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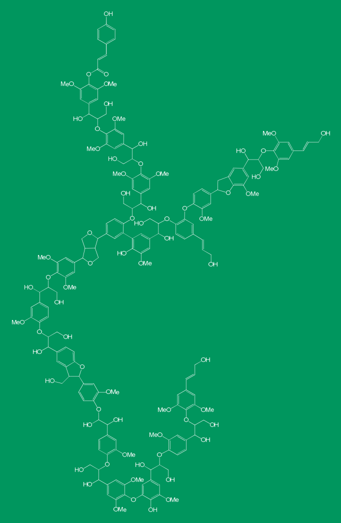
Lignin: Structure and characterization



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Learning outcomes

- Where does lignin come from?
- Principles of lignin biosynthesis
- Classification of lignins
- Importance of lignin to plants
- Distribution of lignin in plant cell walls
- Overview of reactions under thermochemical conditions
- Industrial sources & relevance
- Isolation and characterization
- Current and emerging applications

Occurrence

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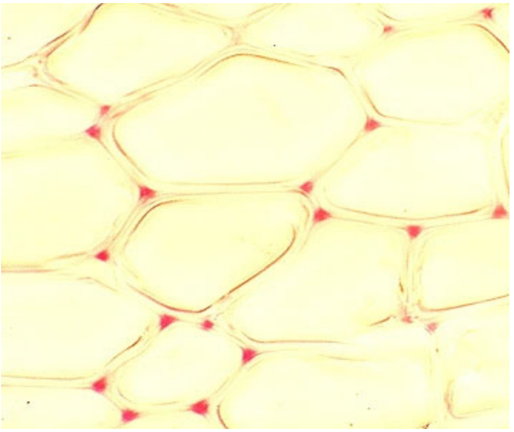
Lignin

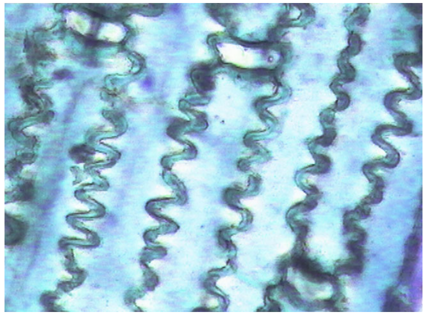
- Main component of plant biomass (in addition to cellulose and hemicelluloses)
- Makes 25-35% of wood dry weight and 15-25% in annual plants
- Structurally, it is a polydisperse mixture of methoxylated polyphenols
- Naturally brown, hence the colour of wood
- Dissolved from wood chips in chemical pulp production: combusted for chemical recovery and heat and power production
- New uses for lignin are sought in materials and chemicals



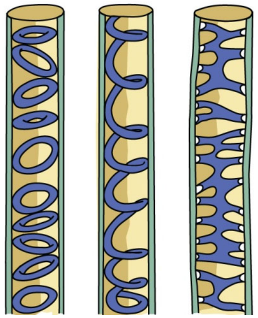
Occurrence and functions in plants

- Lignin is a natural resin that fills spaces between plant cells and strengthens cell walls by covering cellulose microfibrils
- Functions of lignin in plants:
  - Provides strength
  - Barrier against the attack of insects and microbes
  - Enables transportation of water and ions from the soil





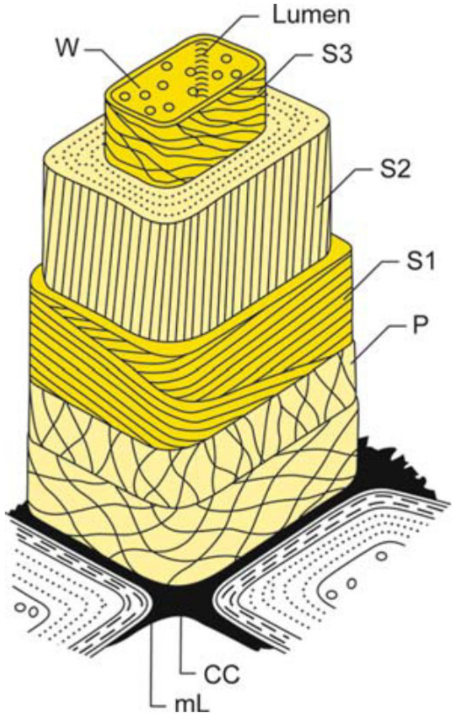
Wheat straw xylem tissue seen in optical microscope



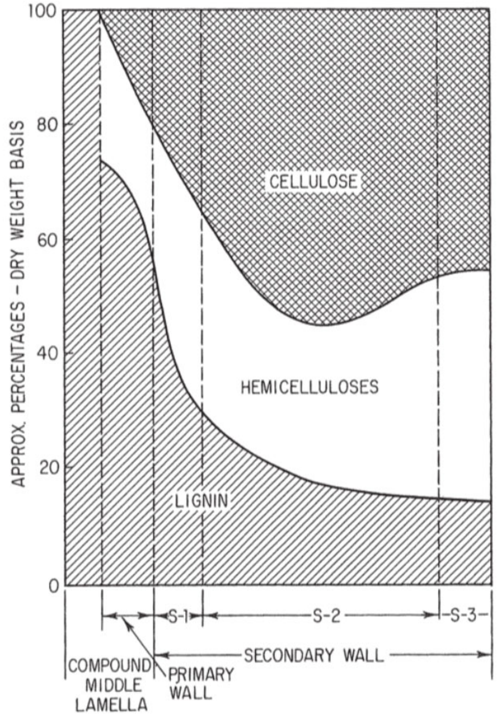
http://sydney.edu.au/science/biology/learning/plant_form_function/revision_modules/2003A_Pmodules/module2/2AD1.shtml
http://elte.prompt.hu/sites/default/files/tananyagok/StructureOfPlantsAndFungi/ch04s04.html

Distribution of lignin in plant cell walls

- CC, ML enriched in lignin, presumably because lignification is initiated there[1]
- Secondary walls contain the largest proportion of the total lignin in cell walls



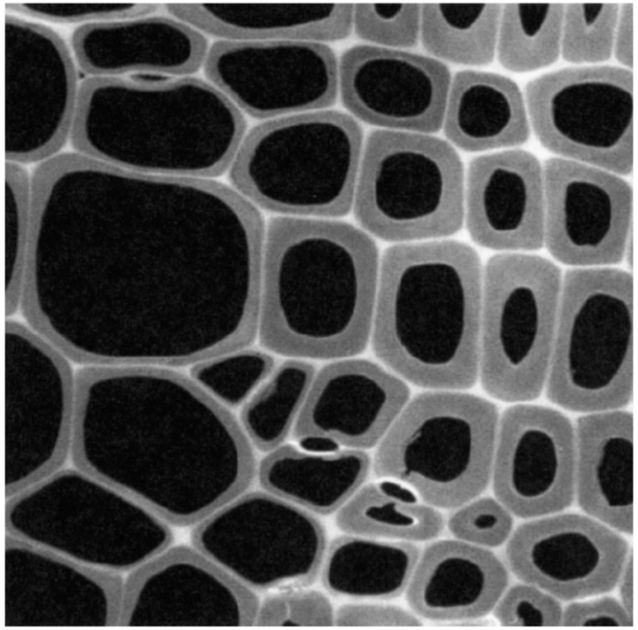
Wood cell wall regions[2]



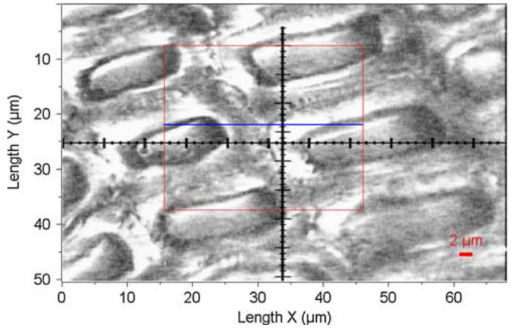
Distribution of lignin in cell walls[3]

[1] Donaldson, Phytochem. 57 (2001) 859–873
[2] Agarwal, Planta (2006) 224:1141–1153 (ref. Sjöström, 1993)
[3] Hale, 1969 ”Structural and physical properties of pulpwood”

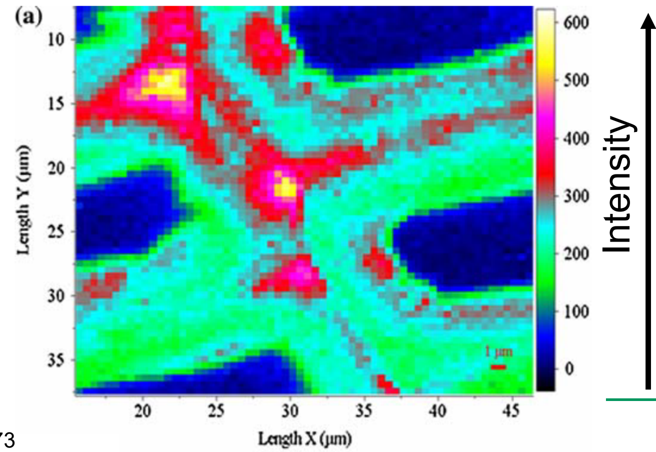
Imaging distribution of lignin in plant cell walls



Bright areas in pine wood arise from autoflurorescense of lignin[1]

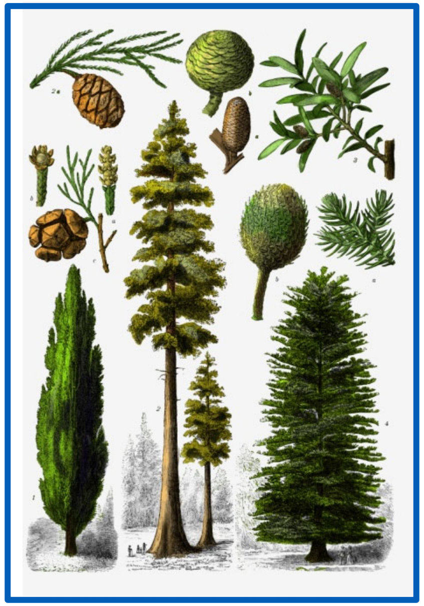


Raman imaging of spruce lignin at the area of six mature cells[2]



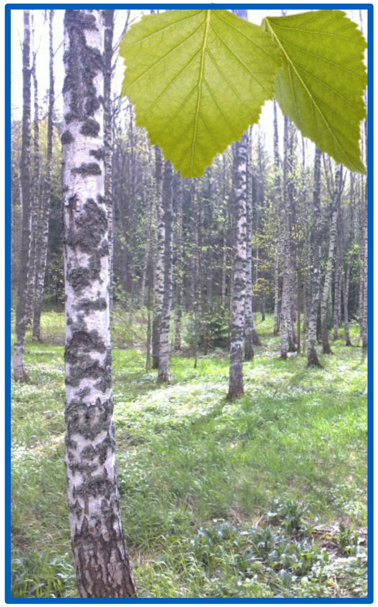
[1] Donaldson, Phytochem. 57 (2001) 859–873
[2] Agarwal, Planta (2006) 224:1141–1153

Botanical classification of lignins



Gymnosperms
”Softwood”

