



Aalto University
School of Chemical
Technology

CHEM-E1150
BIOMASS PRETREATMENT AND FRACTIONATION

LIGNIN CHEMISTRY

Herbert Sixta, 2018

Learning outcome

1. Introduction

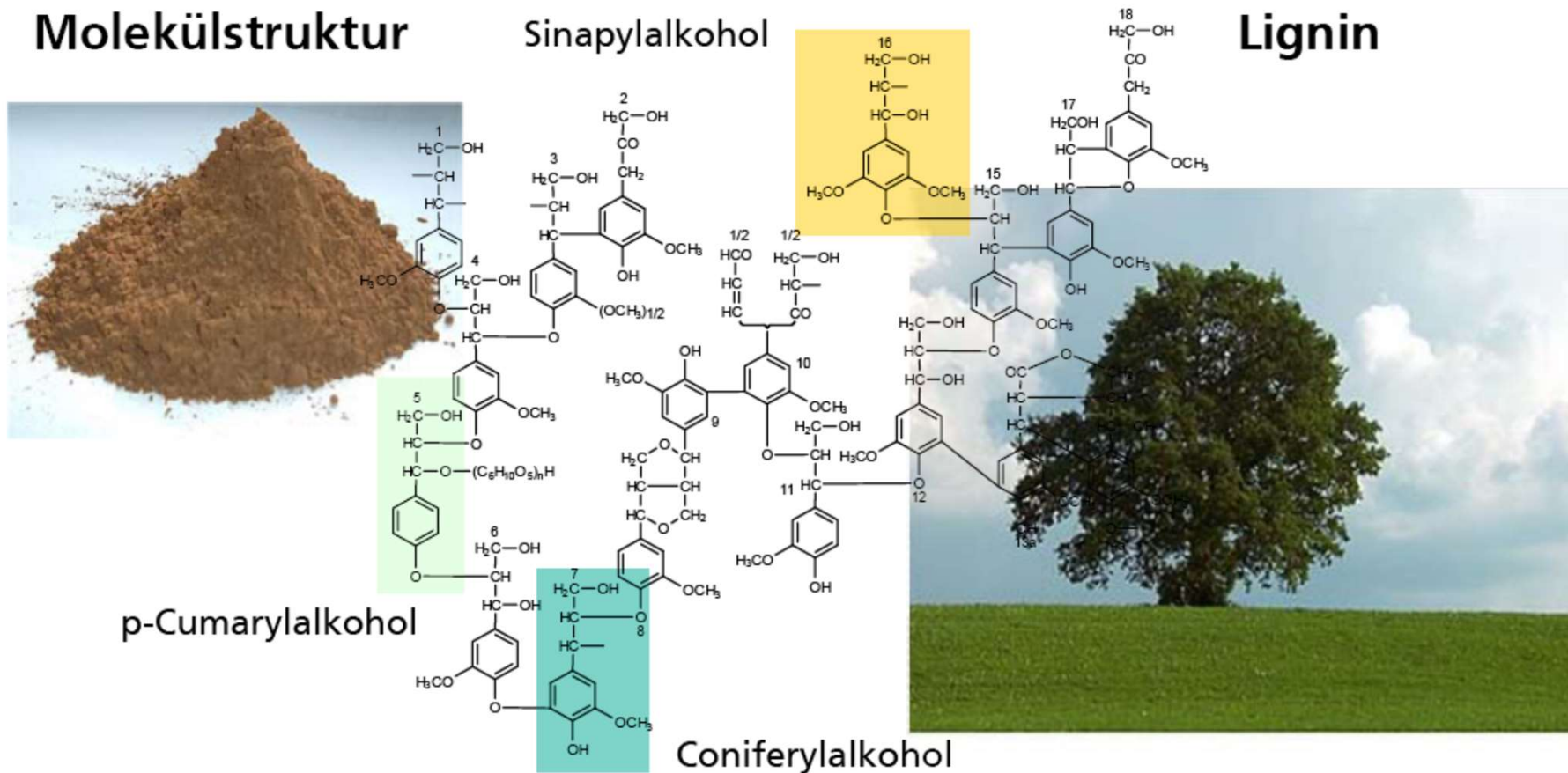
2. Occurrence
3. Biosynthesis
4. Isolation
5. Chemistry of lignin units
6. Lignin-carbohydrate complexes (LCCs)
7. Determining lignin structure by chemical degradation
8. Spectroscopy of lignins

Lignin

Molekülstruktur

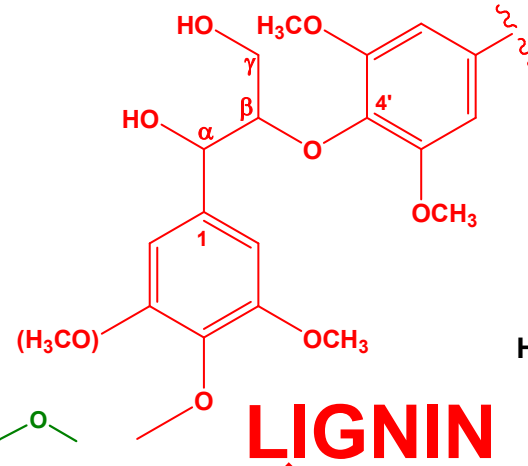
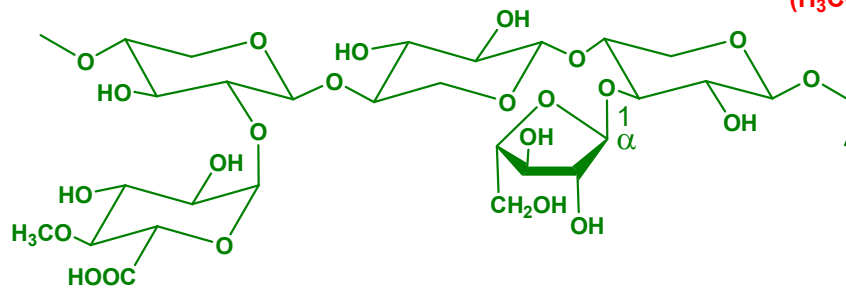
Sinapylalkohol

