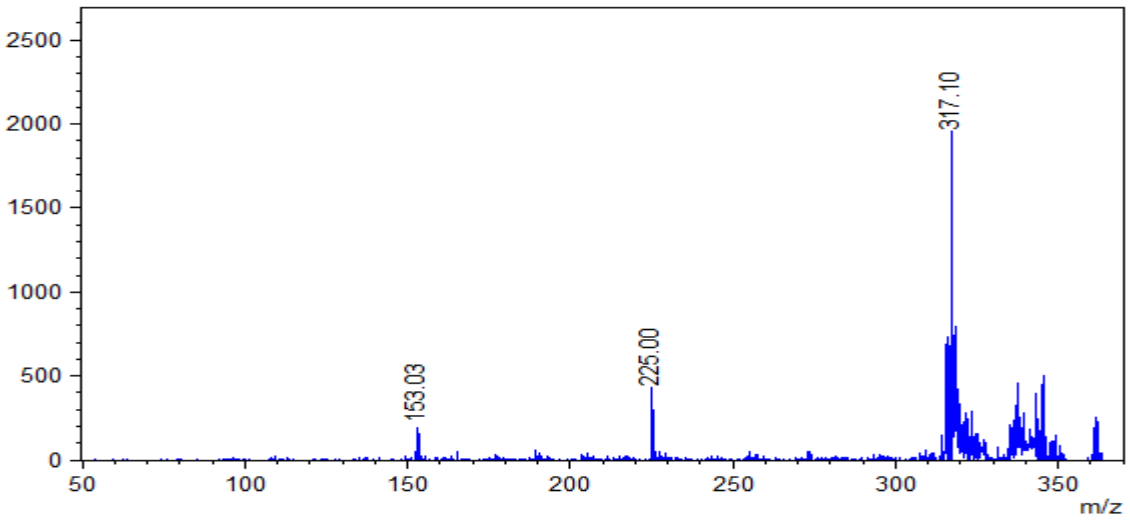
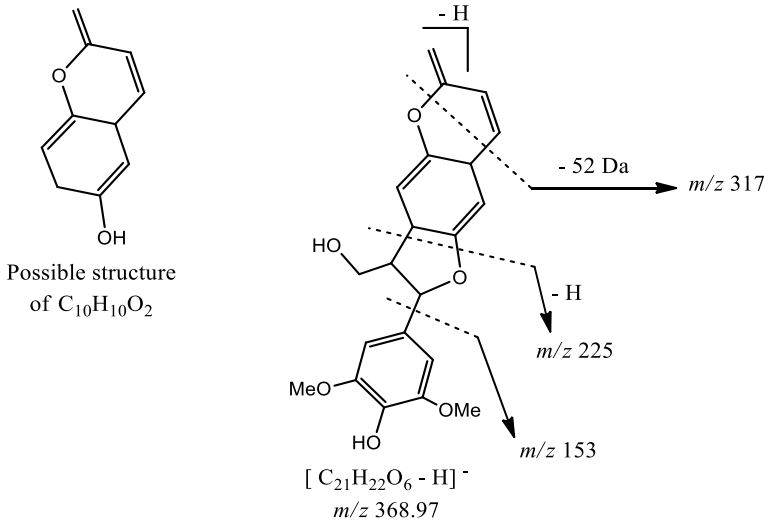


Figure B.13. Product ion scan of the precursor ion at *m/z* 368.97



Scheme B.12. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 368.97

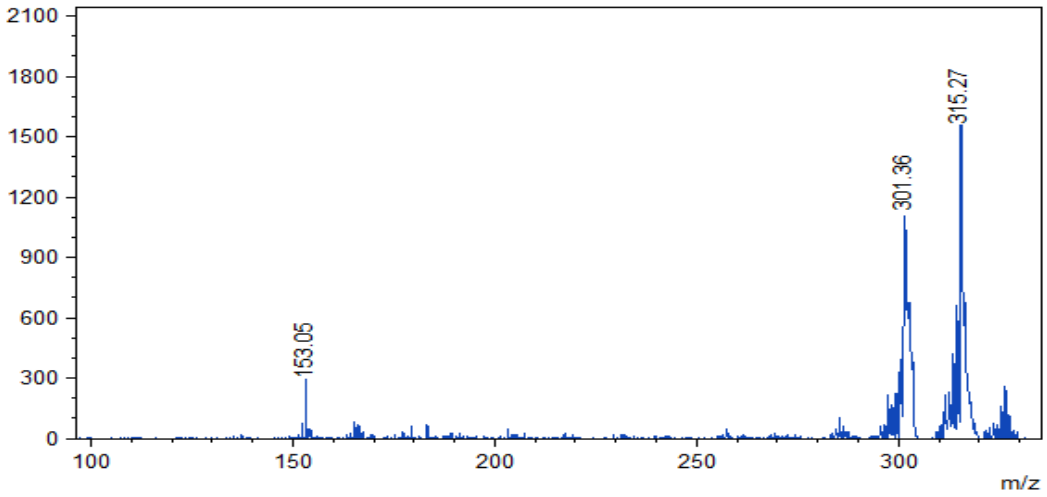
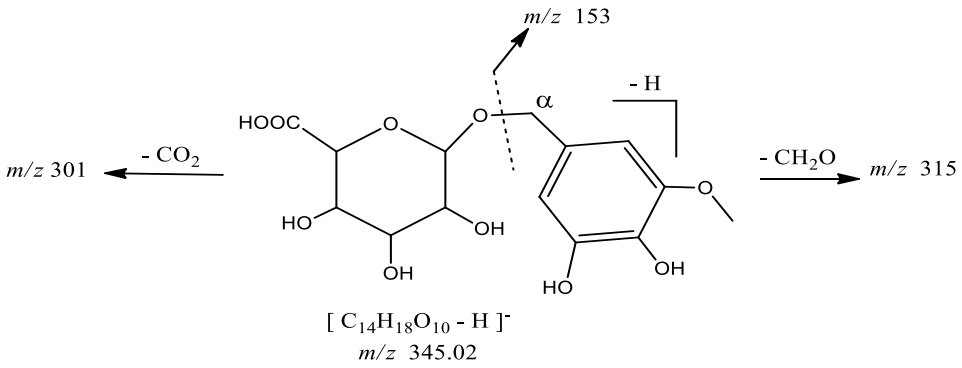