G-lignin



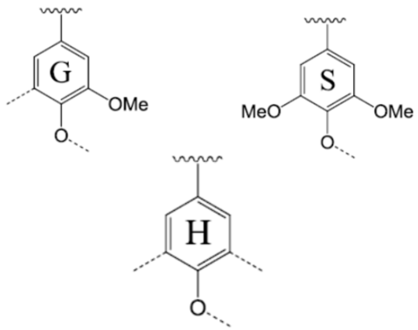
Angiosperm
dicotyledons
”Hardwood”

GS-lignin

Graminoids
”Grasses”

HGS-lignin

Additionally, lignin in softwood compression wood can be classified as **GH lignin**[1-2]



[1] Nimz et al., Holzforschung 35 (1981) 16-26.
[2] Rolando et al., Thioacidolysis, Methods in lignin chemistry, 1992, pp. 334-349
https://www.ecn.nl/uploads/pics/Grote_rol_tarwestro_NW_01.jpg
https://upload.wikimedia.org/wikipedia/commons/6/68/Grass_dsc08672-nevit.jpg
https://upload.wikimedia.org/wikipedia/commons/thumb/4/40/Gymnospermae.jpg/330px-Gymnospermae.jpg
http://62.80.138.132/skolor/skolbilder/filer/e0842627cbc4dc6bf2bbb27b66622986.Julgran.png
http://www.giftofatree.ie/wp-content/uploads/2014/01/Betula-pendula-Youngii-Leaf.jpg

Biosynthesis and structure

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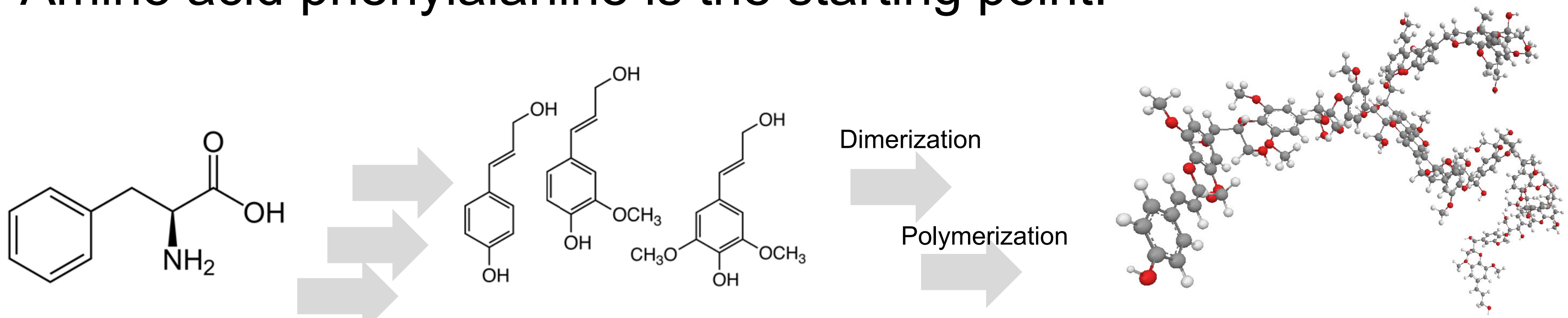
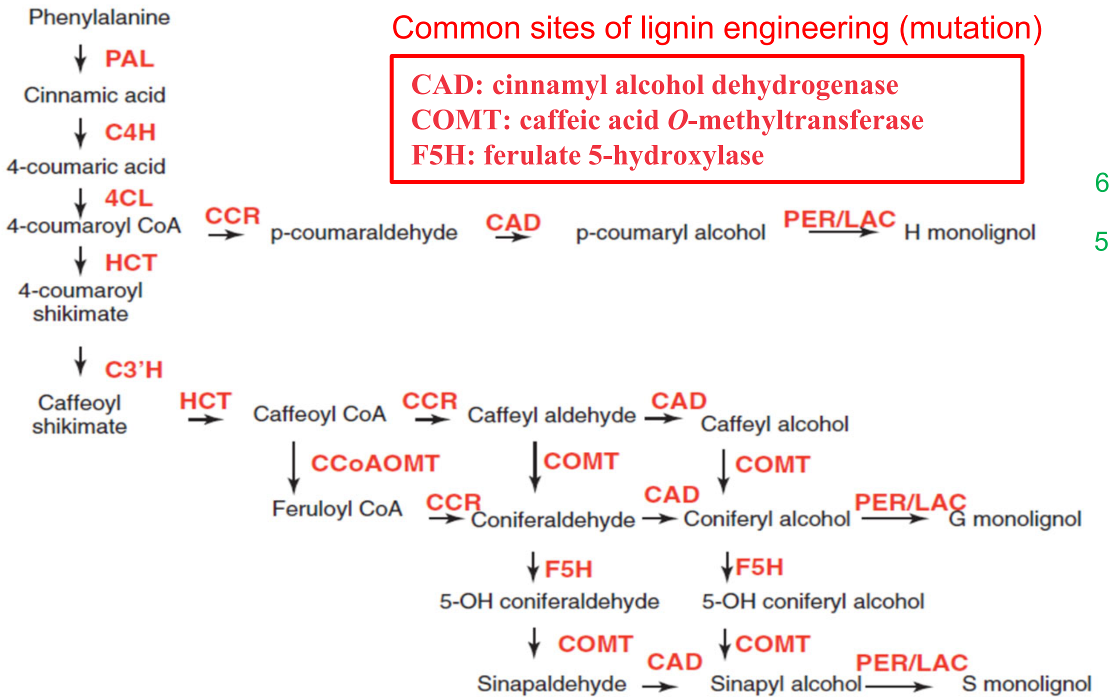
Biosynthesis and the diversity of molecular structures

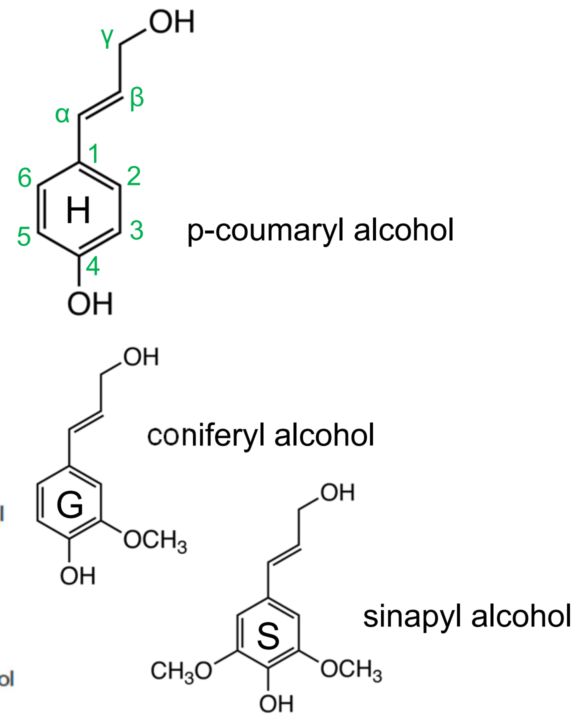
- Growing trees and other plants do not ”just grow” – they are living organisms with subtle control over biosynthesis
- Lignin is synthesized principally from three precursor monomers which are referred to as monolignols
- Amino acid phenylalanine is the starting point:



phenylalanine Monolignols Lignin polymers

Several enzymes are needed in the monolignol synthesis



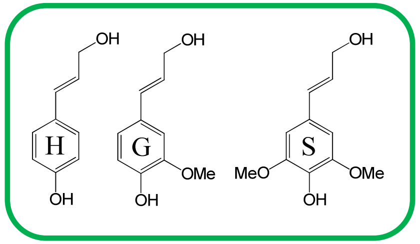


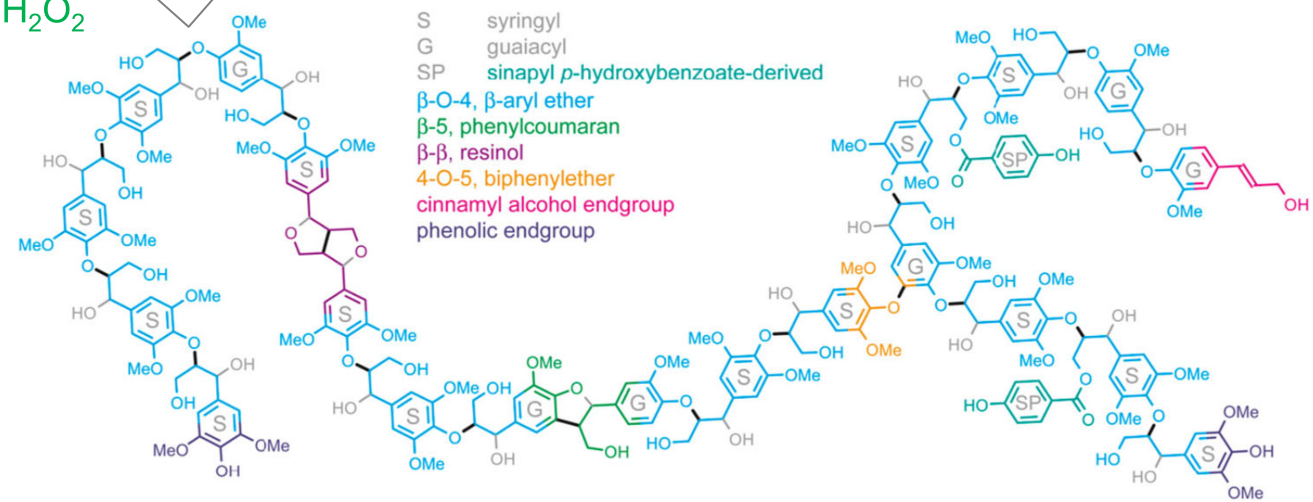
Zhao and Dixon, Trends Plant Sci. 2011

Lignin polymer is formed from monolignols in enzyme-initiated radical polymerization

Note: lignification occurs under chemical control i.e. it does not use template control (such as in protein synthesis)