Lignin



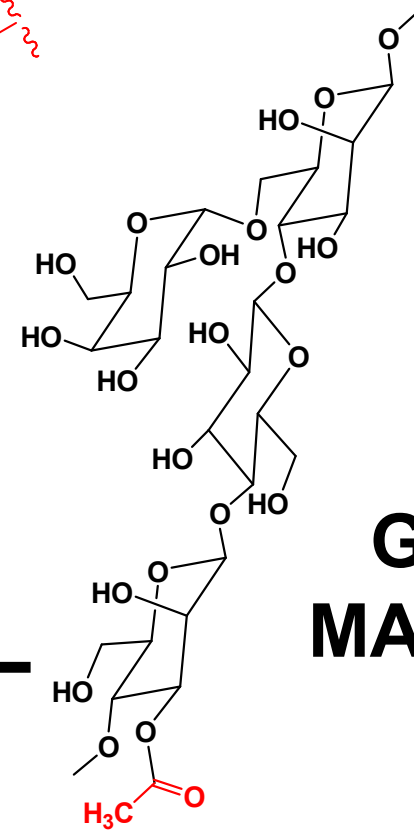
Quelle: O. Faix, BFH Hamburg

XYLAN

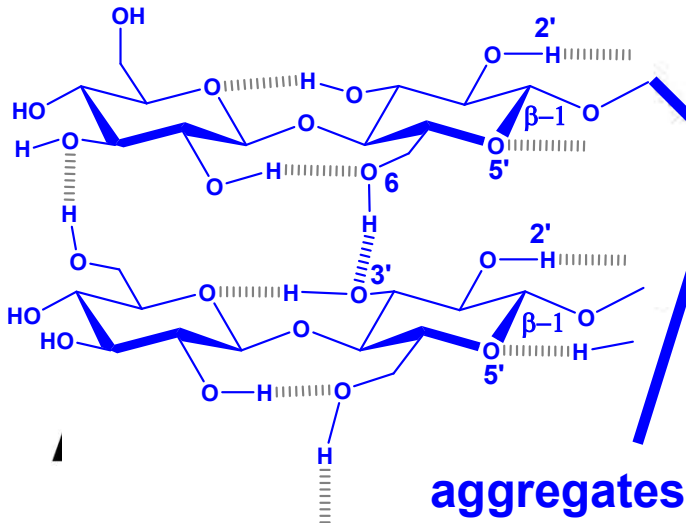


LIGNIN

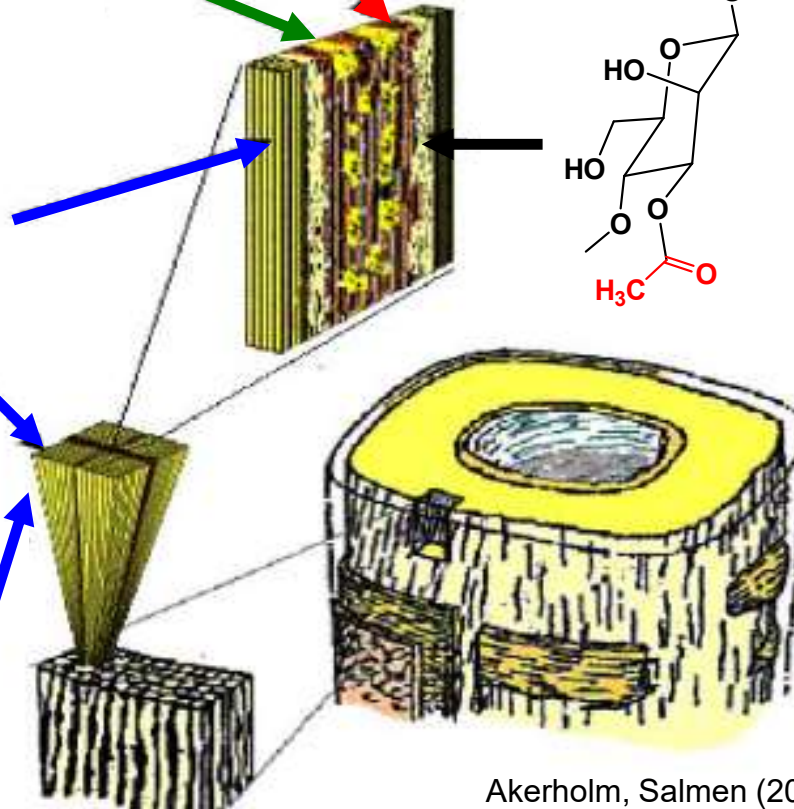
**GAL
GLUCO
MANNAN**



CELLULOSE



aggregates



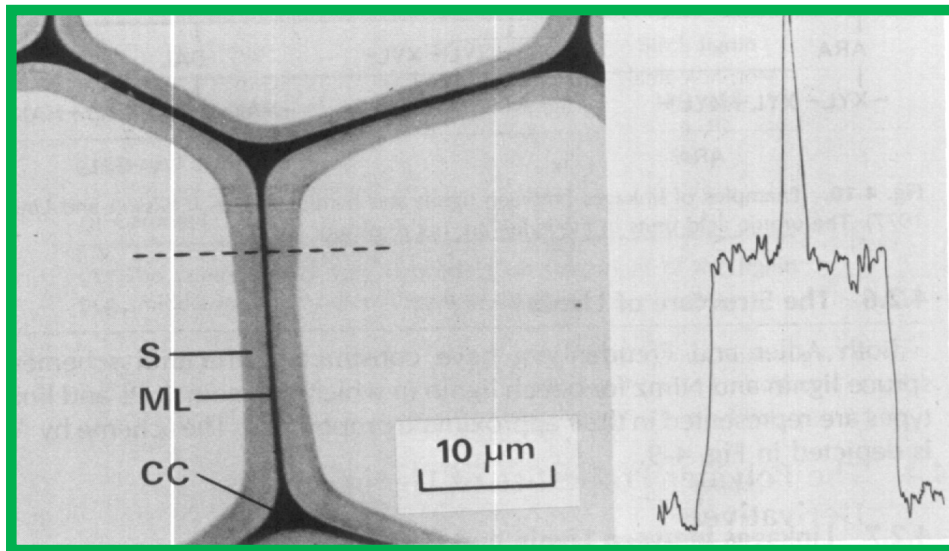
Lignin Distribution

Concentration:

- o Middle lamella: 70%
- o Secondary wall: 23%

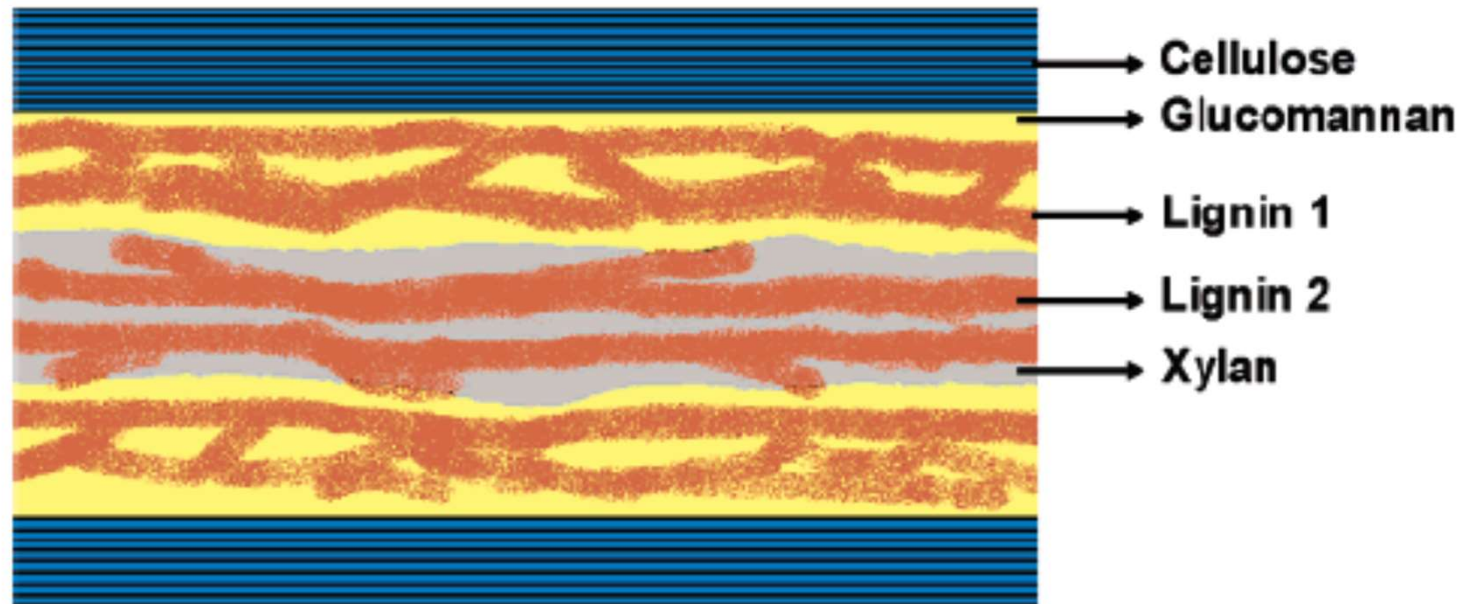
Amount:

- o Cell corner: 12%
- o Middle lamella: 16%
- o Secondary wall: 72%



Proposal for a schematic view of a fibre wall segment
according to the findings of LCC fractionation:

Proposal for heterogeneous Lignin-carbohydrate patterns^o



Learning outcome

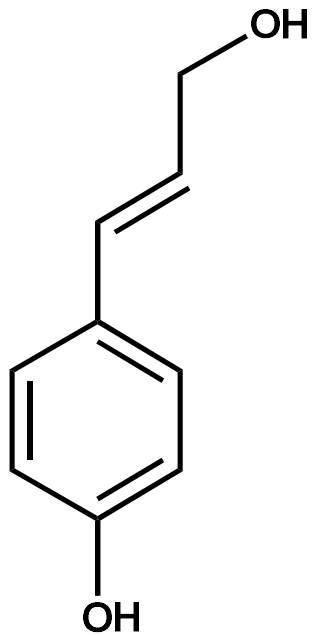
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Occurrence

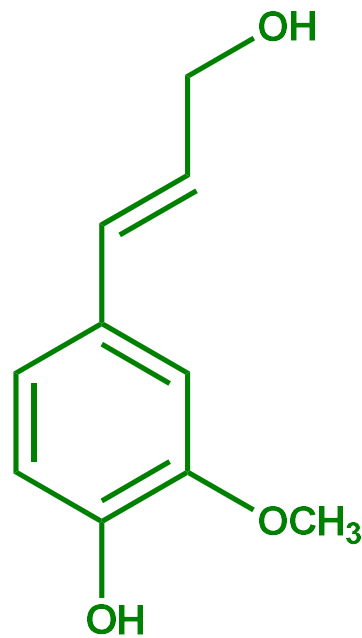
Second most abundant natural polymer on earth:

- Softwood: 26 – 32% Lignin (Klason)
 - Compression wood: 35-40%
- Hardwood: 20 – 28% Lignin (Klason)
 - Tension wood: 20 – 25%
 - Tropical hardwoods: > 30%