Figure B.14. Product ion scan of the precursor ion at *m/z* 345.02



Scheme B.13. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 345.02

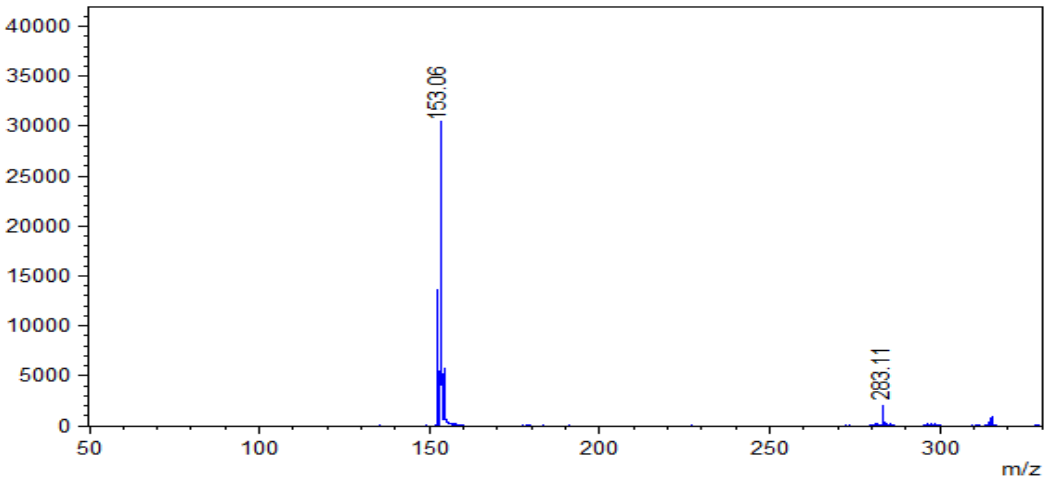
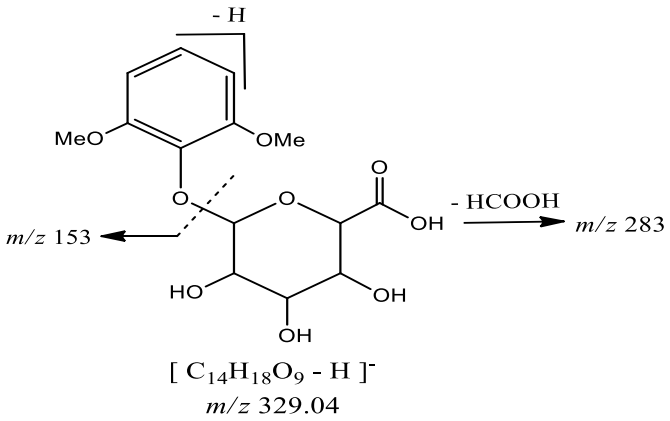


Figure B.15. Product ion scan of the precursor ion at *m/z* 329.04



Scheme B.14. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 329.04

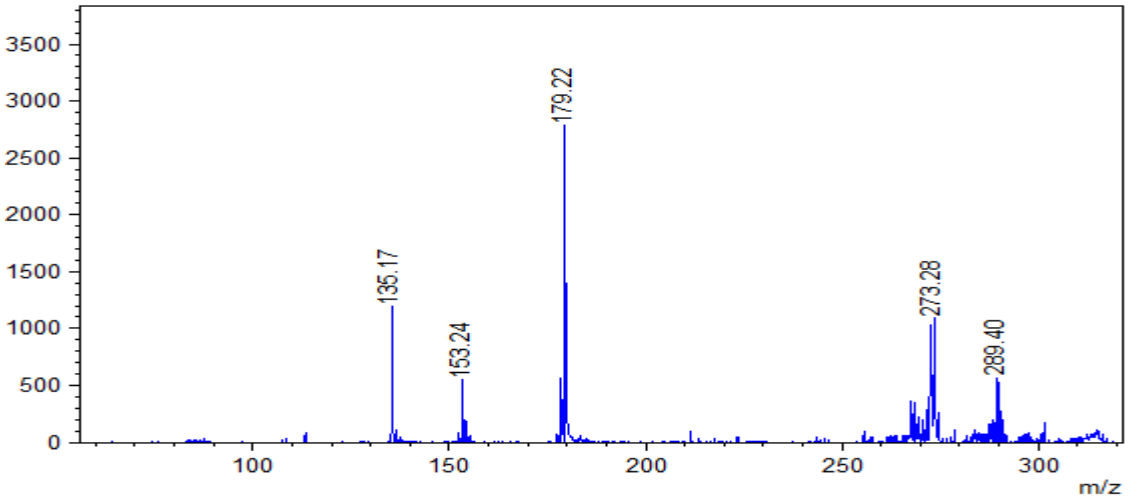
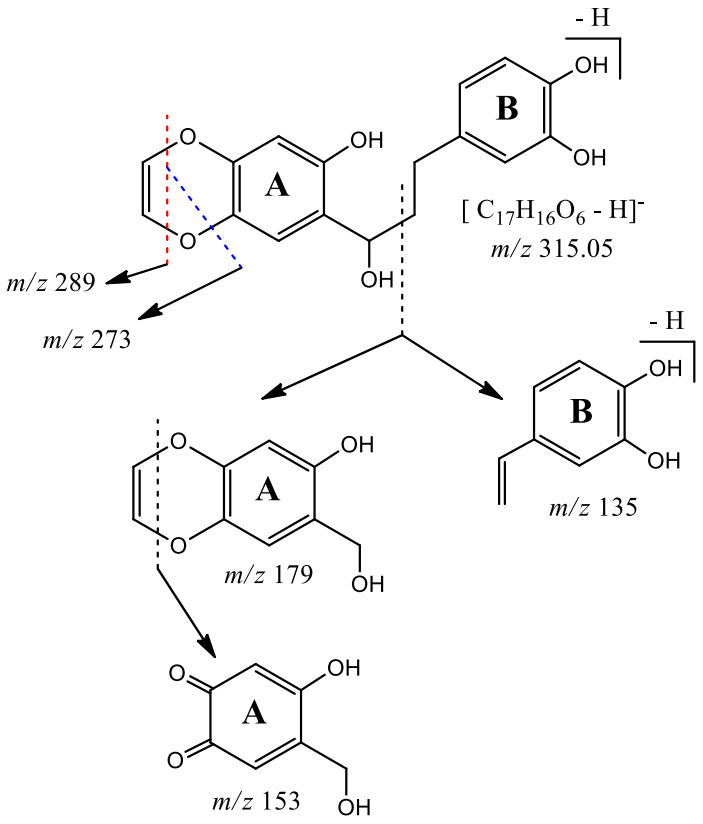