Laccase or Peroxidase + H_2O_2

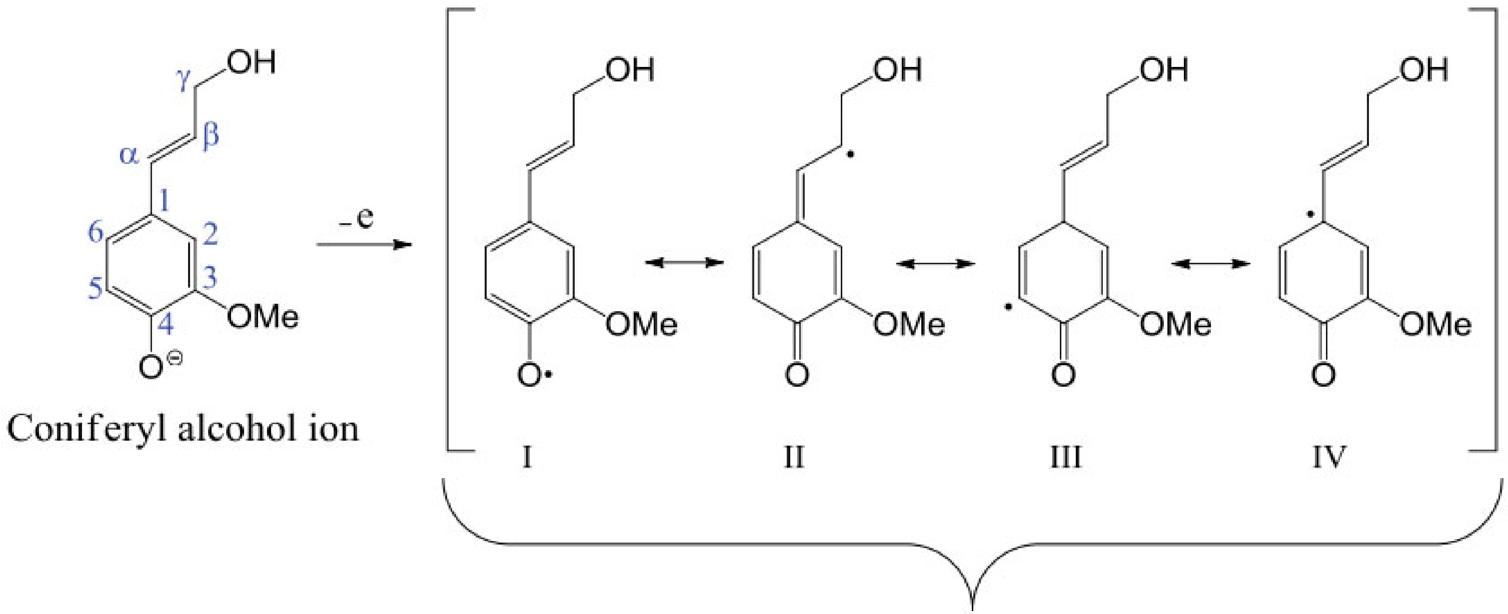




- **Result = mixtures of molecules (not one well-defined compound)**

Vanholme et al., Plant Physiol. 153 (2010) 895−905.

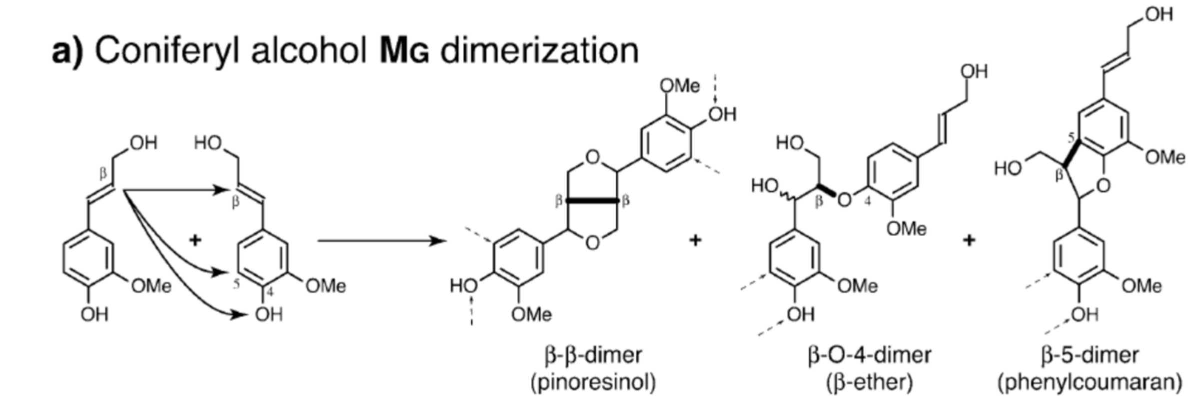
Example of radical delocalization



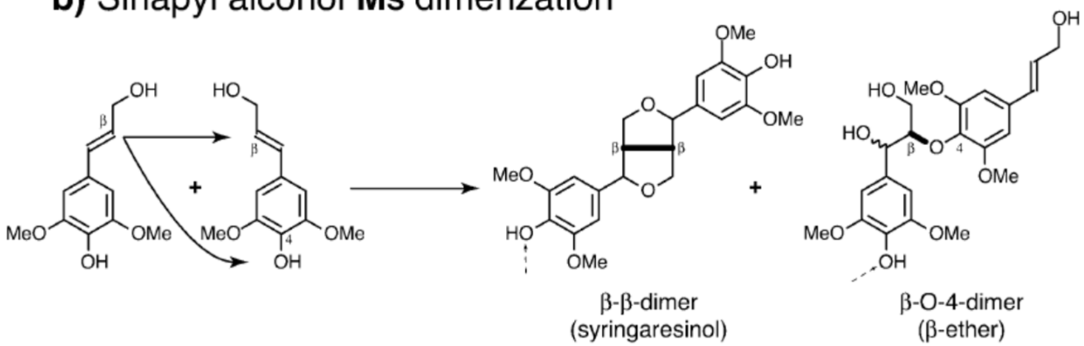
Polymerization of lignin by radical coupling

Dimerization reactions of monolignols

a) Coniferyl alcohol **MG** dimerization



b) Sinapyl alcohol **Ms** dimerization

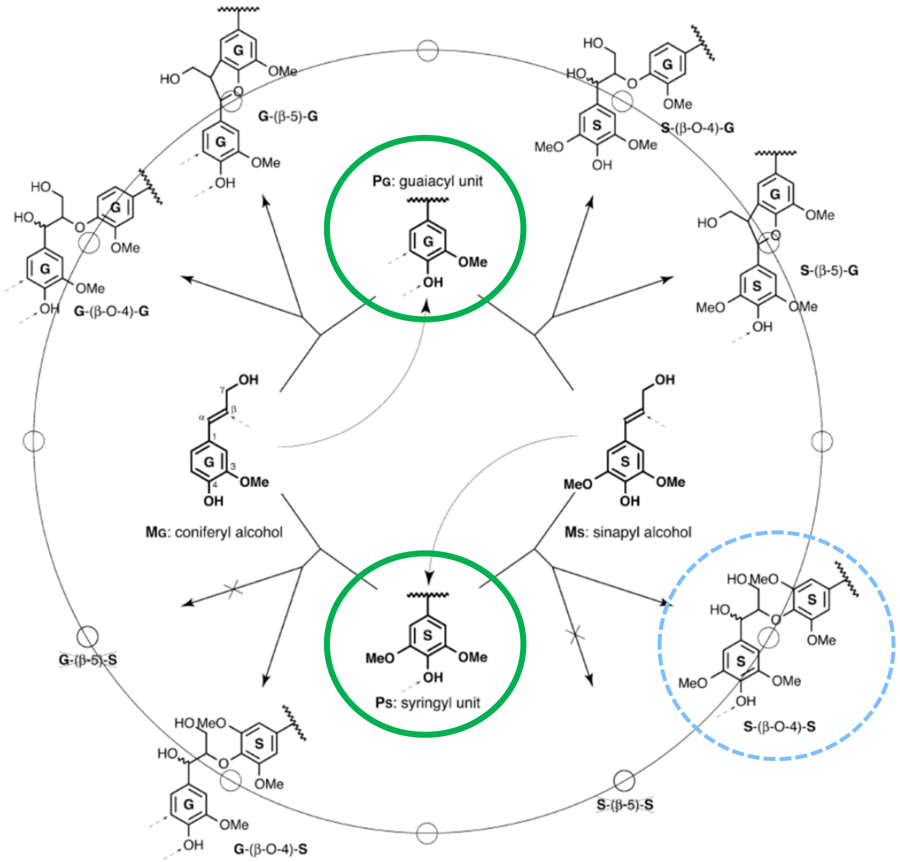


Ralph et al., Phytochem. Rev. 3: 29–60, 2004.

Polymerization reactions of monolignols

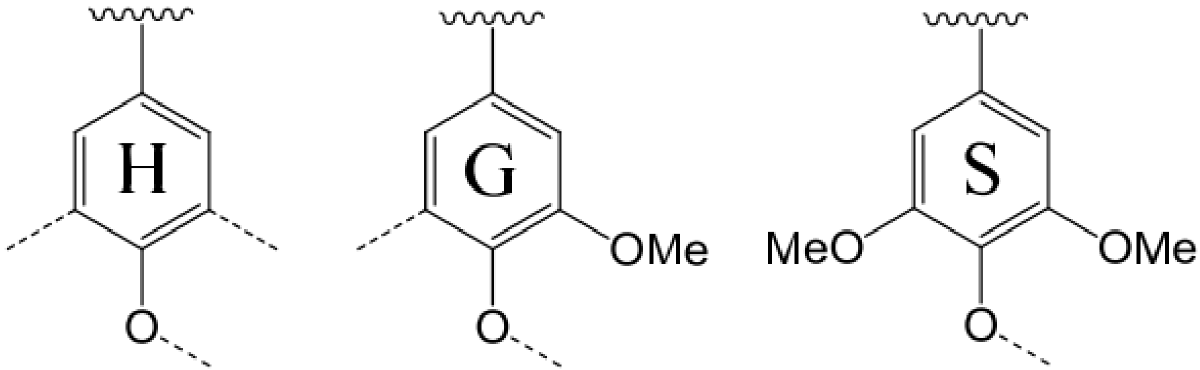
Radical coupling:

- Monolignols react endwise with the growing polymer
- The resulting structure of the lignin macromolecule is “random” in the sense that several linkage types are possible at each step
- S-S coupling is an exception since this predominantly gives rise to β-O-4 units



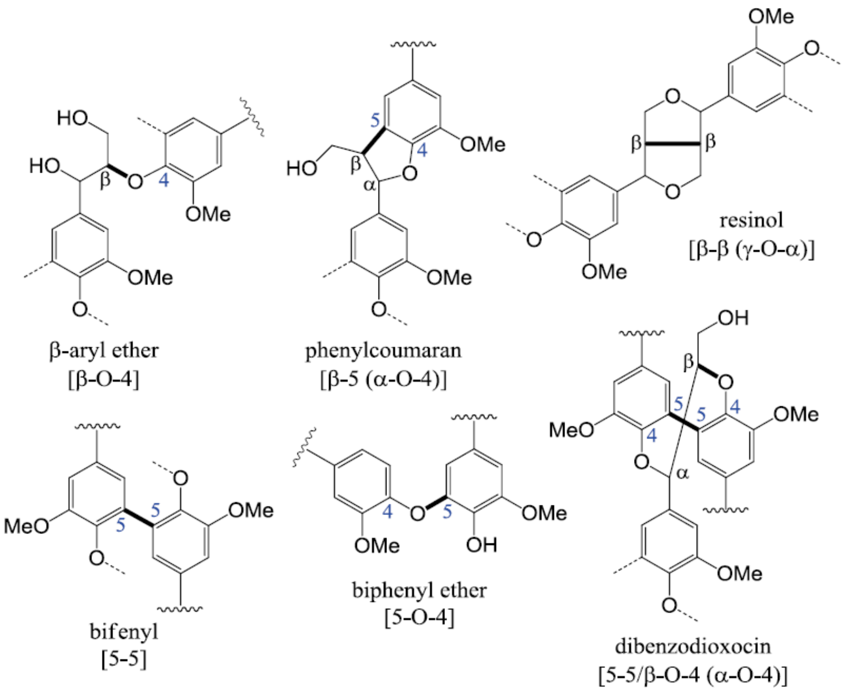
Ralph et al., Phytochem. Rev. 3: 29–60, 2004.