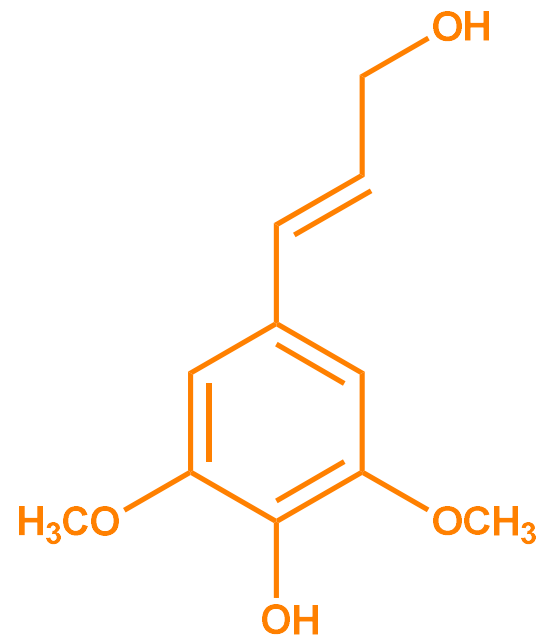
Lignin Precursors



p-coumaryl alcohol



coniferyl alcohol

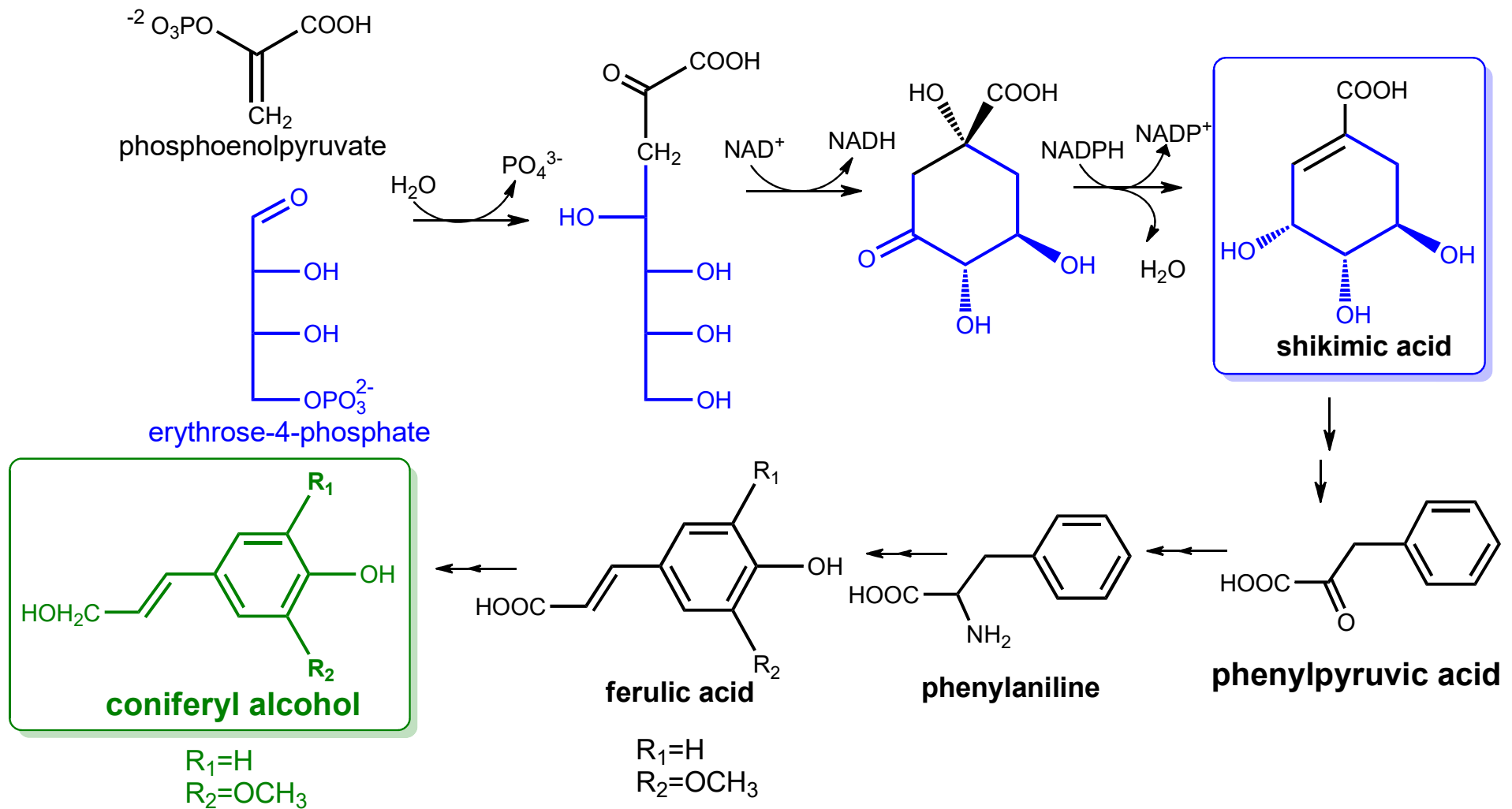


sinapyl alcohol

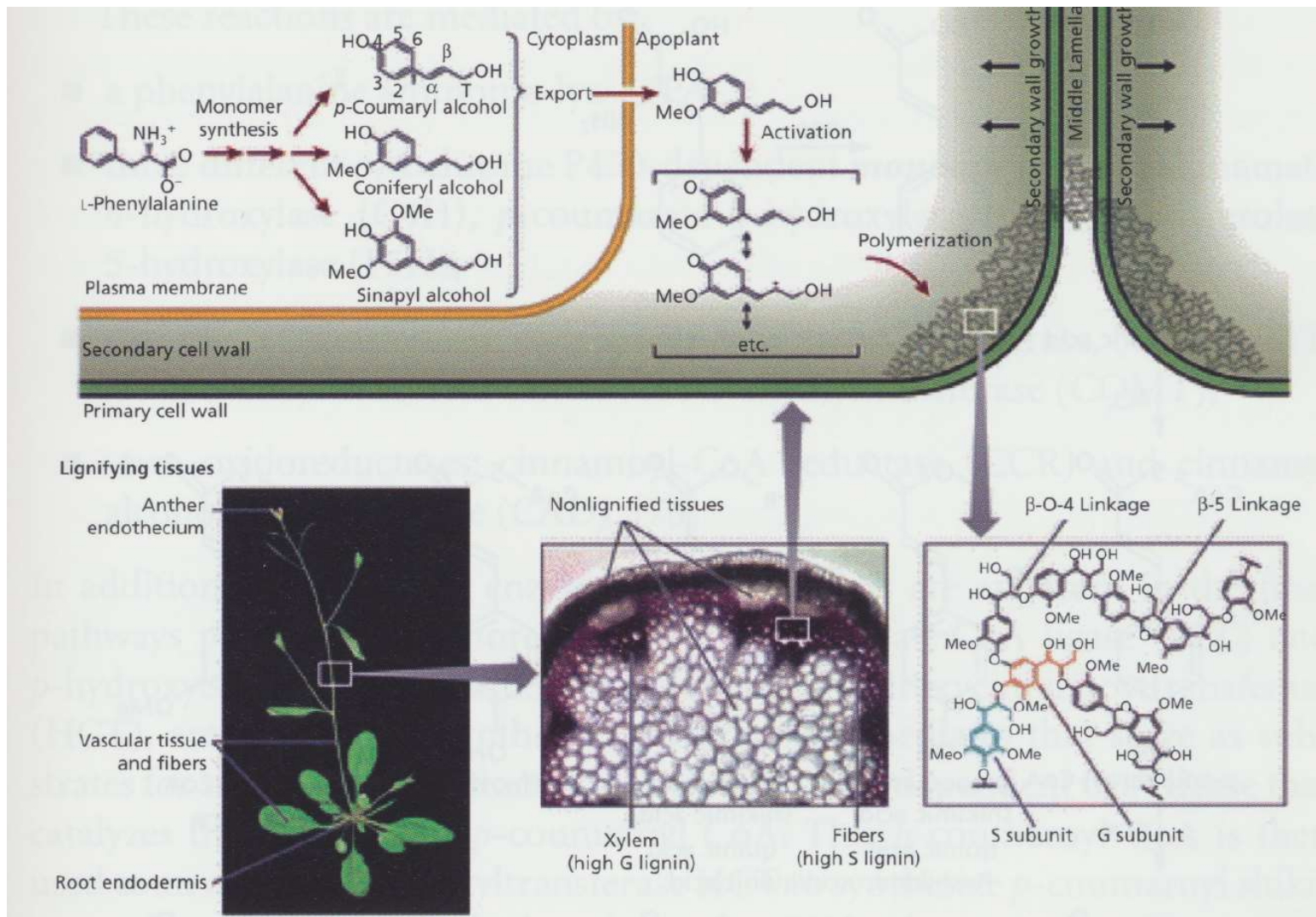
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Biosynthesis of Precursors