Figure B.16. Product ion scan of the precursor anion at *m/z* 315.05



Scheme B.15. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 315.05

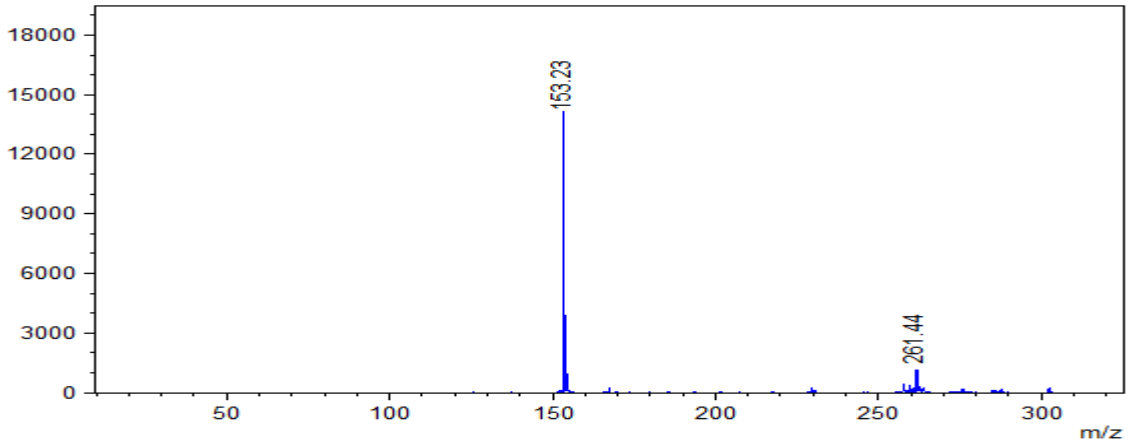
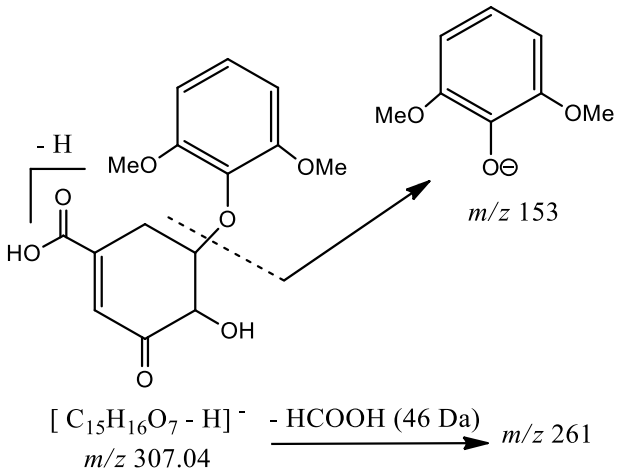


Figure B.17. Product ion scan of the precursor ion *11* at *m/z* 307.04



Scheme B.16. High energy CID-MS/MS fragmentation mechanism of the precursor ion at *m/z* 307.04

B.4. Positive Ion Mode APPI-QqTOF-MS and Low-Energy CID-MS/MS

The Oak VRL complex mixture was investigated by positive ion mode APPI- QqTOF-MS (Figure B.18). This study led to the identification of eight major protonated lignin oligomers: neolignan cedrusin *(1)*, five different aryltetralin lignans dimers *(2-6)*, one lignan-dehydroshikimic acid complex *(7)*, and a lignan trimer *(8)*. These identified structures were supported by low-energy CID-MS/MS analyses and/or fragmentation mechanisms, as shown in some selected schemes from this work (Figure B.19-B.23 and Scheme B.17-B.21)