Naming of the units in the lignin polymer



p-hydroxyphenyl (H) Guaiacyl (G) Syringyl (S)

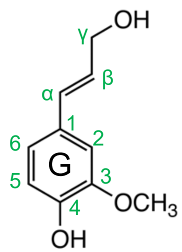
Major structural units of lignin polymer



- Bolded bonds are formed in radical coupling and other in dimerization reactions

Linkage type	Dimer structure	Approximate percentage
β-*O*-4	Phenylpropane β-aryl ether	45–50
α-*O*-4	Phenylpropane α-aryl ether	6–8
β-5	Phenylcoumaran	9–12
5–5	Biphenyl and dibenzodioxocin	18–25
4-*O*-5	Diaryl ether	4–8
β-1	1,2-Diaryl propane	7–10
β–β	β-β-Linked structures	3

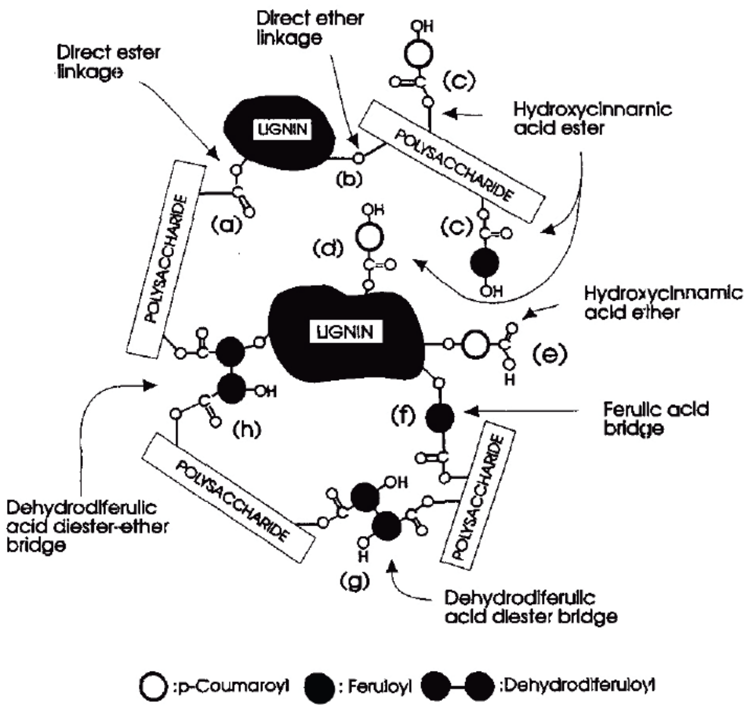
- Units linked to another phenylpropane unit from the aryl ring positions 3 and 5 (2 and 6) are referred to as ”condensed linkages”



Sipponen Doctoral thesis 2015
Chakar and Ragauskas Ind.Crops Prod. 20 (2004) 131-141.

Covalent linkages between lignin and polysaccharides

- Lignin-carbohydrate complexes (LCCs) contain ether and ester bonds
- Linkages are mainly between hemicelluloses and lignin
- LCCs contribute to the cell wall integrity and difficulty of delignifying residual lignin[1]
- Lignin carbohydrate network (LCN) must be disrupted for enzymatic saccharification of plant biomass



A schematic illustration of LC-linkages in grasses[2]

[1] Lawoko, Doctoral thesis, 2005
[2] Iiyama et al., Plant Physiol. 104 (1994) 315–320.

Fundamental properties and reactivity

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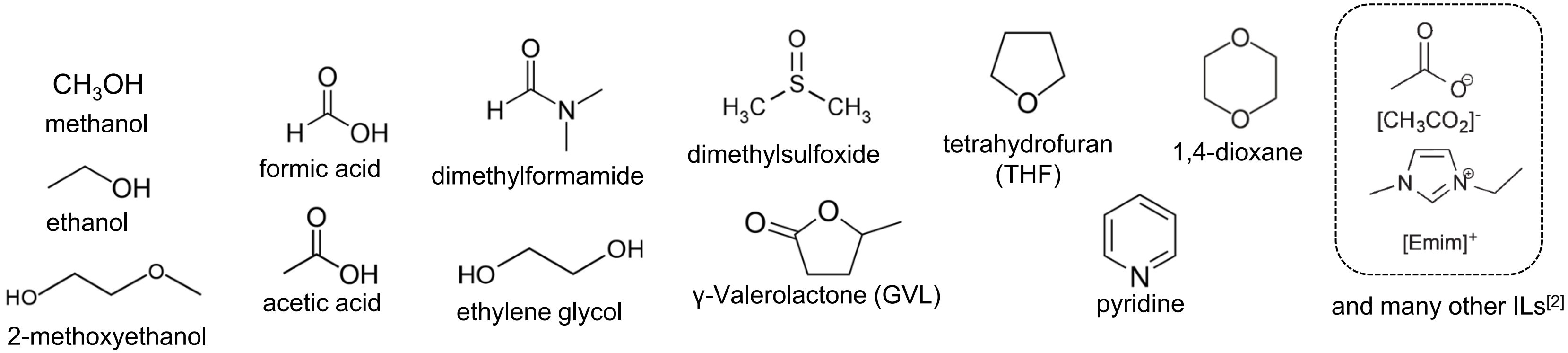
Industrial relevance

- Lignin is removed from fibers during cellulose pulp production
  - Provides surplus energy for the pulp mills
- Kraft, soda, sulfite, organosolv processes rely on distinct reactions
- Lignin is a structural constraint and inhibitor in biochemical conversion of plant biomass into ethanol etc.
- Biorefineries produce soluble and insoluble lignin
  - A spectrum of different hydrothermal and thermochemical lignocellulose pretreatment processes are under development

Fundamental properties and reactivity

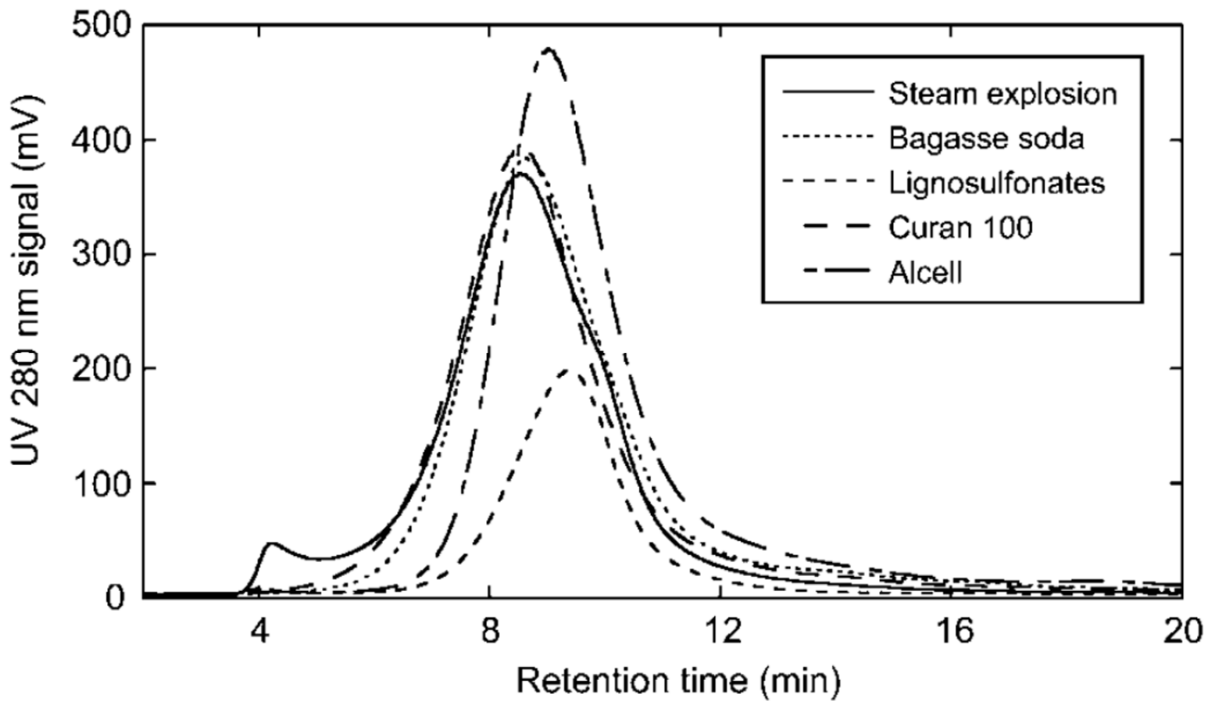
- Lignins are negatively charged in aqueous solutions
  - pKa of phenolic hydroxyl groups vary between 7–10,[1] depending on the neighbouring substituents; carboxylic acids have generally pKa < 4
- Solubility of lignin is important for industrial utilization and analytical work
  - Lignins are usually soluble in alkaline water and often (but not always) also in polar organic solvents and ionic liquids such as:



[1] Ragnar et al., J. Wood Chem. Technol. 20, 277–305
[2] Hart et al., Green Chem., 2015, 17, 214–218

Polydispersity of lignin

- When isolated from wood, lignin contains molecules at various molecular weights
- Small lignin oligomers are naturally present also in plant biomass