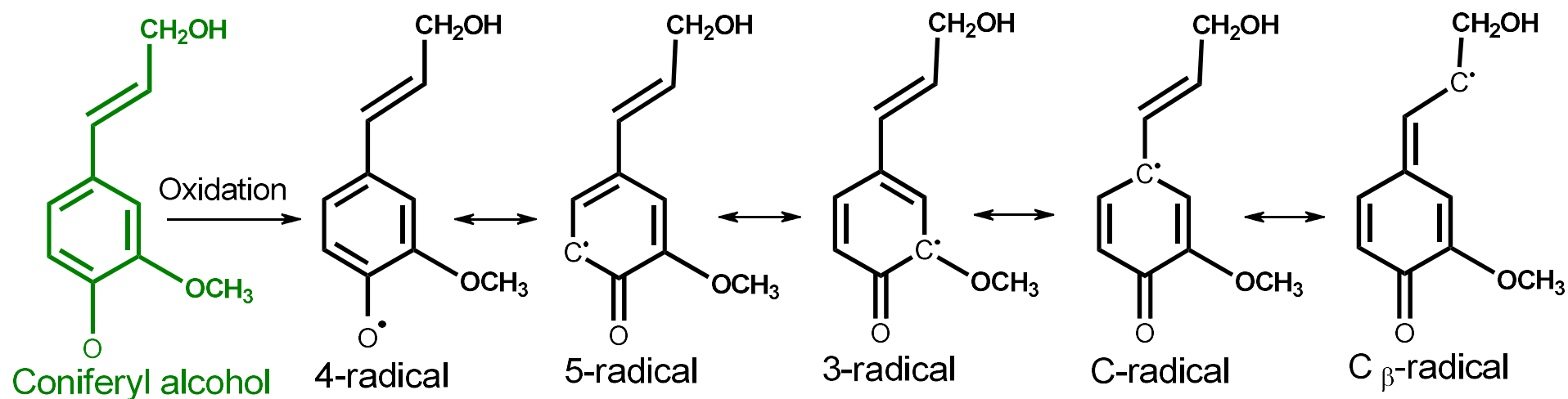


Biosynthesis of Precursors

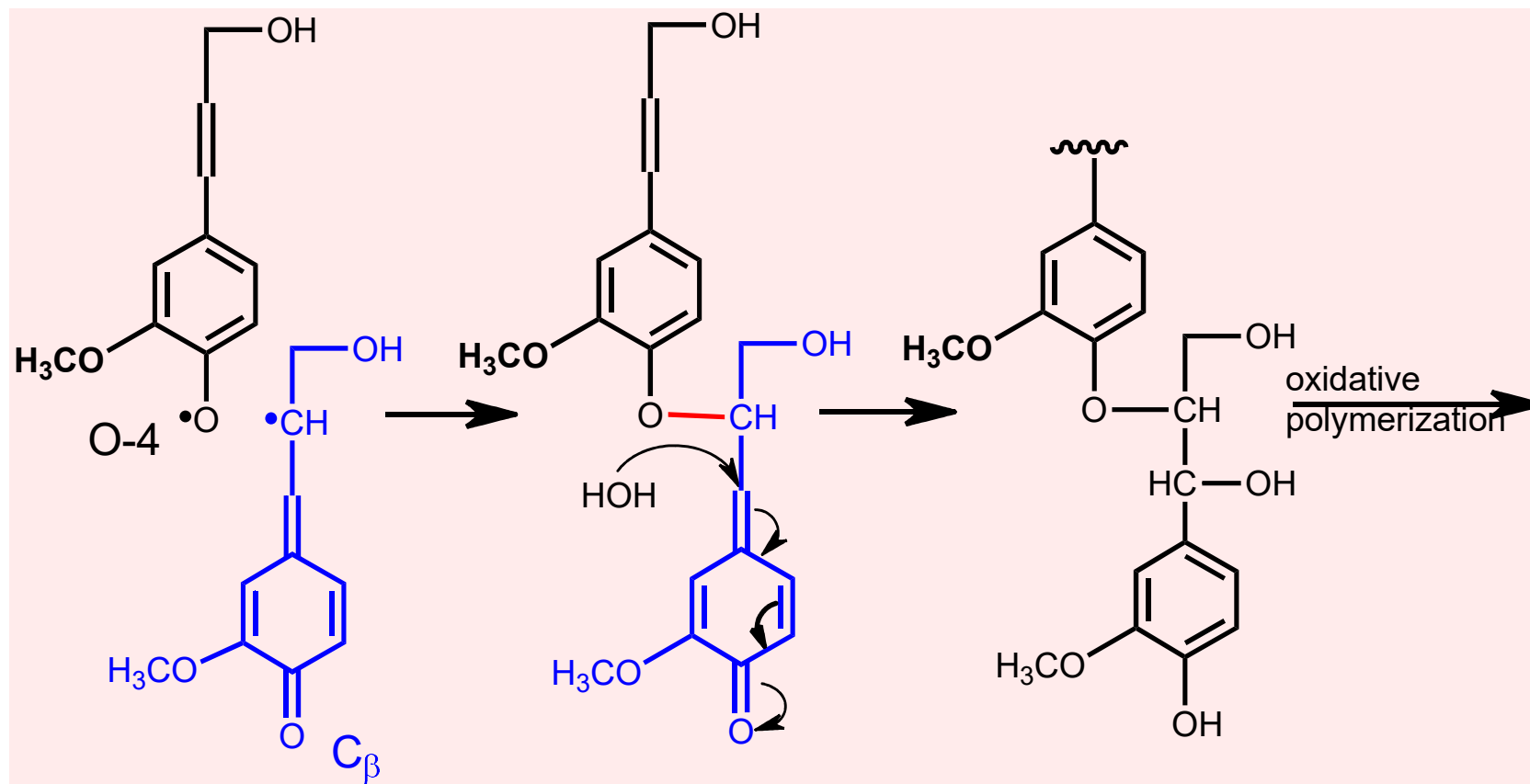


Monolignols in the cytoplasm, translocated to the apoplast and are synthesized and polymerized into lignin

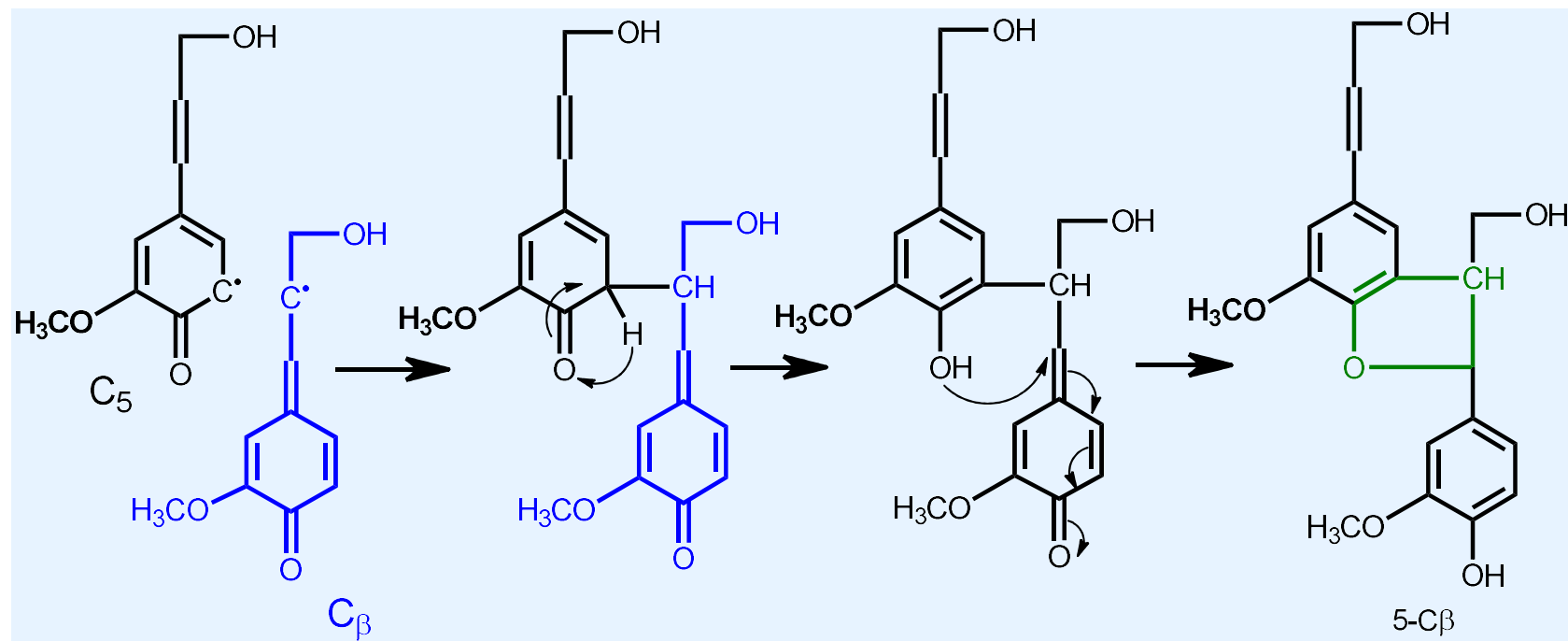
Phenolic radical formation



C_β -O₄ Bond formations: Head-to-tail coupling



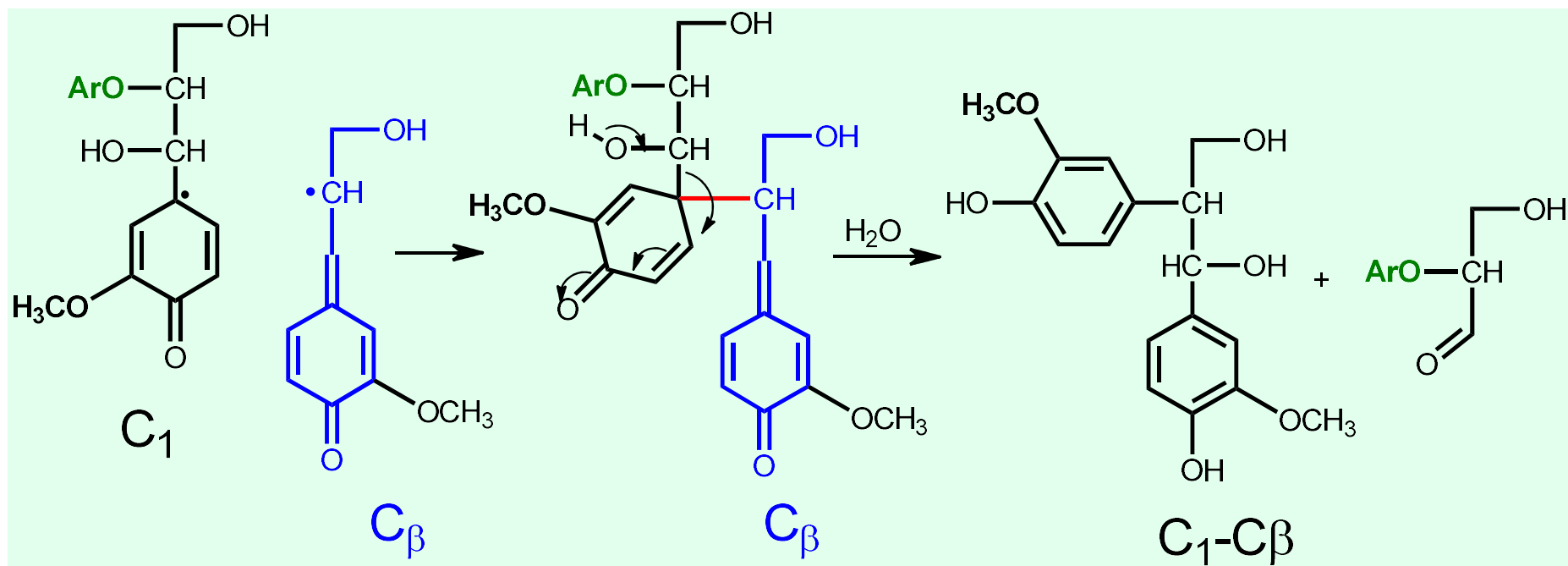
C₅-C_β-Bond formations



enolization

Michael Addition

C₁-C_β-Bond formations



addition of a nucleophile to an α,β unsaturated carbonyl

Lignin units

Softwood

Guaiacyl-Lignin (**G**) from *trans*-Coniferyl alcohol

Hardwood:

Syringyl-Lignin (**S**) from *trans*-Sinapyl alcohol:

S/G = 1:1-3:1

Beech:G:S:H = 56:40:4

Annual plants:

also *trans*-Coumaryl alcohol: Hydroxyphenylpropene units (**H**)

Elemental composition of lignin

Species	C %	H %	O %	OCH ₃ %	Molar composition as C ₉ -Unit
Picea abies	63,8	6,0	29,7	15,8	C ₉ H _{8,36} O _{2,51} (OCH ₃) _{0,95}
Picea mariana	63,7	6,3	29,4	15,4	C ₉ H _{9,00} O _{2,51} (OCH ₃) _{0,93}
Pinus silvestris	64,0	6,1	29,8	15,7	C ₉ H _{8.54} O _{2,52} (OCH ₃) _{0,94}
Tsuga heterophylla	63,4	6,3	29,8	15,7	C ₉ H _{9.00} O _{2,55} (OCH ₃) _{0,94}
Betula verrucosa	58,8	6,5	34,0	21,5	C ₉ H _{9.46} O _{3.06} (OCH ₃) _{1.49}
Populus tremula	60,4	6,2	33,0	21,4	C ₉ H _{8.56} O _{2.84} (OCH ₃) _{1.43}
Fagus sylvatica	60,3	6,3	33,4	21,4	C ₉ H _{8.78} O _{2.90} (OCH ₃) _{1.43}

Macromolecular properties

Softwood **M**illed - **W**ood - **L**ignin (**MWL**):

$M_n = 15.000 - 20.000$ (DP 75 - 100)

Molar mass of hardwood MWL slightly lower

PDI = $M_w/M_n = 2.3 - 3.5$

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Acidolysis

1. Mild alkaline pre-extraction to remove condensed tannins (optional)*.
2. Pulp sample refluxed under nitrogen with 0.1M HCl in dioxane-water 82:18 at 88°C (azeotrope)
3. Filtration and washing of pulp (3*300 mL)
4. Addition of water to the filtrate which was then film evaporated (min water volume to control acidity)

*Evtuguin, D.V et al.: J.Agric.Food.Chem. **2001**,49,4252-4261

°Gellerstedt,G et al, J.Wood Chem.Technol. **1994**, 14, 467-482.