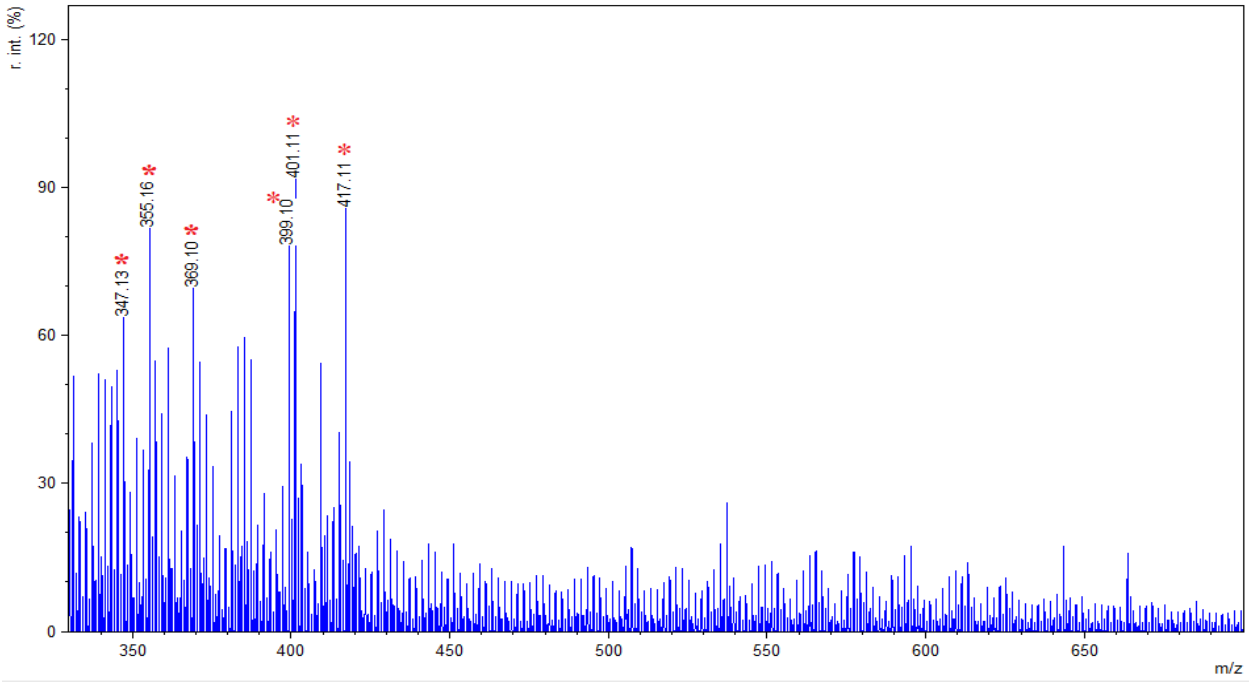


Figure B.18a. APPI-QqTOF-MS of the Oak lignin from *m/z* 340-700

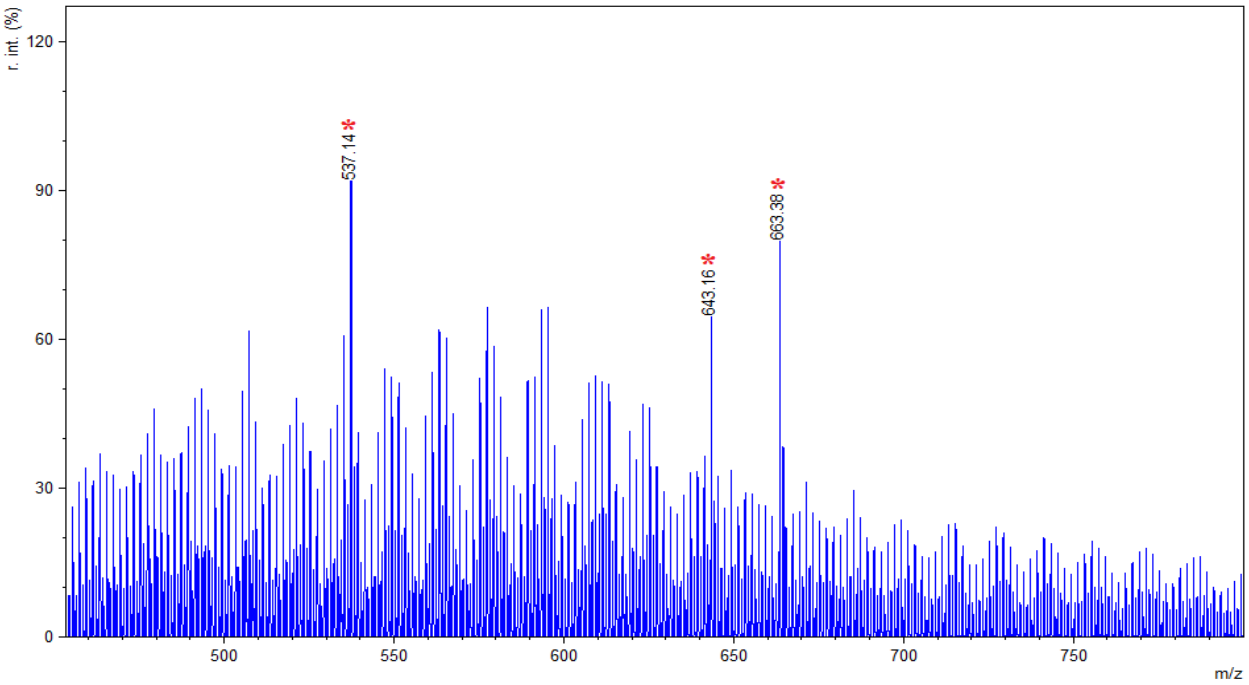


Figure B.18b. APPI-QqTOF-MS of the Oak lignin from *m/z* 450-800

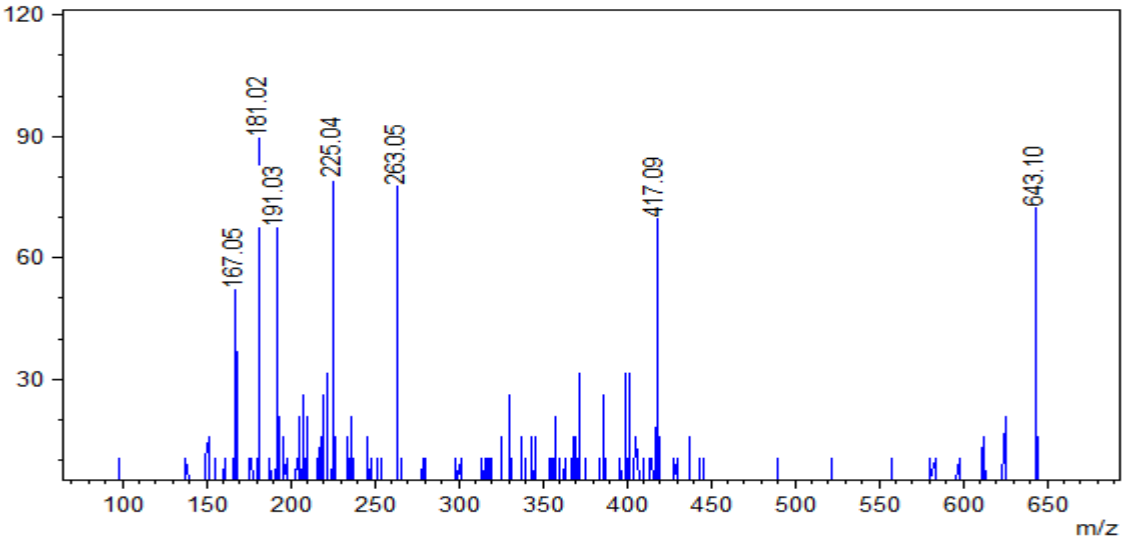
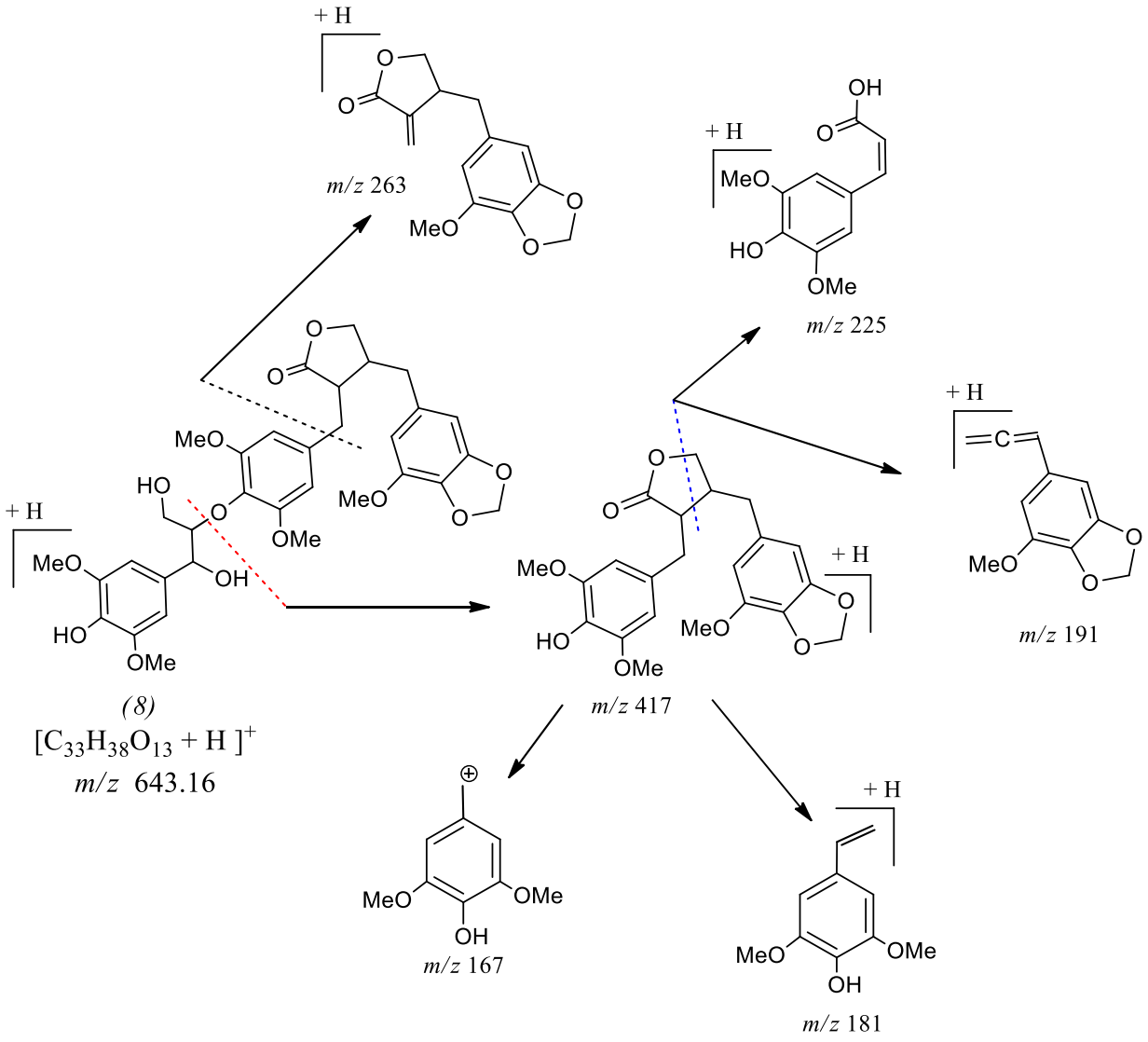