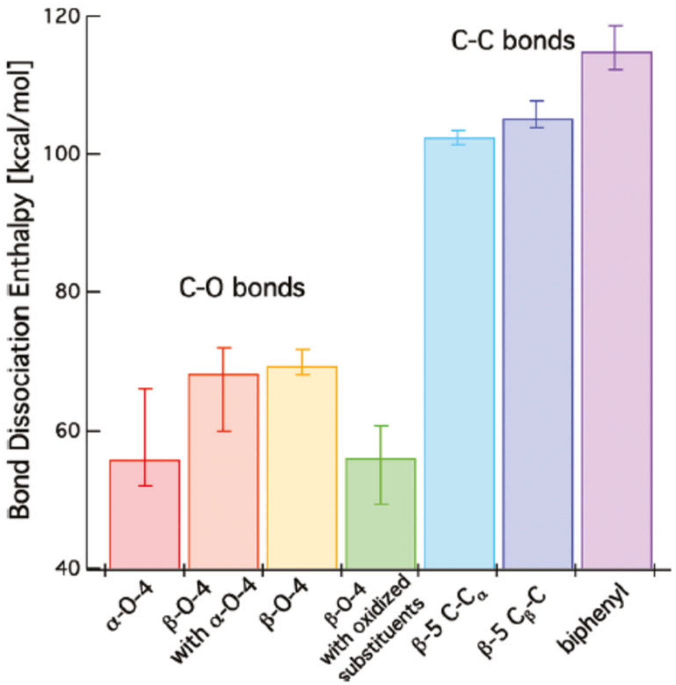


Molecular weight increases logarithmically

Baumberger et al., Holzforschung 61 (2007) 459–468

Stability of covalent bonds in lignin

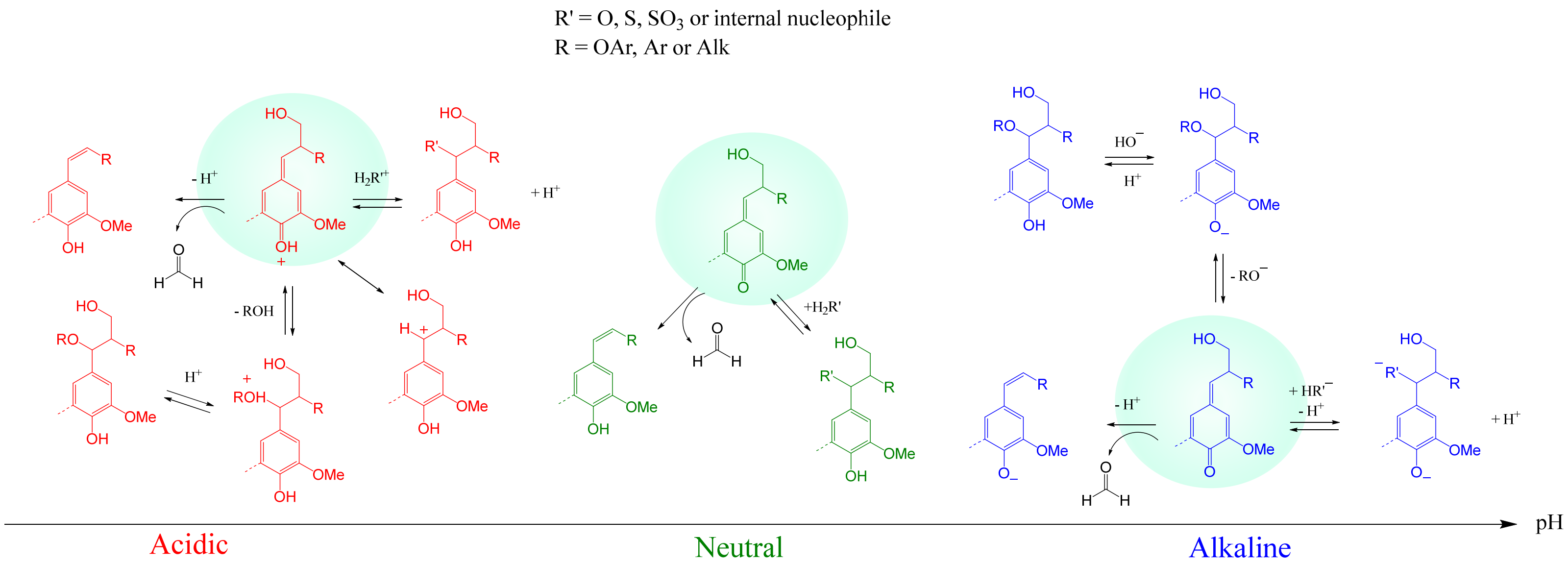
- **Bond dissociation enthalpies (BDE) based on density functional theory calculations:**
  - α–O–4 in GG pinoresinol, 284 kJ/mol[1] β–O–4 (native) HG, 292 kJ/mol[2] SS 295 kJ/mol[3]
  - C–O are weaker than C–C bonds
  - Side-chain oxidation decreases BDE
- **Note that lignin is more reactive in thermochemical conditions because of:**
  - Formation of reactive quinone methide intermediates
  - Neighbouring group participation



Kim et al., 2011

[1] Elder, Energy Fuels 2014, 28, 1175–1182
[2] Younker et al., ChemPhysChem 2011, 12, 3556 – 3565
[3] Kim et al., J. Phys. Chem. Lett. 2011, 2, 2846–2852

Quinone methide intermediates are important reactive forms of lignin



Reactions of lignin through the quinone methide intermediate in acidic, neutral and alkaline media.[1]

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[1] J. Gierer, Wood Sci. Technol., 1985, 19, 289–312.

Lignin during pulping

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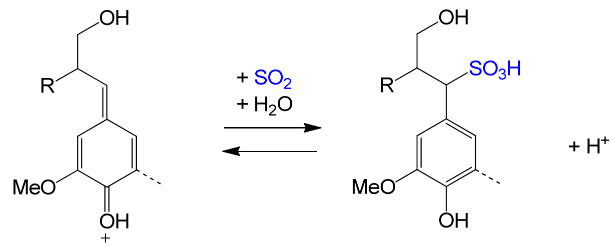
Sulfonation of lignin

- Formation in acid sulphite pulping
- Sulfoxyl groups (pKa<2) give water-solubility within a broad pH region
- Lignosulfonates are industrial chemicals
  - Plasticizers and additives in concrete and cement production
  - Viscosity reducing additive in oil drilling mud
  - Dispersants in pesticides, dyes, carbon black
  - Vanillin production (example follows later)

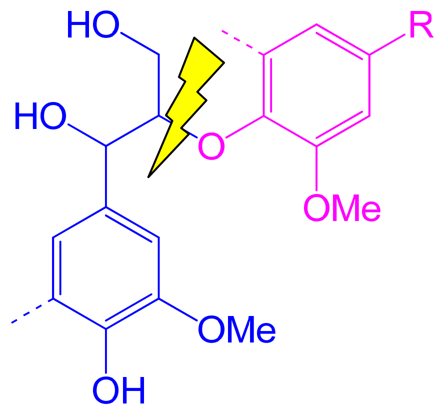


Sulfonation of lignin[1]

[1] J. Gierer, Wood Sci. Technol., 1985, 19, 289–312.

Cleavage of β–O–4 bonds in pulping processes

- The most important scission reaction in lignin chemistry, why?
  - Because β-O-4 bonds are so abundant and thermochemically labile
- Basis of delignification (depolymerisation → dissolution) in cellulose pulp production
  - Increases the content of phenolic hydroxyls
  - Side reactions form new C–C bonds



β-O-4 cleavage during kraft cooking

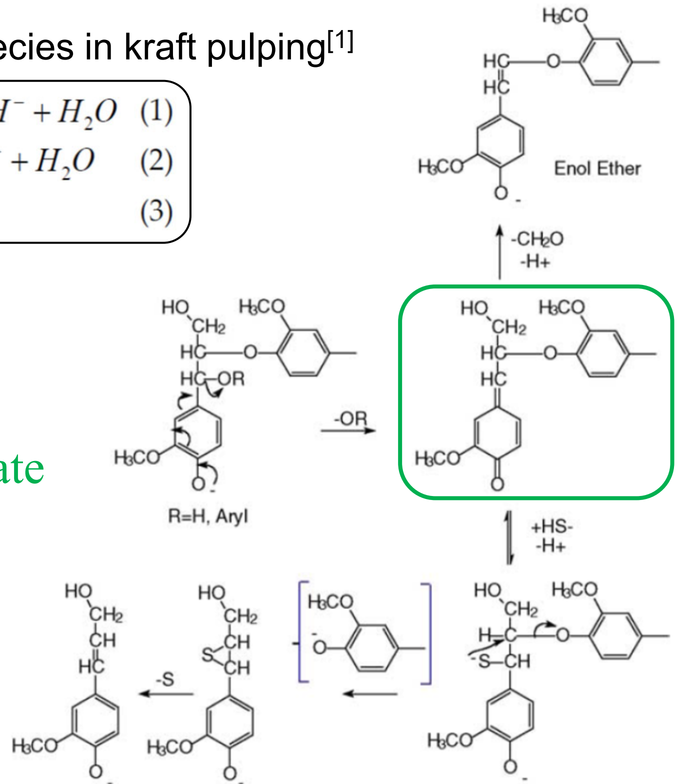
Formation of active species in kraft pulping[1]

$$NaOH + H_2O \leftrightarrow Na^+ + OH^- + H_2O \quad (1)$$
$$Na_2S + H_2O \leftrightarrow 2Na^+ + S^{2-} + H_2O \quad (2)$$
$$S^{2-} + H_2O \leftrightarrow HS^- + OH^- \quad (3)$$

Formation of the reactive quinone methide intermediate

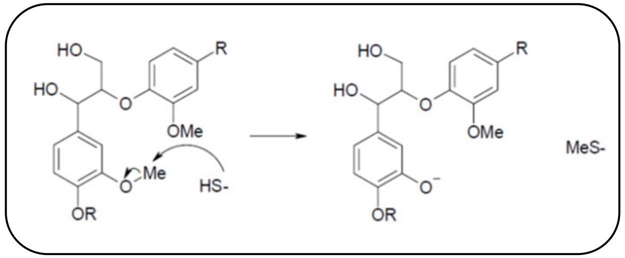
Sulfidolytic cleavage of β-aryl ether bonds

Elimination of formaldehyde



A simplified reaction scheme of kraft cooking[2]

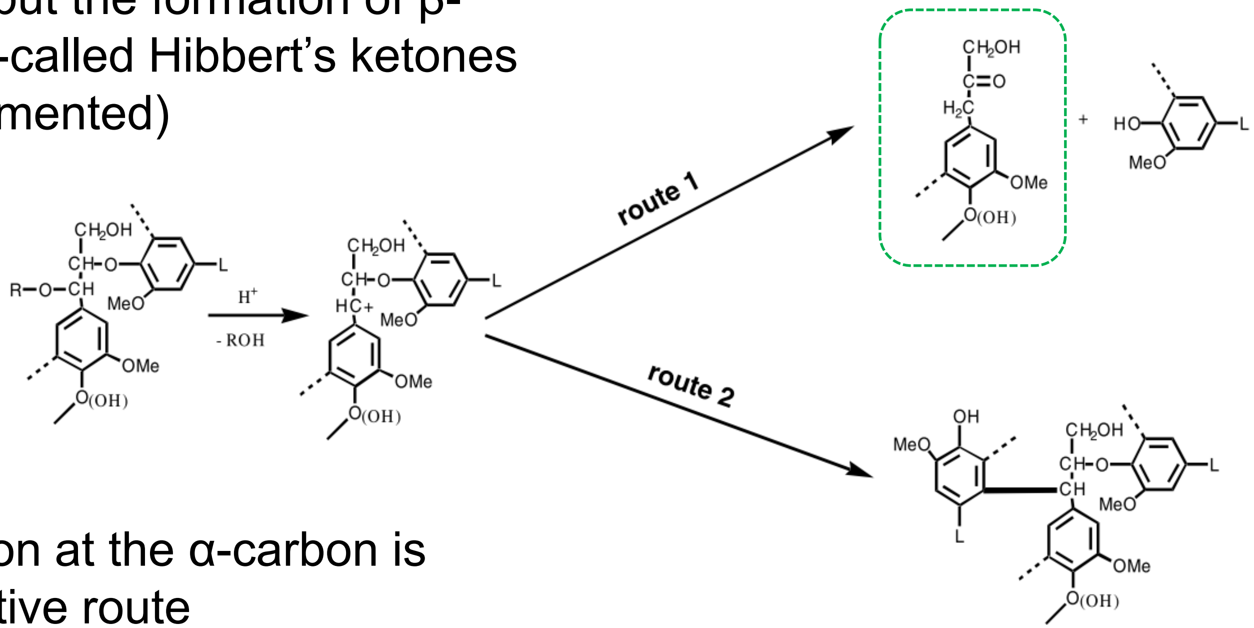
- The cooking takes place at 160-180 ºC and lasts 1-2 h
- Demethylation and condensation occur also[1]



[1] Vuorinen, CHEMISTRY OF PULPING AND BLEACHING, https://mycourses.aalto.fi/pluginfile.php/140064/mod_resource/content/1/Supporting%20material%201.pdf
[2] Chakar and Ragauskas, Ind. Crops Prod. 20 (2004) 131-141.