Acidolysis

5. Cooling overnight to achieve **lignin precipitation**.
6. Filtration, freeze-drying.
7. Soxhlet extraction with pentane to remove traces of low-molecular weight material.

Experimental Results

Eucalyptus globulus wood chips, Soda AQ cooking

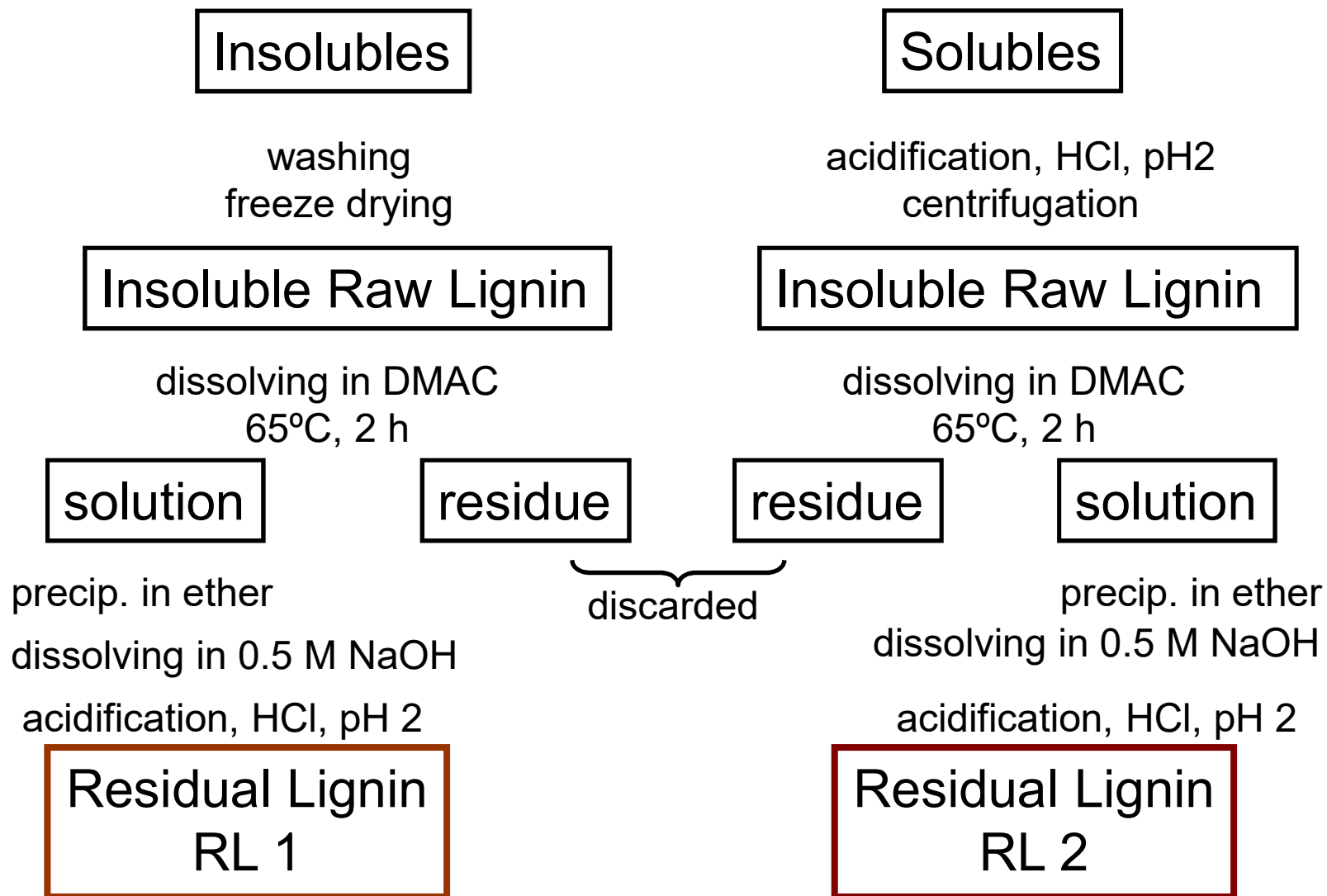
Parameter	Pulp	AcE	Acidolysis
Total Kappa	14.0	13.5	2.4
Ox-Dem kappa	7.4	6.5	1.4
HexA-kappa	2.3	2.4	n.a.

Lignin	Sugar	Acidolysis					OMe	C900 formula
	wt%	C	H	N	O	S	%	
AcE	1.2	58.4	5.7	0.2	29.7	0.2	17.6	C ₉₀₀ H ₈₃₃ O ₂₇₀ (OCH ₃) ₁₁₈

V. Gehmayr, J. Guetsch, M. Leschinsky, G. Zuckerstaetter and H. Sixta. New aspects of residual lignin isolation. *Holzforschung*, Vol. 64, pp. 417–420, 2010

Enzymatic Hydrolysis of Pulp

1. Grinding in a Jokro mill for 20-30 min
2. Stirring a fiber suspension at 5% cons with acetate buffer, pH 4.5
3. Addition of **Celluclast enzymes**, ~ 750 NCU/g, 3 d at 40°C;
4. Separation of the insoluble fractions by centrifugation



Two-Stage method (EMAL)

1. **First step:** mild **enzymatic** treatment to remove hemicelluloses (see above).
2. **Second step:** mild **acidolysis** step, applied with 0.05 M HCl to cleave all carbohydrate linkages.
3. Because of the relatively low enzyme/pulp ratio used in this step, only small amounts of proteins coexist, thus requiring no protease purification.

Dissolved Wood Lignin (DWL)

1. Milling in cutting mill (40 mesh), extraction in acetone:water=9:1, drying at 0.003 mbar.
2. Ball milling under N₂, 48 h, dissolution in 30 mL **2 vol DMSO + 1 vol NMI** per g substrate.
3. Precipitation in 600 mL **9 vol dioxane** + **1 vol water**, centrifugation at 5000 rpm to separate cellulose, distillation of solvent, recovery of raw lignin.

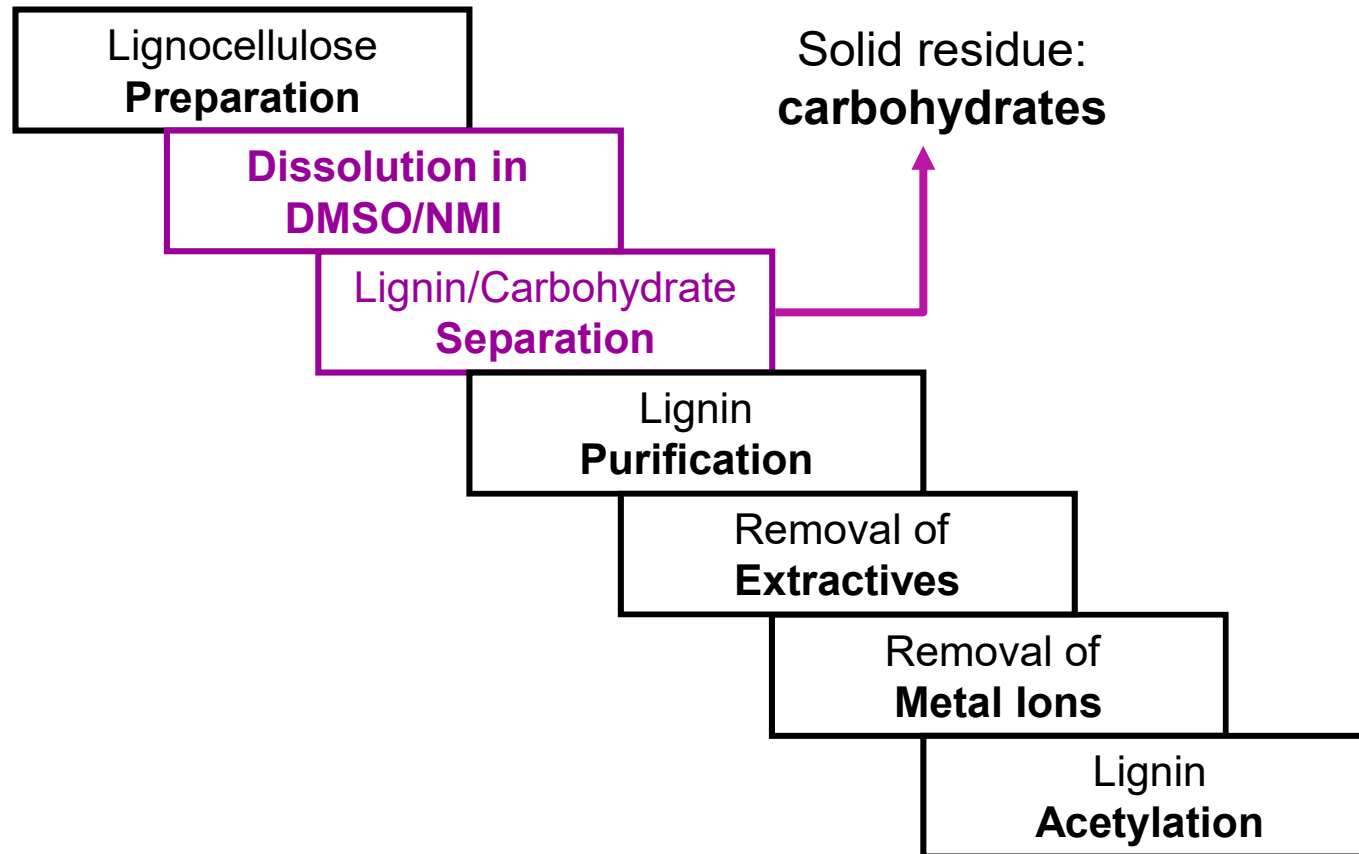
Dissolved Wood Lignin (DWL)

4. Dissolution of raw lignin in **75%HOAc**, centrifugation, filtration, precipitation in water, centrifugation at 13000 rpm, drying of purified lignin

5. **DCM- and acetone** extractions in **ASE**.