Figure B.19. Product ion scan of the protonated precursor ion *8* at *m/z* 643.16



Scheme B.17. Low energy CID-MS/MS fragmentation mechanism of the protonated precursor ion at *m/z* 643.16

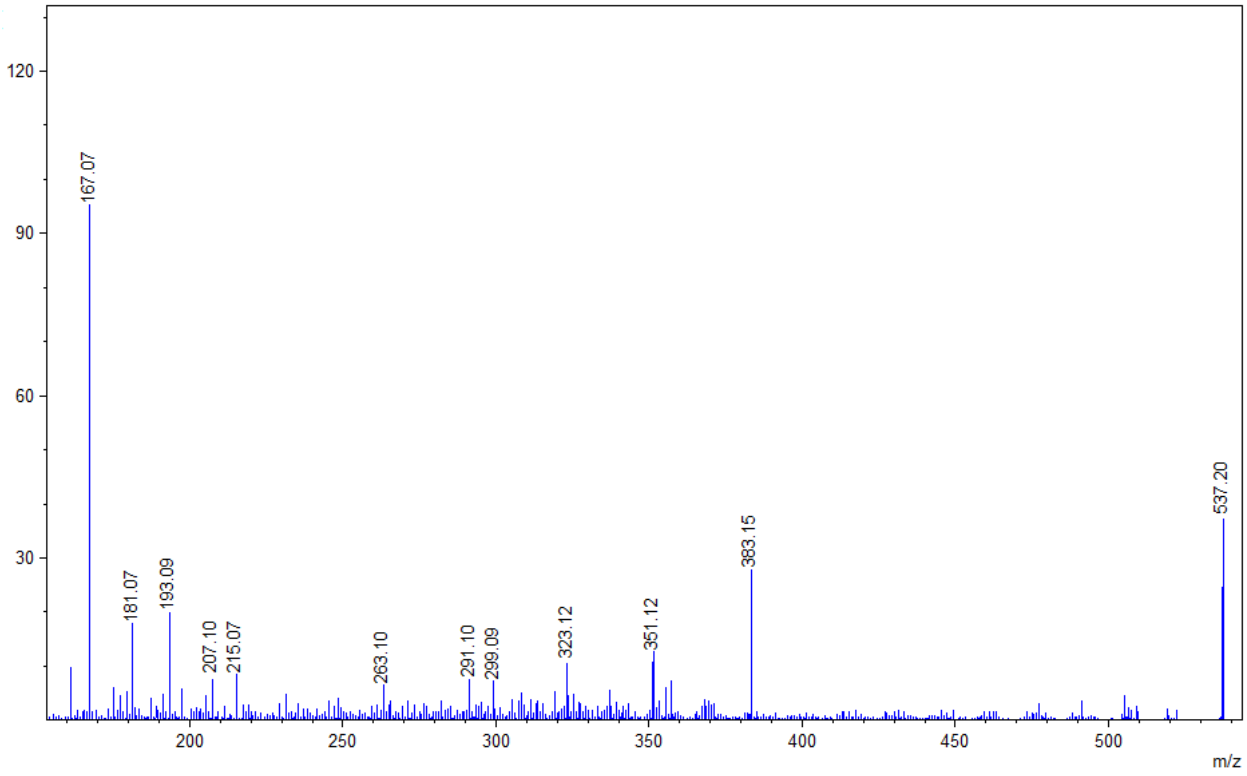
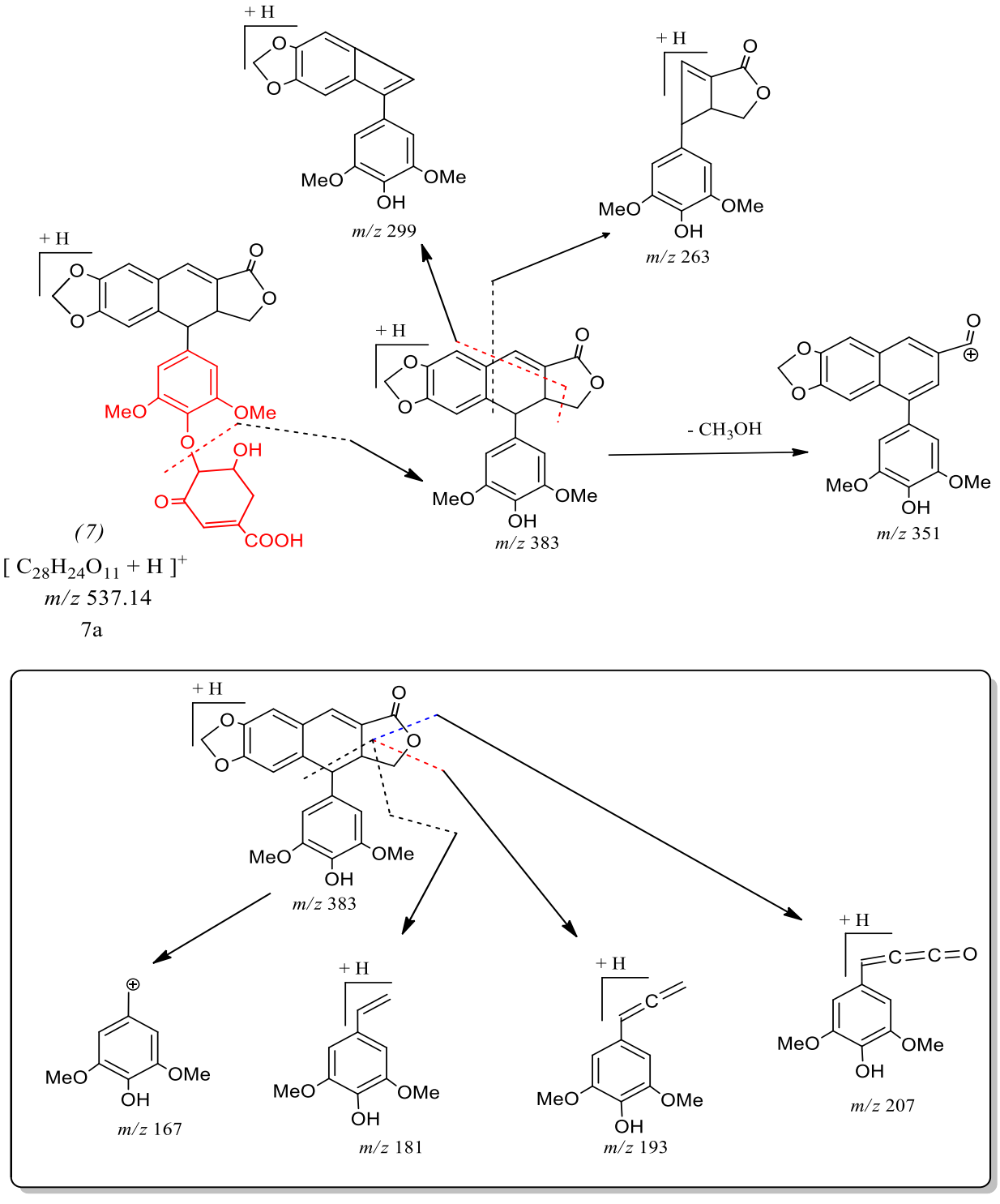