β-O-4 cleavage in acidic conditions

- Exact mechanism had not been fully elucidated, but the formation of β-ketones (so-called Hibbert's ketones is well documented)

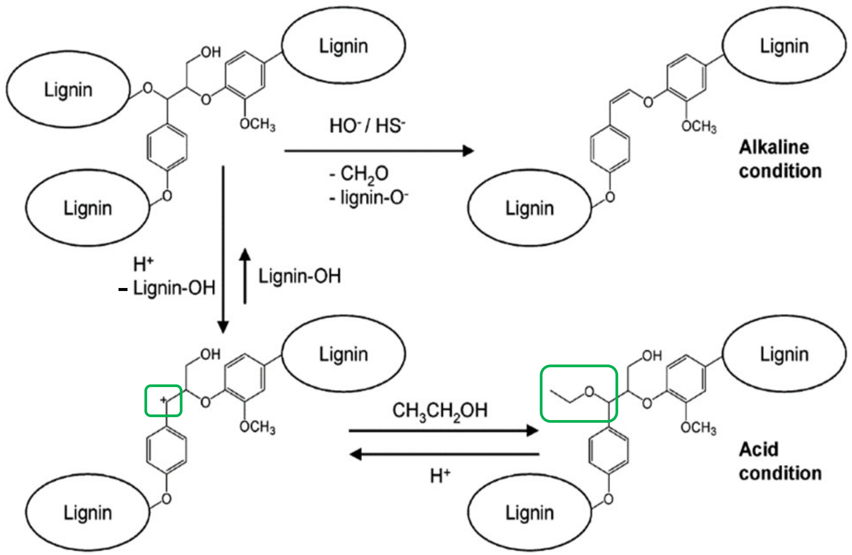


- Condensation at the α-carbon is the competitive route

Li et al., Bioresour. Technol. 98 (2007) 3061–3068

Organosolv pulping: ethanol-water process as an example

- Ethylation of the carbocation at Cα reduces formation of condensed linkages
- This is one reason why organosolv lignins have low Mw, Tg, and dissolve well in organic solvents



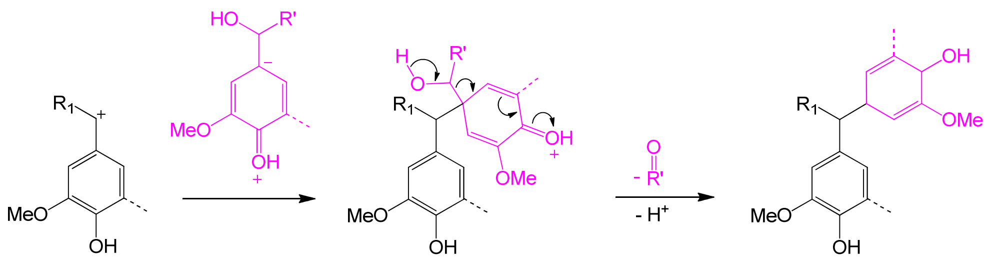
Proposed ethylation of lignin in organosolv pulping[1]

[1] Kubo and Kaddla, Macromolecules 2004, 37, 6904-6911

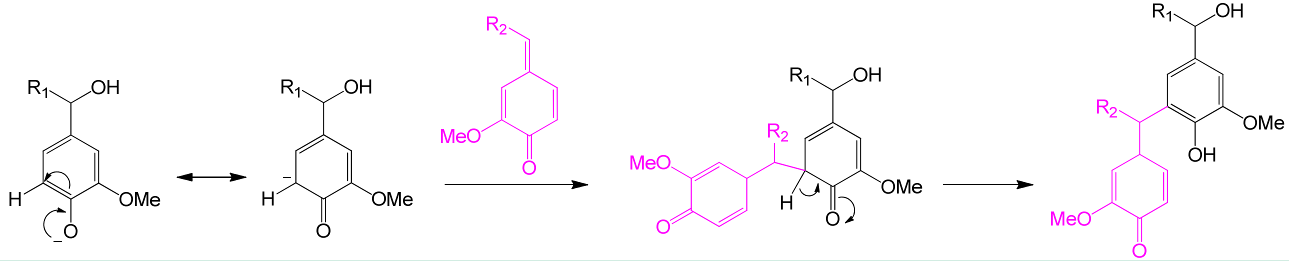
Formation of chemically stable ”condensed” C–C linkages

- Generally, condensed linkages reduce solubility and increase molecular weight of lignin
- Occurs either in acidic or alkaline media, for example:

Acidic media:



alkaline media:



Gierer, Wood Sci. Tecnol. 19 (1985) 289-312

Isolation

Isolation and purification of lignin

Industrial lignin production

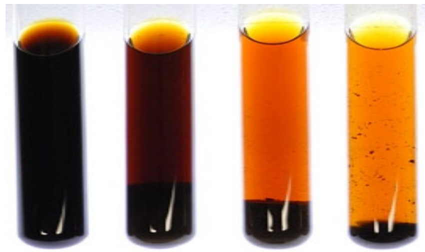
- Lignosulfonates (~1100 kt per year)
  - Borregaard LignoTech (Norway) is the main producer
- Kraft (sulfate) lignin (~100 kt per year)
  - WestRock (formerly MeadWestvaco) (US)
  - 2013: Domtar, North Carolina (US)
  - 2015: Stora Enso, Sunila (Finland)
  - 2016: West Fraser LignoForce 30 t/day demonstration plant

Stora Enso's Sunila mill

http://www.hs.fi/webkuva/taysi/700/1374823934135?ts=763

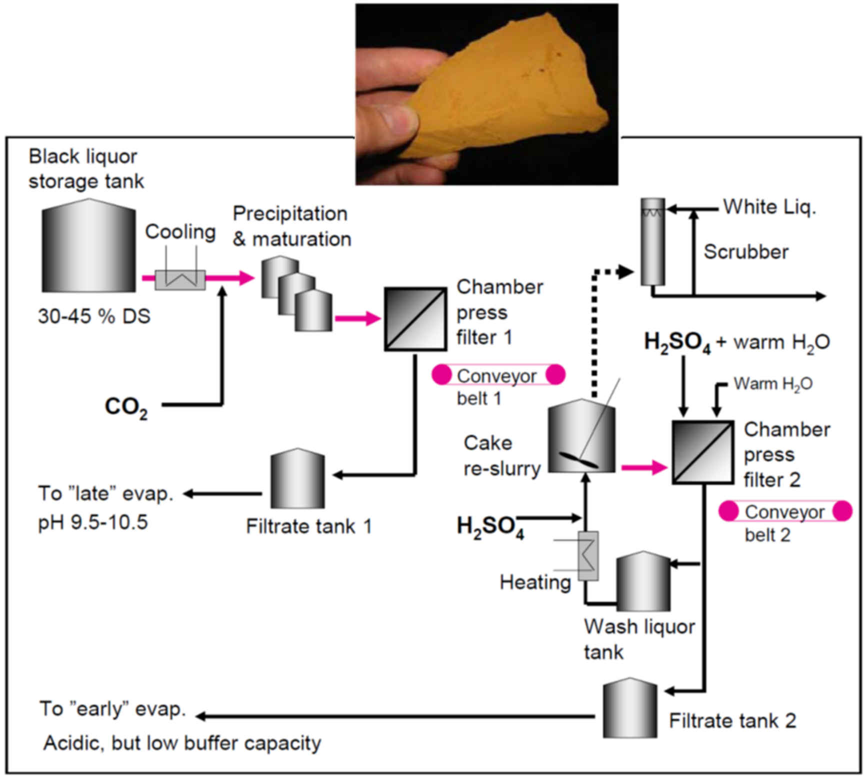
Isolation of lignin from spent pulping liquors

- Acid precipitation from alkaline solution: recall that lignin contains acidic groups which ionize above pH 4 and pH 7−10: precipitation occurs when anionic charge is reduced so that aggregation occurs
- Complexation with metals or cationic polymers
- Anti-solvent precipitation: lignin soluble in organic solvent (ethanol) precipitates when mixed with a miscible fluid (water)
- Membrane technology using small pore sizes ~1 kDa



Commercial lignin isolation processes

- Based on the capacity of CO_2 to reduce pH and precipitate lignin at pH 10
  - Carbon dioxide precipitation of lignin from pine kraft black liquor[1]
- Lignoboost technology was further developed by Innventia and acquired by Valmet
- LignoForce technology oxidizes lignin before CO_2 precipitation[2]

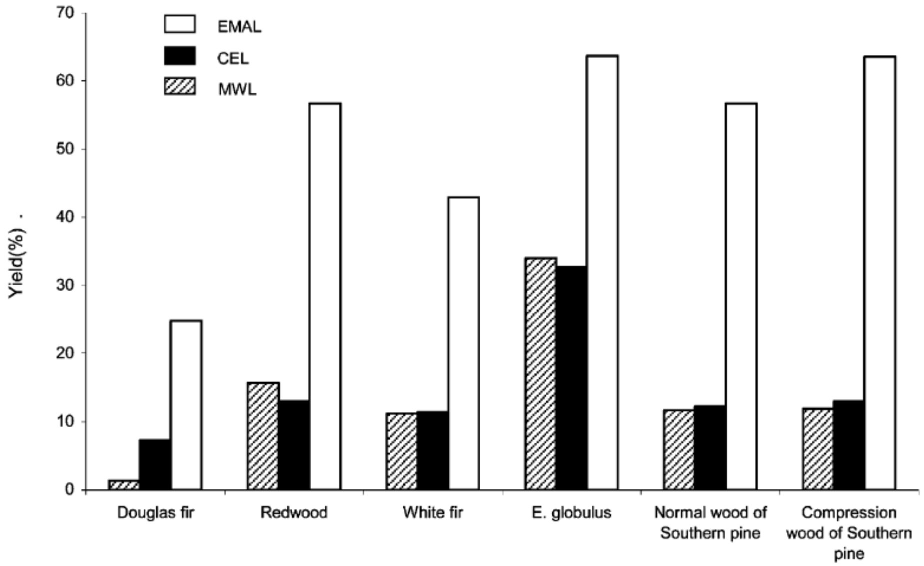


Tomani, Cellulose Chem. Technol., 44 (1-3), 53-58 (2010)

[1] Alén et al., Tappi 62: 11 (1979) 108-110.
[2] https://www.lemaitrepapetier.ca/images/stories/documents/articles%20techniques/J-FOR_Vol2-issue4-ART1-The%20Lignoforce%20System.pdf

Analytical lignin isolation methods

- Isolation of lignin from wood for characterization or as a model material causes always some structural alteration
- In the Milled Wood Lignin (MWL) method, wood is ground in a ball mill and subsequently extracted with aqueous Dioxane
- Cellulolytic enzyme lignin (CEL) and Enzymatic Mild Acidolysis Lignin (EMAL) procedures remove polysaccharides using hydrolytic enzymes before extraction of lignin with (acidic) aq. dioxane



Guerra et al., J. Agric. Food Chem., 2006, 54, 5939–5947
Guerra et al., J. Agric. Food Chem., 2006, 54, 9696–9705