6. To remove metallic impurities, lignin is dissolved in 5 vol acetone + 1 vol water, a chelating ion exchanger, Amberlite IR748C, was added, shaking overnight, filtrated, washed, freeze-dried.

Dissolved Wood Lignin (DWL)



Fasching, M., Schröder, P., Wollboldt, R.P., Weber, H.K., Sixta, H.: *Holzforschung* 62, 15-23, 2008

Learning outcome

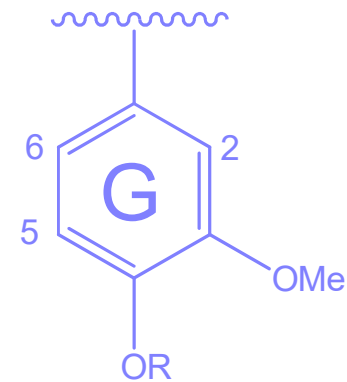
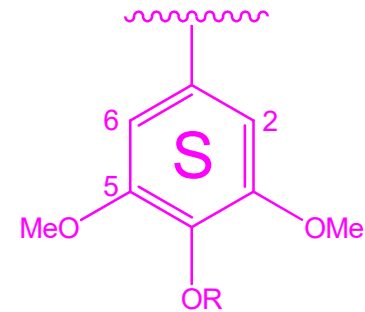
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Chemistry of lignin units - Bonding patterns

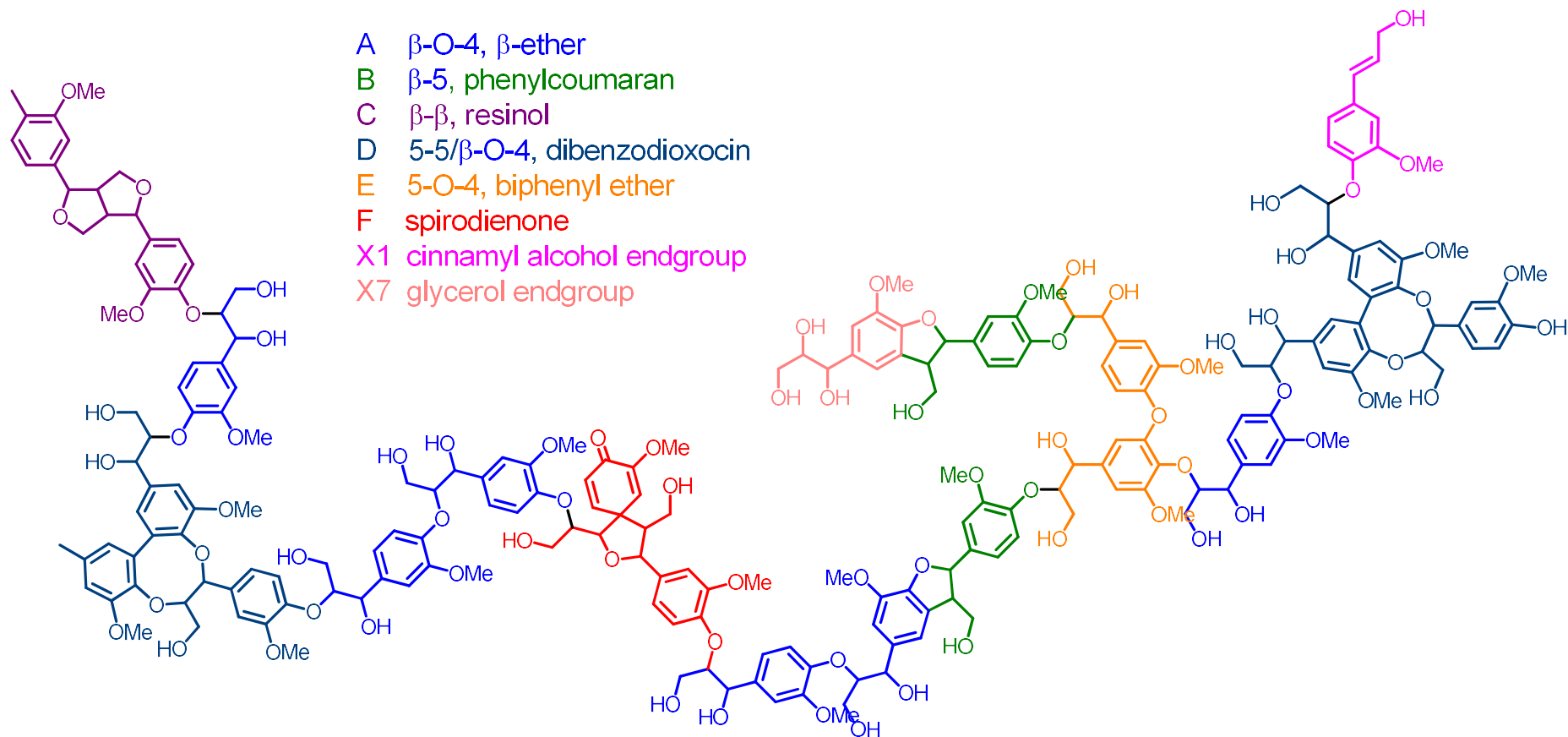
- β -O-aryl ether linkage
- Noncyclic benzyl aryl ethers
- Biphenyl, dibenzodioxocin, diaryl ether
- β -5-linkage: phenylcoumaran structure
- β - β -linkage: pino- and syringaresinol structures
- 1-aryl-2-aryloxy-1-propane units
- β -1 structures
- β -6 or α -6 linkage

Important Lignin Structures

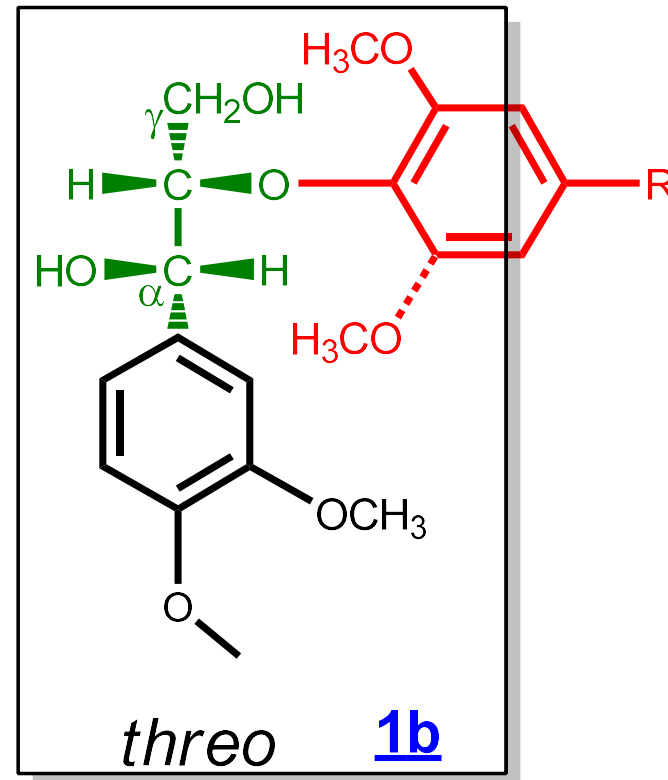
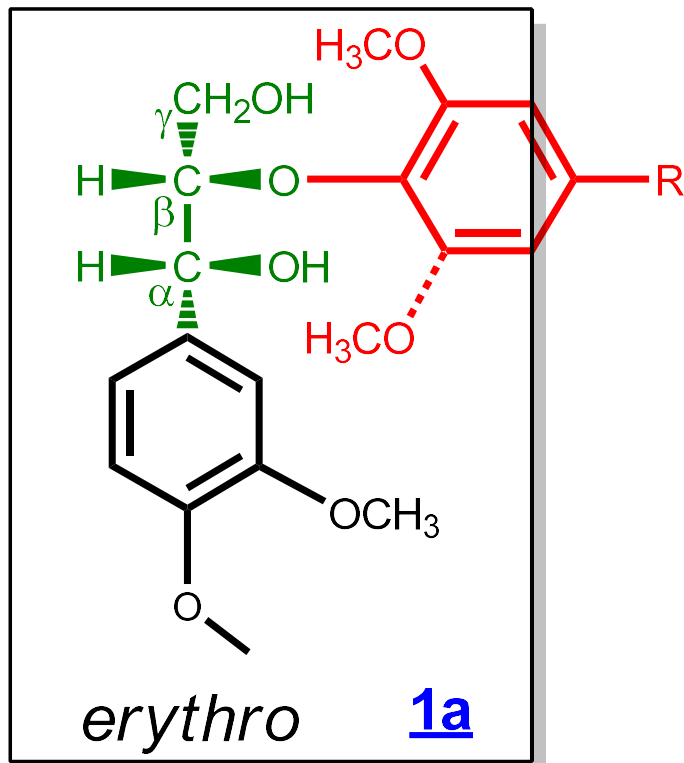
- Syringyl-to-guaiacyl ratio (S/G)
- Phenolic-OH
- Condensed structures
- Carboxyl groups
- Lignin-carbohydrate complexes (LCC)