Figure B.20. Product ion scan of the protonated precursor ion *7* at *m/z* 537.14



Scheme B.18. Low energy CID-MS/MS fragmentation mechanism of the protonated precursor ion at *m/z* 537.14.

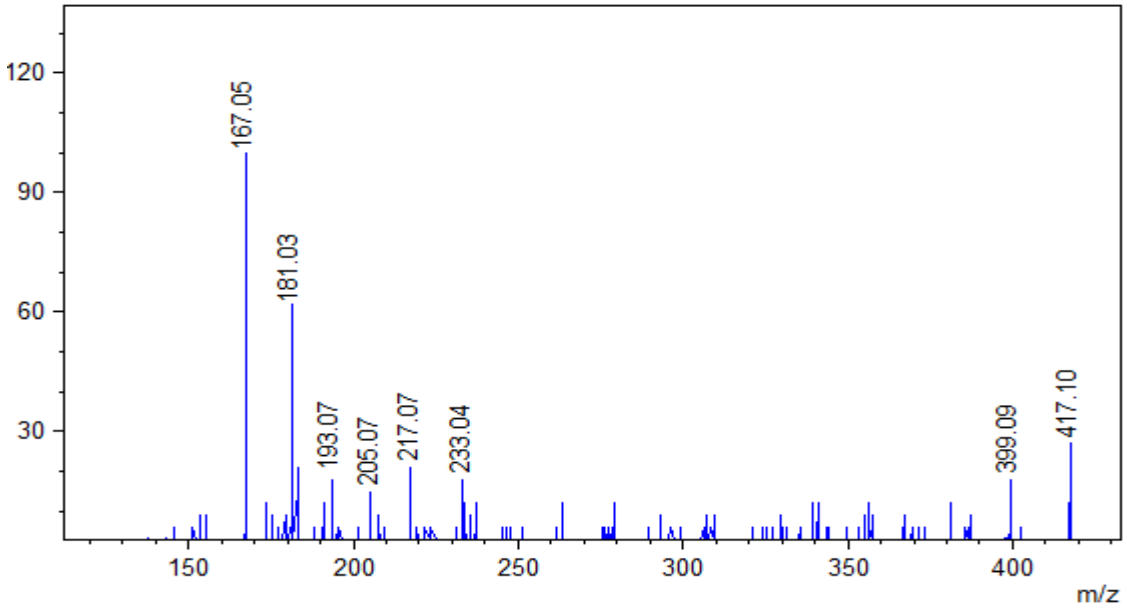
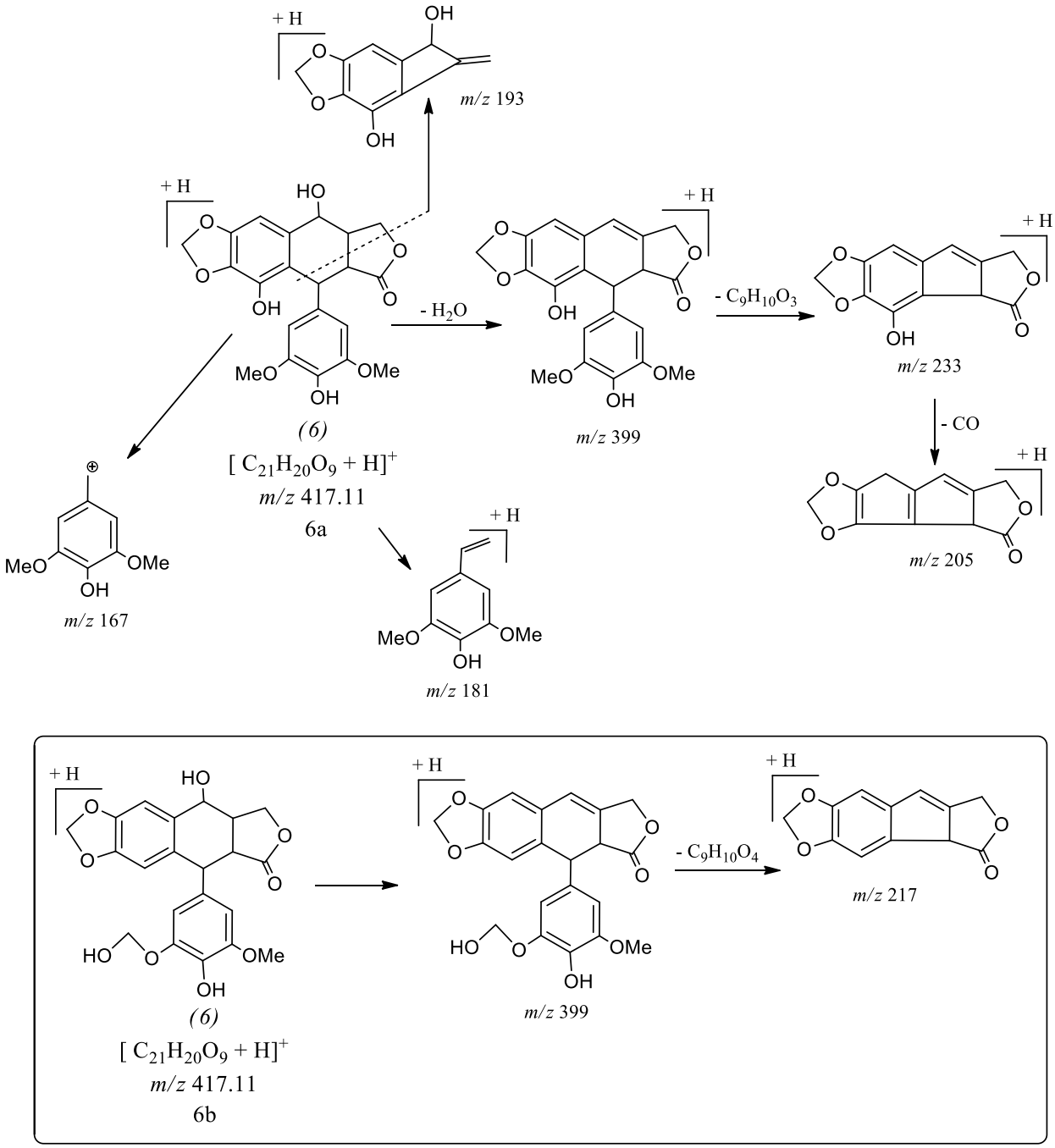