Lignin derivatizations

- In order to analyze structure of lignin or to render it more suitable to applications, chemical or anzymatic derivatizations are used
- Some common chemical derivatizations are:
  - Acetylation
  - Methylation
  - Carboxymethylation
  - Cationization
  - Cross-linking
- Various other organic compounds such as fatty acids can be grafted to lignin
- The enzyme laccase has been used to oxidize and cross-link lignins

Characterization

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Tools for characterization of lignin

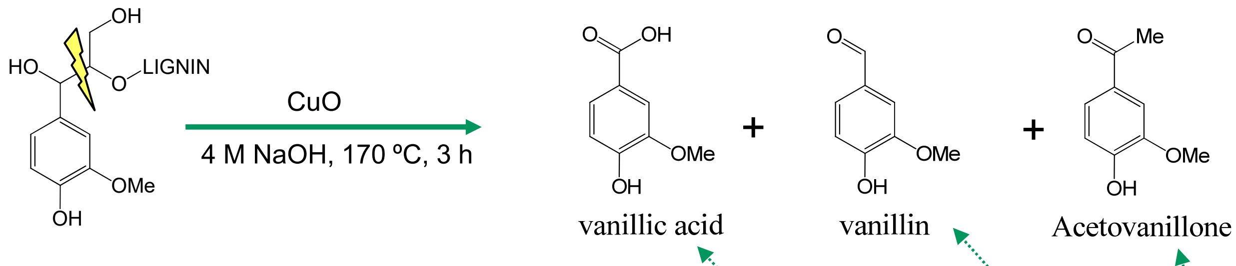
- Combination of wet chemistry with spectroscopy, chromatography, and various other techniques
- Understanding the organic chemistry in use may help developing possible routes for chemical and materials production from lignin
- Characterization in liquid or solid state?
  - **Liquid state**: functional groups, molecular weight, interunit linkages **Solid state**: material properties , functional groups and elemental composition, imaging of the physical structure
- A few illustrative examples follow

Solution-state analysis of lignin

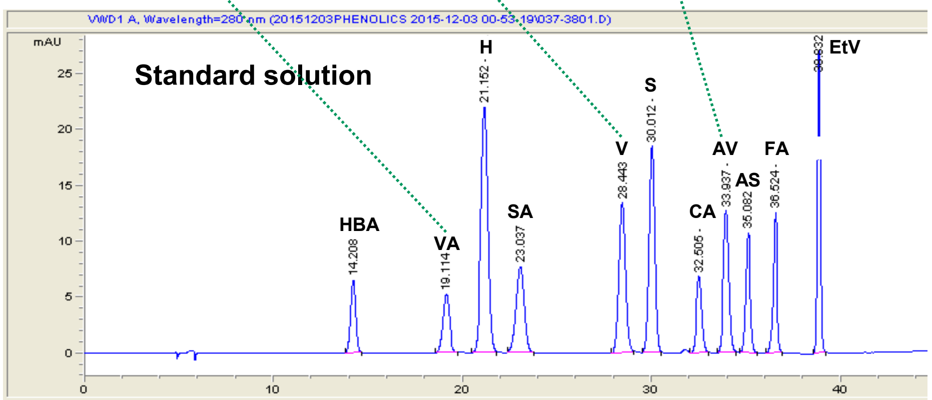
- **Chemical degradations**
  - Oxidations: ozonolysis, permanganate oxidation, nitrobenzene oxidation, **cupric oxide oxidation**
  - Reductive treatments: **thioacidolysis**, DFRC
  - Chromatography is used to quantify the products
- **NMR spectroscopy**
- **Size-exclusion chromatography**

Cupric oxide oxidation

- A classical oxidative degradation method still in use today due to its simplicity
- Reaction mechanism involves radical oxidation

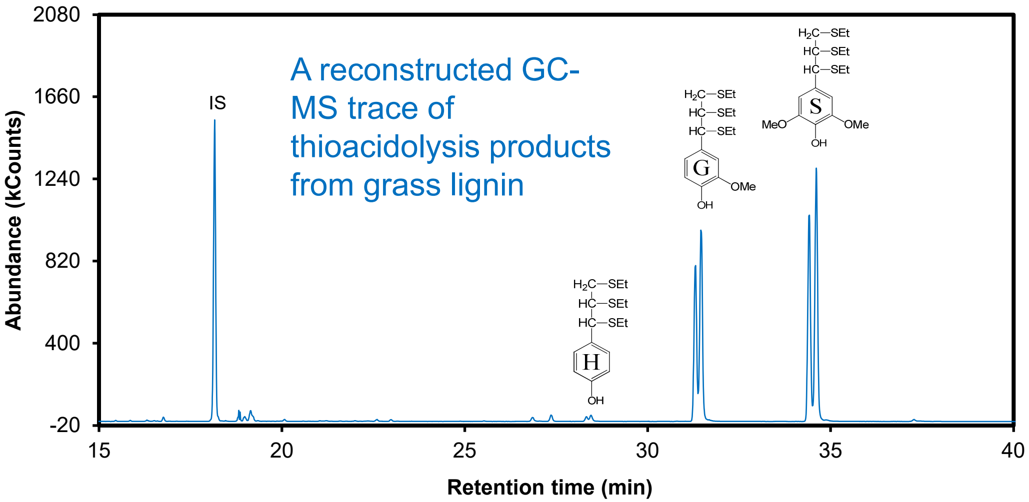


Note that regardless of the lignin source vanillin is always the main product

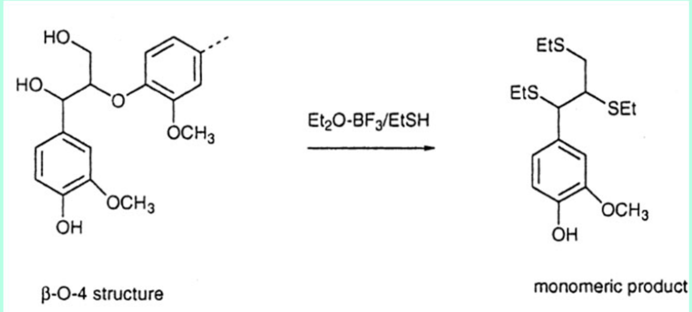


Thioacidolysis

- Comparable information as from CuO oxidation, but more specific to b-O-4 cleavage and yields less degradation side-products
- Solvolytic cleavage of lignin in the presence of ethanethiol
- Malodorous (EtSH) and harmful (Et_2O-BF_3) reagents limit applicability



Primary reaction in thioacidolysis:



Brunow G (2001) Methods to Reveal the Structure of Lignin. In: Hofrichter M & Steinbüchel A, (ed), Lignin, Humic Substances and Coal, Vol 1 (pp. 89–116). Wiley-VHC, Weinheim.

Sipponen, Doctoral thesis 2015

^{13}C NMR

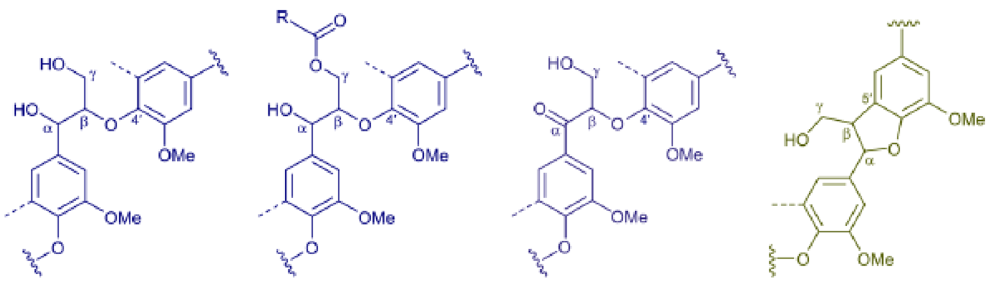
- Nuclear Magnetic Resonance (NMR) spectroscopy measures chemical shifts (changes in frequency compared to the reference frequency) when the atom is subjected to a magnetic pulse at similar frequency as its natural resonance frequency
- ^{13}C NMR delivers a wealth of quantitative structural information:[1]
- However, time consuming in data collection and analysis

β-O-4 total	
Pino/syringylresinol (F_α)[b]	Primary aliphatic OH
Phenylcoumarane (E_α)	Secondary aliphatic OH
Sugars (C_1)	5-free phenolic OH
$S_{2,6}$	5-subst. phenolic OH
G_2	Ar-H
H_4	Oxygenated aliphatic
Degree of condensation	Saturated aliphatic
OMe	
Non-conjugated CO	EtO–
Conjugated CO	Alkyl-O-Alkyl
Non-conjugated COOR	Side chain length
Conjugated COOR	Demethylation degree
Total OH	

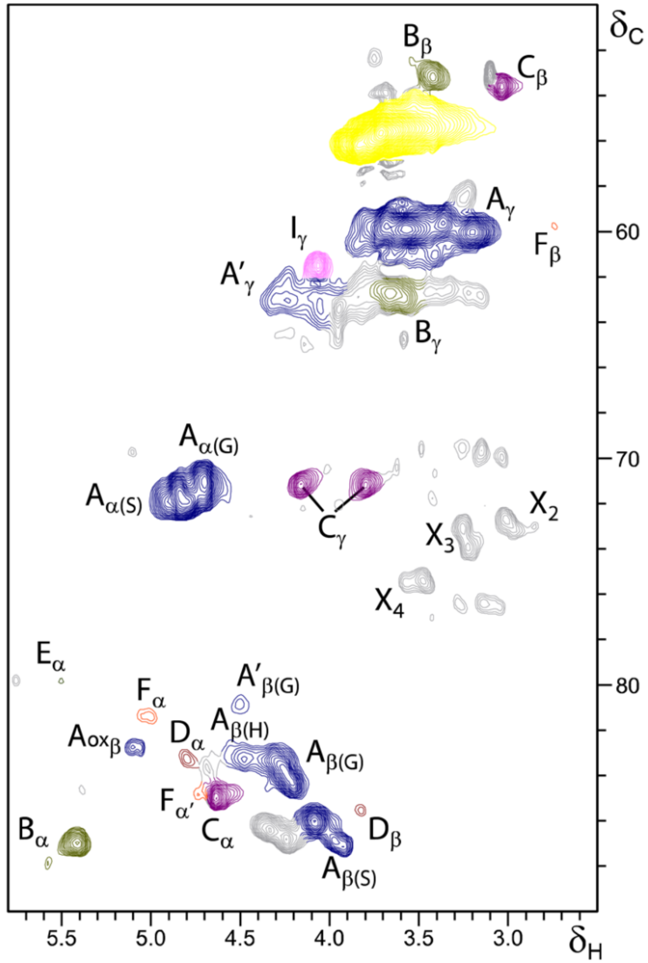
[1] Balakshin and Capanema, RSC Adv., 2015, 5, 87187-87199

HSQC NMR (2D NMR)

- Integration of ^{1}H and ^{13}C cross peaks gives semiquantitative information of the lignin interunit linkages as well as linkages to carbohydrates