Lignin Polymer Model for Softwood

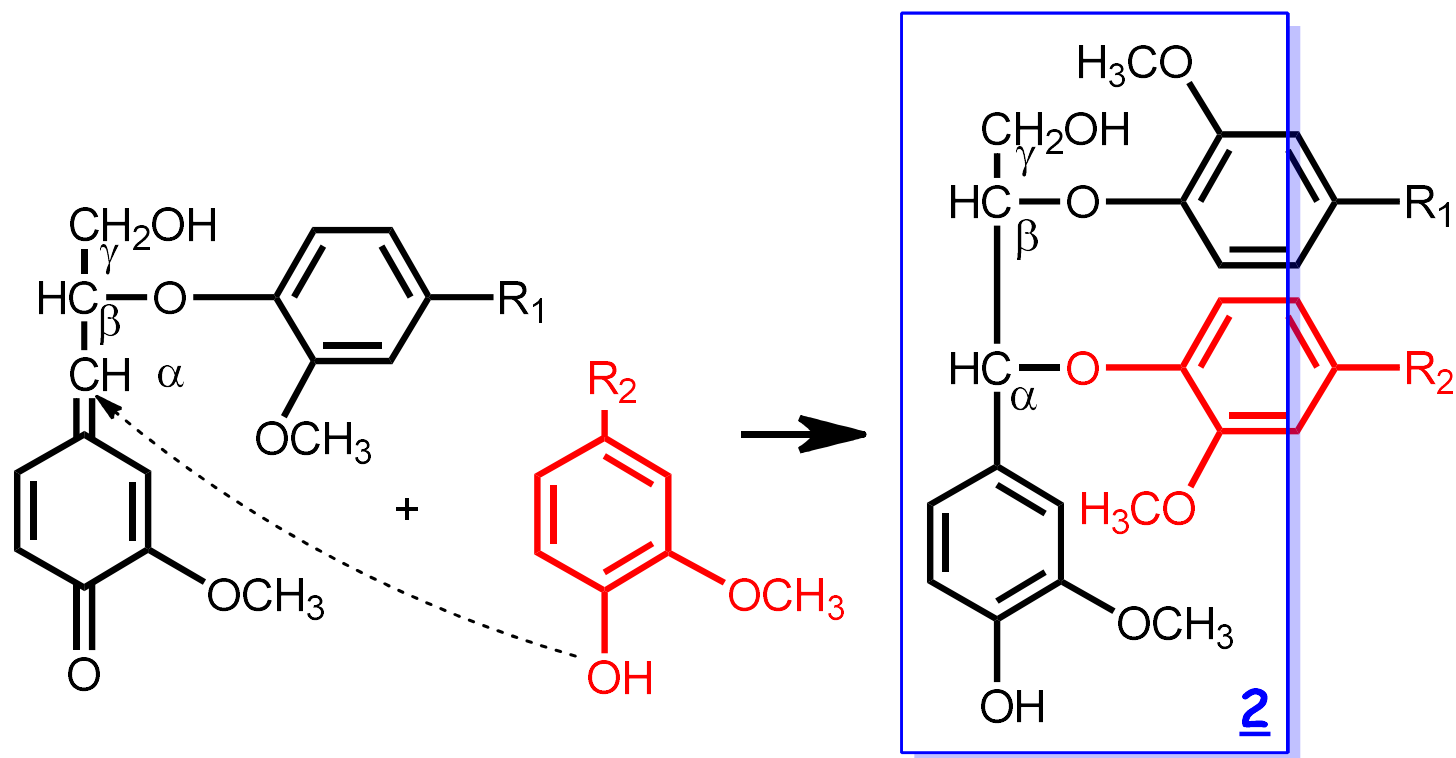


β -O-aryl ether linkage



Equal amounts of the diastereomeric forms present in softwood lignins, predominance of the *erythro* form in hardwood lignin

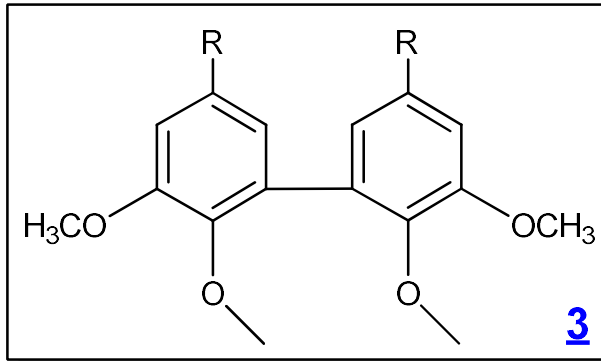
Noncyclic benzyl aryl ethers



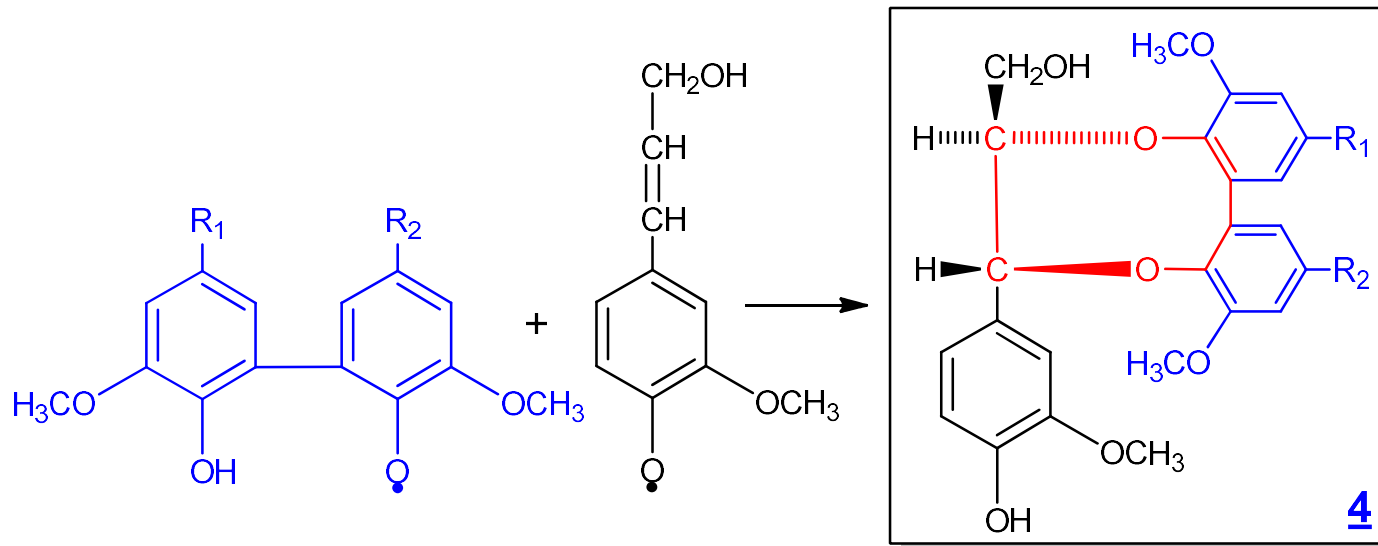
Noncyclic α -, β -diarylethers present in **very small amounts** in MWL.

In neutral solns., addn. of phenols predominant reaction. Under slightly acidic conditions the noncyclic benzyl aryl ethers were found to be stable.

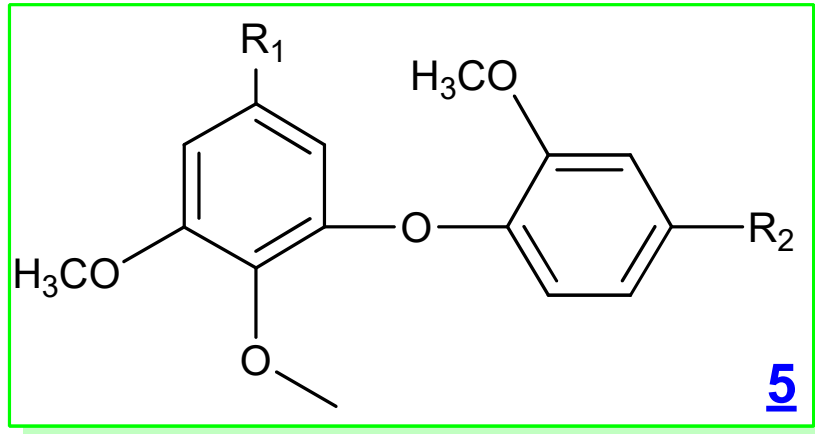
Biphenyl, dibenzodioxocin structures



Biphenyl type in softwood (3),
proved by permanganate oxidation.
Significant amounts of
dibenzodioxocin structures (4)