Figure B.21. Product ion scan of the protonated precursor ion at *m/z* 417.11



Scheme B.19. Low energy CID-MS/MS fragmentation mechanism of the protonated precursor ion at *m/z* 417.11

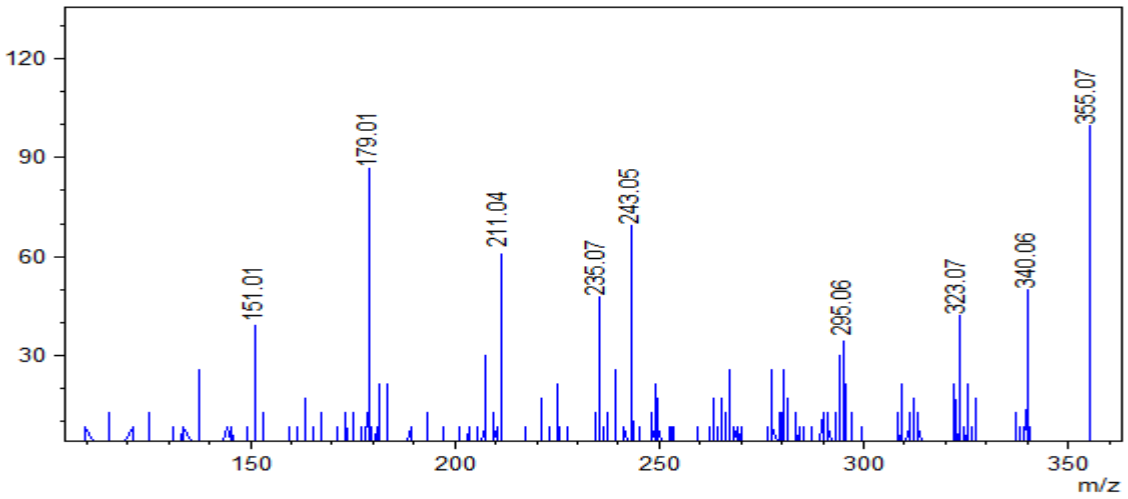
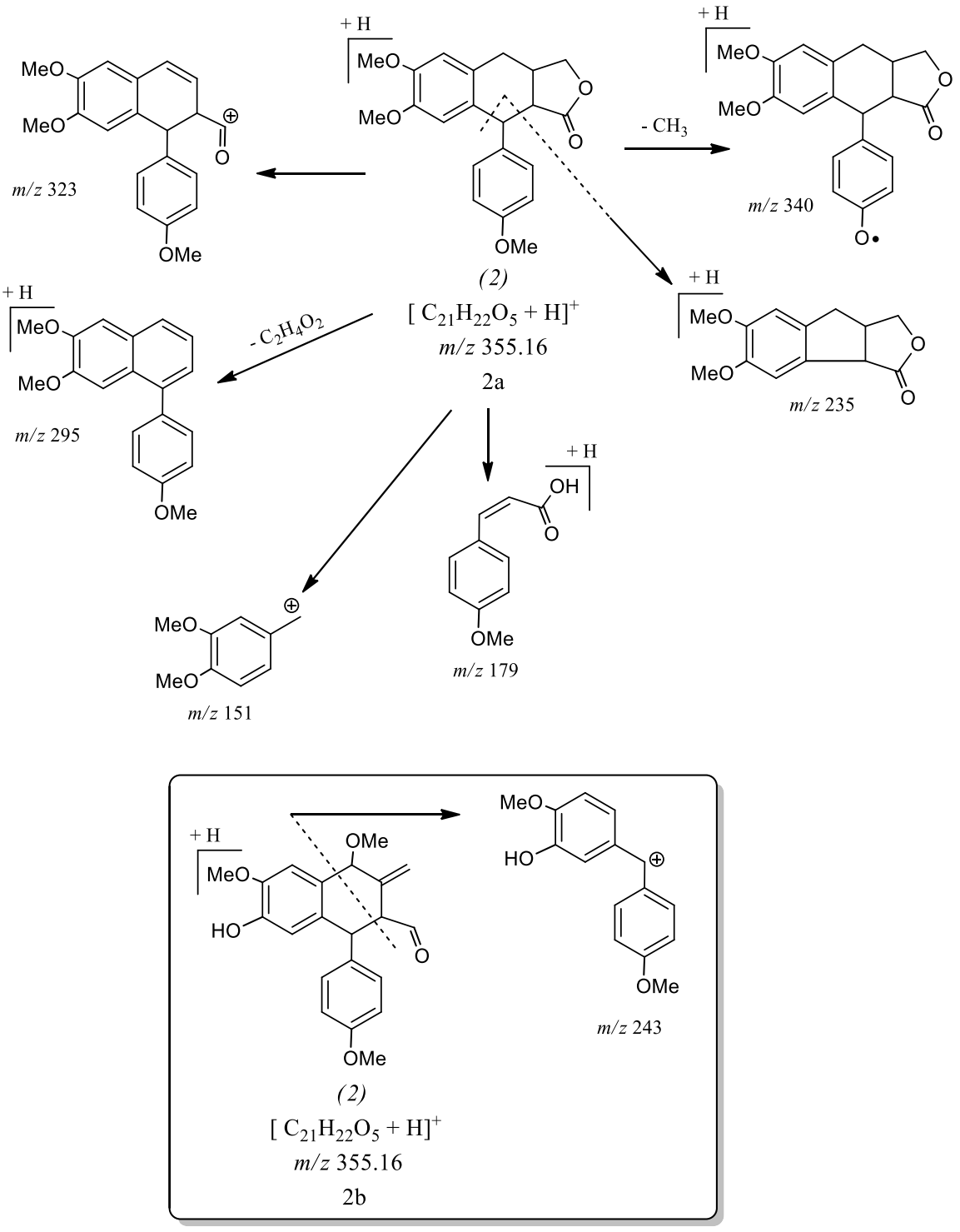