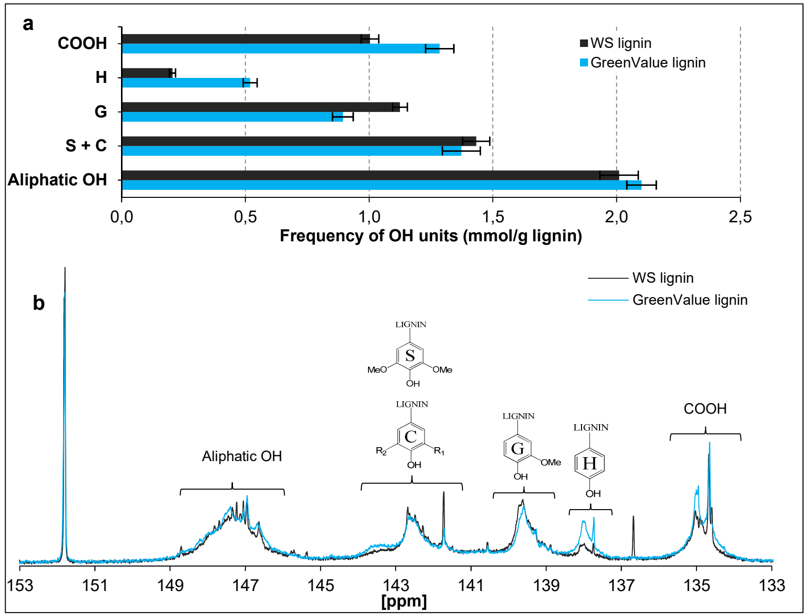
A A′ A_{ox} B

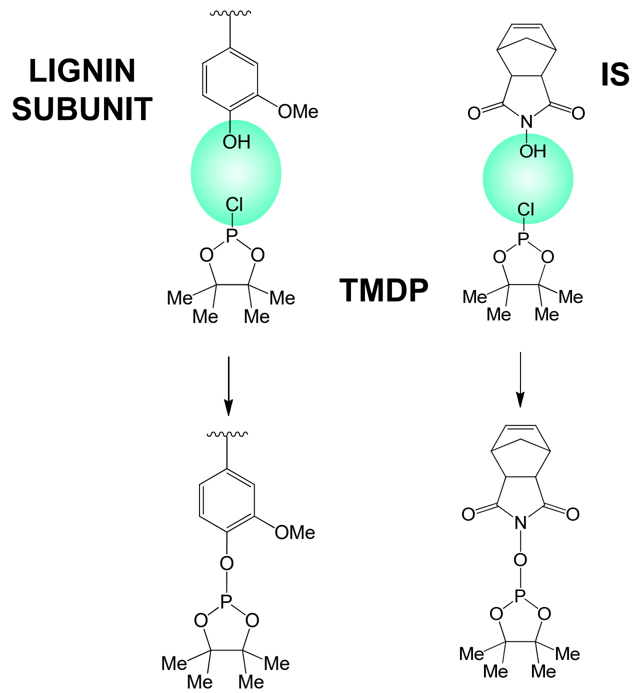


Del Río et al., J. Agric. Food Chem. 60 (2012) 5922−5935.

^{31}P NMR, a powerful method for OH groups

- Derivatization of lignin in solution with a P-donor which is quantitatively measured against internal standard





TMDP = 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane
IS = N-Hydroxy-5-norbornene-2,3-dicarboxylic acid imide

Size exclusion chromatography

- Separation of molecules based on their hydrodynamic radii (size in solution)
- Organic and aqueous elution systems are in use
- Sample must dissolve fully and should not associate with the particles in the column packing material
- Molecular weight is calculated relative to known Mw standards or directly using laser scattering or viscometer

Analysis of lignin as a product

- **Complex structure, but all-inclusive analysis is not necessary**
- **Key properties in relation to the application important**
- **Real application tests needed also**
- **Distinction of a high purity lignin from a low purity one:**
  - *Low amounts of contaminating carbohydrates, sulphur, inorganics*
  - *Good solubility and material properties*

Applications

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Applications of lignin

- $2 \cdot 10^{10}$ tons of lignin is produced annually in Nature [1]
- Only a fraction of the total is used in products, why?
  - Lack of regular (sterochemical) structure
  - Heterogeneous repeating units (H, G, S and their linkage patterns)
  - Poor understanding of how the degree of polymerization and structure change when lignin is isolated from cell walls
- However, recent advances in lignin isolation and characterization hold promise for increased use of lignin in materials

[1] Funaoka, Macromol. Symp. 2003, 201, 213−221

R&D for commercialization of lignin is undergoing rapid growth

New kind of gold from Nordic forests

Lignin is an organic material that binds the fibres and cells of wood. The word lignin may not be familiar to many, but there is a lot of anticipation and excitement around this material. Why?

PHOTO: Vesa Laitinen

Experts say that lignin may eventually be one of the new renewable materials extracted out of the green gold of the Nordic forests. Stora Enso's Sunila Pulp Mill in Finland will, in the future, extract lignin from pine and spruce.

Following the biorefinery investment announcement made in July, there is an air of excitement at the mill.

The first dedicated biorefinery investment is expected to be completed in early 2015. Mill Director **Olli-Pekka Reunanen** is pleased, and with good reason. Installing the new machinery in the architectural milieu that dates back to 1938 is an enjoyable challenge for the mill staff. "We won't be building new buildings; instead, the idle buildings in the mill area will be used to house the machinery required for the biorefinery," explains Reunanen.

Prior to making the 32-million-euro investment decision, Stora Enso carefully looked into areas where lignin extraction would be profitable. Sunila is Stora Enso's first, but probably not its last, biorefinery dedicated investment.

Stora Enso has been researching lignin and its attributes for more than a decade.

"Lignin is a challenging and complex material; it's an honour to be a pioneer in this area. At the same time, we are taking a leap into new and previously uncharted markets, but Stora Enso is in a good position to develop commercially viable applications from lignin," says **Mikael Hannus** of the Biomaterials's Biorefinery and Bioenergy unit.

The initial markets are anticipated in, for example, the construction and automotive industries, where lignin offers a sustainable alternative to the phenols used in plywood and wood-panelling glues and the polyols used in foams. Other applications are also under development.

rethink.

storaenso

Lignin – a vast source of opportunities for innovative minds

The Biofore Company UPM

Lignin is an ideal bio-based substitute for various petro-based products used today.

Now, for the first time, high-quality lignin is commercially available in large quantities, ready to be used in a broad range of applications.

UPM has developed profound know-how and IP regarding the chemistry of lignin from various sources as well as lignin analysis, fractionation, purification, activation, modification, and formulation. Together with our partners we are committed to develop our innovative lignin-based products further.

Lignin works in favor of both environment and economy

Features and benefits of UPM's lignin-based products:

- 100% bio-based – UPM's lignin-based products are exclusively based on renewable raw materials
- Excellent performance – stringent quality control and a proprietary activation technology make UPM's lignin-based products reliable and effective
- Reduced dependency on fossil raw materials – lignin-based products can help to increase security of supply while enabling a smooth transition to renewable raw materials
- Cost savings – based on their outstanding performance, UPM's lignin-based products can help realize cost savings in a variety of applications;
- Versatility – lignin can be used in a broad range of applications, ranging from composites, through a broad range of resin systems, to raw material for carbon fibers

http://www.storaenso.com/rethink/new-kind-of-gold-from-nordic-forests
http://www.upmbiochemicals.com/lignin/Pages/Default.aspx

Current uses of lignin

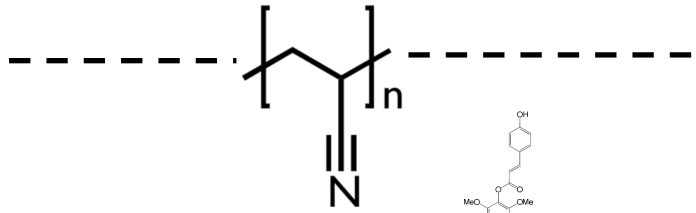
- **Energy (it has value!) 26.7 MJ/kg (HHV)**
- **Vanillin, 3% from softwood (~10% of lignin)**
  - *Today, 20% of vanillin is produced from lignin and 80% from crude oil using the guaiacol route*
- **Pyrolysis of lignin → bio-oil (15−25%), gas (20−50%), char (30−60%)**
- **Aromatic chemicals (BTX)**
- **Composites and polymer blends**
- **Micro- and nano-scale structures (particles, capsules, sheets etc.) for novel applications**
- **High-performance materials such as carbon fibres are also under development**

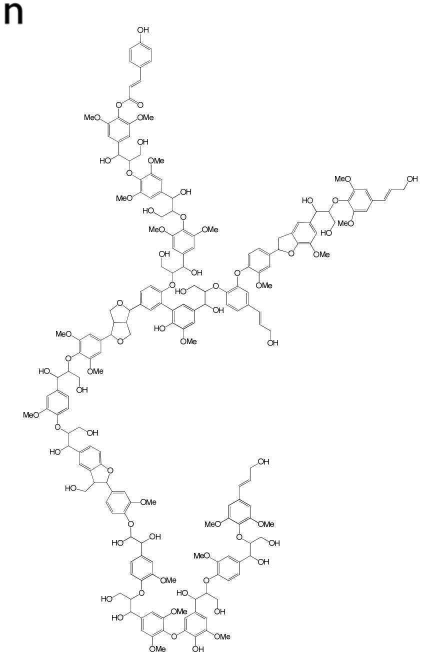
Carbon fibers from lignin

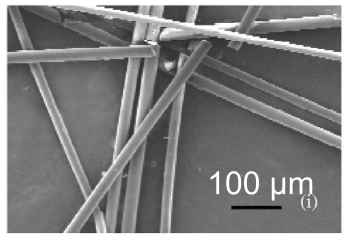
- Carbon fibers are high-value materials currently produced from polyacrylonitrile
- Lignin is more affordable raw material, but less linear polymer with predominant ether linkages

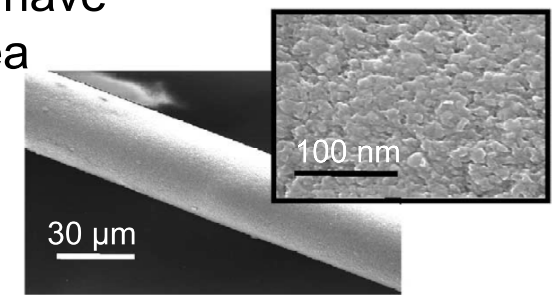
→ Challenges the production of carbon fibres with sufficient strength properties

→ However, this is promising results have been obtained in this research area









Kadla et al., Carbon 40 (2002) 2913–2920

Summary of keywords

dimerization

aliphatic

condensation

oxidation / reduction

radical coupling

phenolic

quinone methide intermediate

polydisperse

condensed / uncondensed units

methoxyl

branched

monolignols

β-O-4

heterogeneous

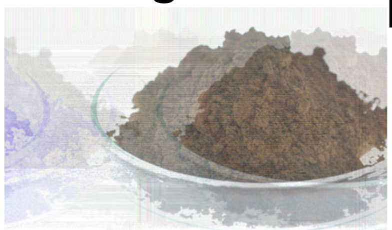
aromatic

anionic

lignin

p-hydroxyphenyl

lignosulfonate



H/G/S

guaiacyl

kraft lignin

syringyl