Figure B.22. Product ion scan of the protonated precursor ion *2* at *m/z* 355.16



Scheme B.20. Low energy CID-MS/MS fragmentation mechanism of the protonated precursor ion at *m/z* 355.16

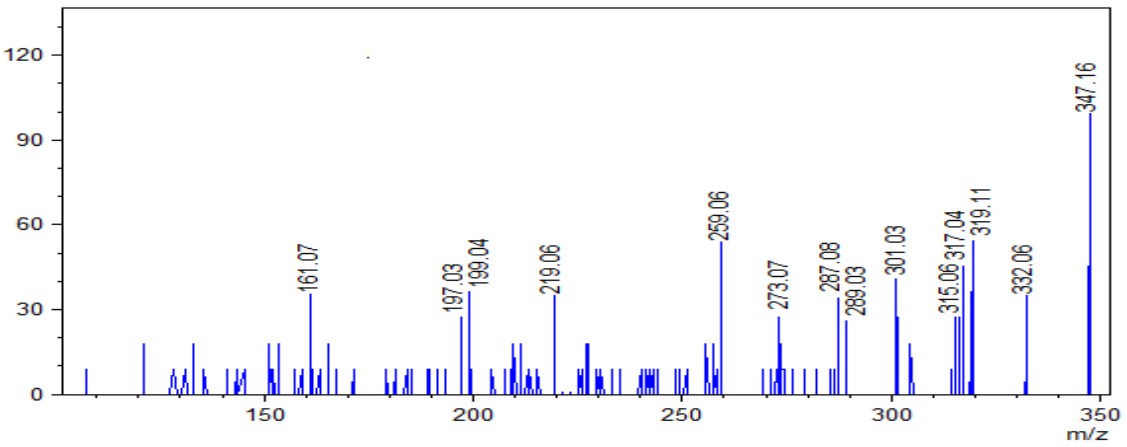
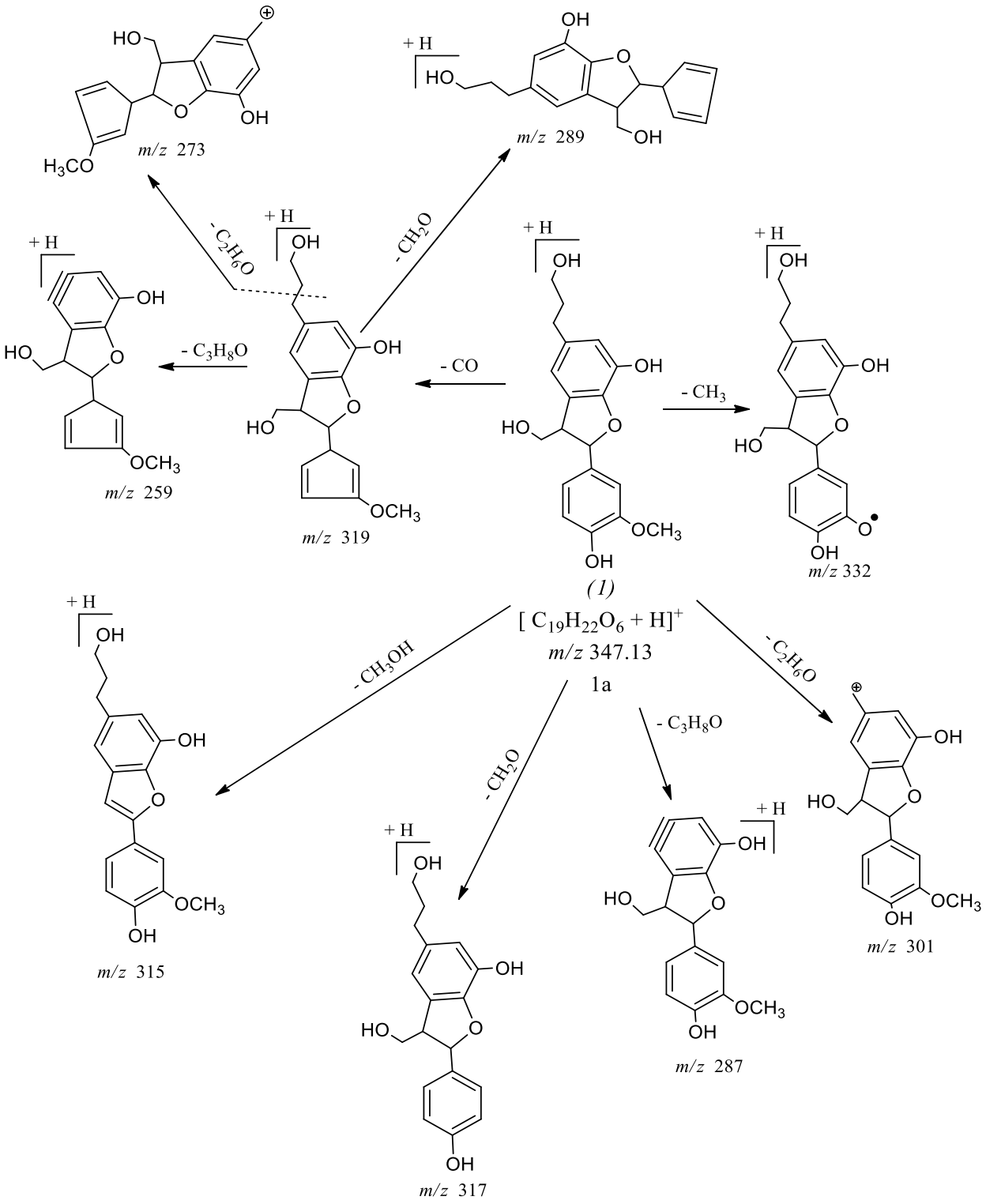


Figure B.23. Product ion scan of the protonated precursor ion at *m/z* 347.13



Scheme B.21. Low energy CID-MS/MS fragmentation mechanism of the protonated precursor ion at *m/z* 347.13

References for Appendix B

1. Mikhael, A., Jurcic, K., Fridgen, T. D., Delmas, M., & Banoub, J. (2020). Matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry (negative ion mode) of French Oak lignin: A novel series of lignin and tricin derivatives attached to carbohydrate and shikimic acid moieties. *Rapid Communications in Mass Spectrometry*, *34*(18), e8841.

2. Mikhael, A., Fridgen, T. D., Delmas, M., & Banoub, J. (2021). Top–down lignomics analysis of the French oak lignin by atmospheric pressure photoionization and electrospray ionization quadrupole time-of-flight tandem mass spectrometry: Identification of a novel series of lignans. *Journal of Mass Spectrometry*, *56*(1), e4676.