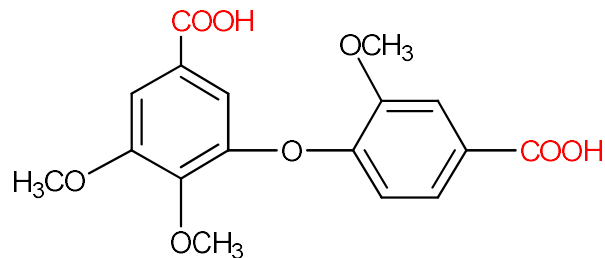


Diaryl ether structures

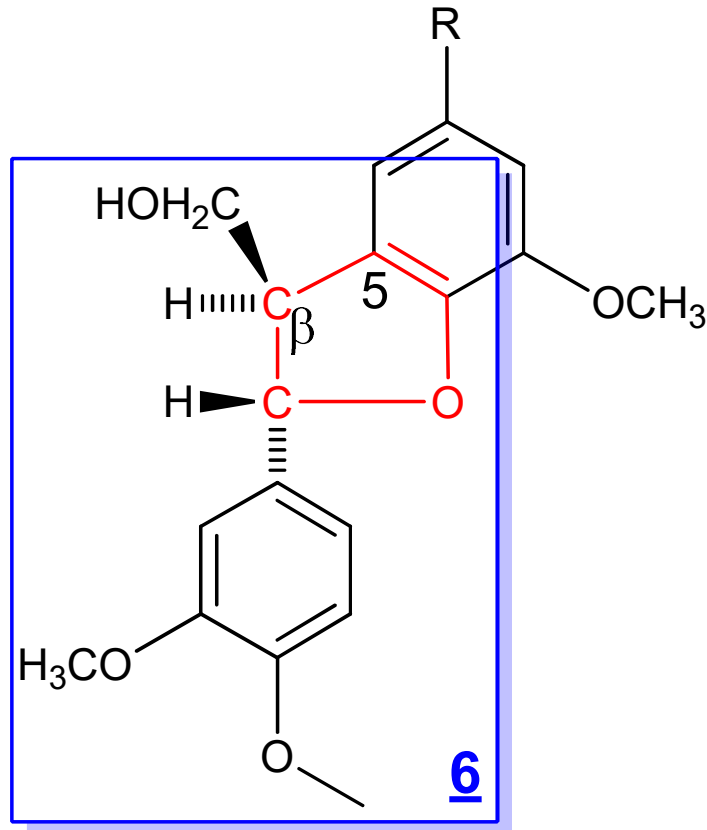


More in hardwood than in softwood lignins

Occurrence of **diaryl ether** structures (5) in lignin was proven by permanganate oxidation. .

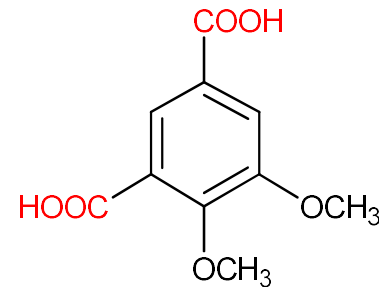


Phenylcoumaran, β -5 linkage



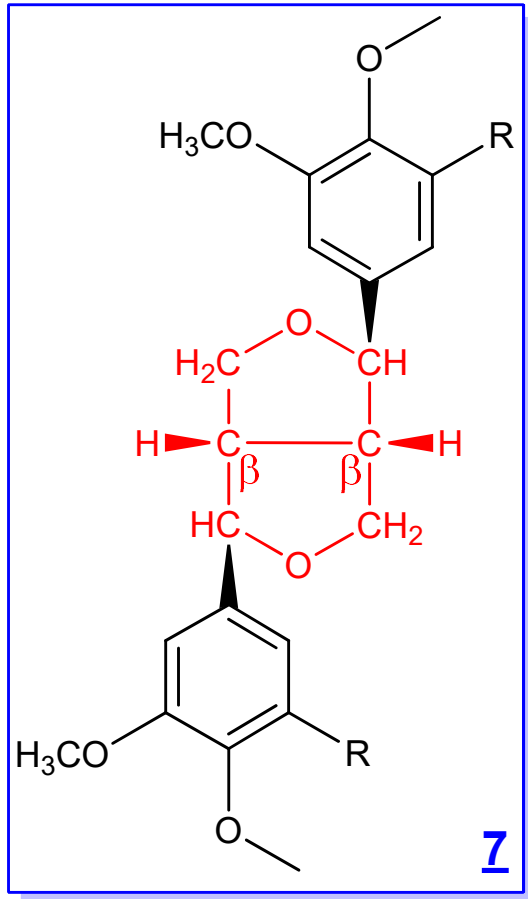
β -5 linked unit are present in phenylcoumaran structures.

Structure proven by permanganate oxidation product (4,5-dimethyl-isophthalic acid*).



Phenylcoumaran structures in lignin primarily *trans*-configuration (¹H NMR).

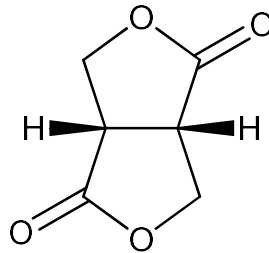
Pinoresinol, Syringaresinol, β - β linkage



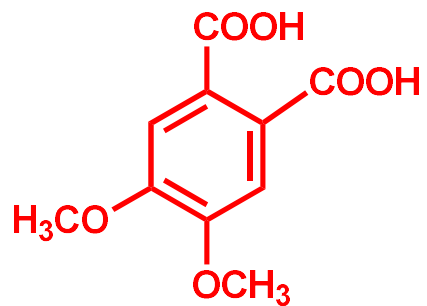
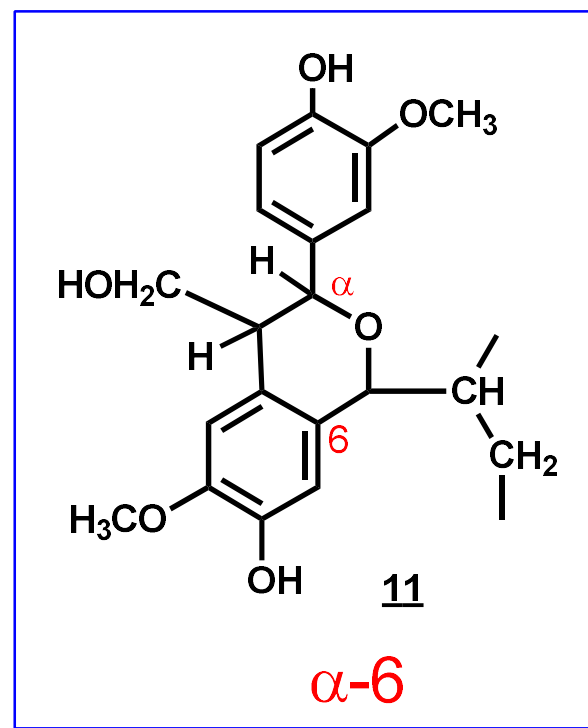
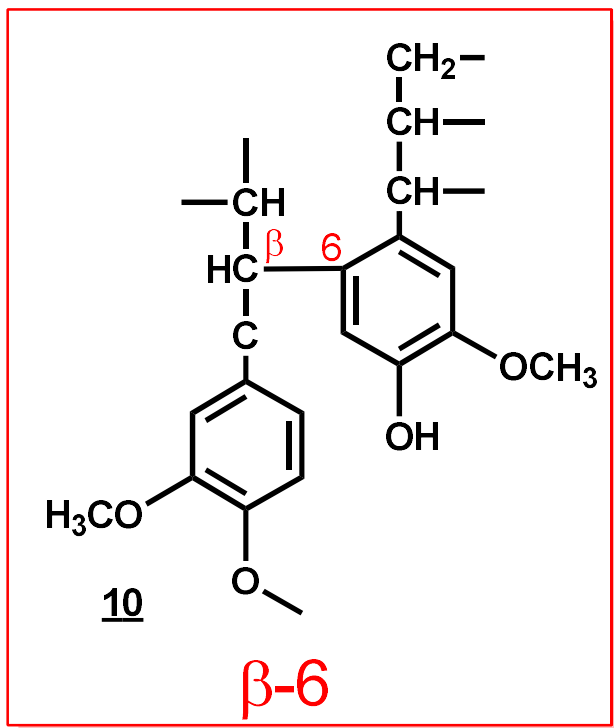
Pinoresinol ($\text{R}=\text{H}$)

Syringaresinol ($\text{R}=\text{OCH}_3$)

Structures proven by
isolation of dilactone(*)
from lignins degraded by
nitric acid

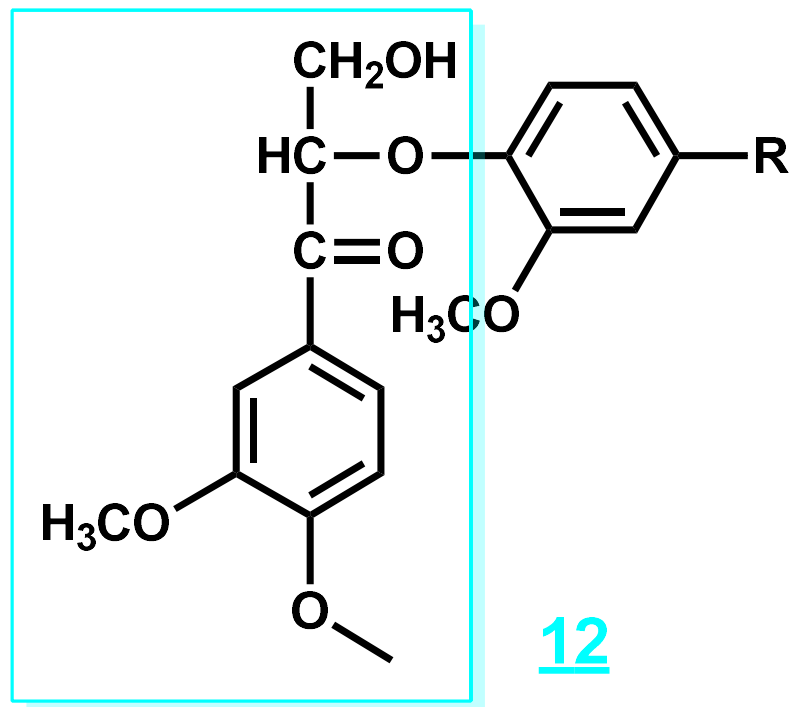


β -6 and α -6 structures



β -6 in birch lignin 0.02/ C_6C_3 and in spruce lignin 0.03/ C_6C_3

1-Aryl-2-aryloxy-1-propanone units



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Small amounts.

Occurrence of arylketone units both in softwood and hardwood lignins (NMR).

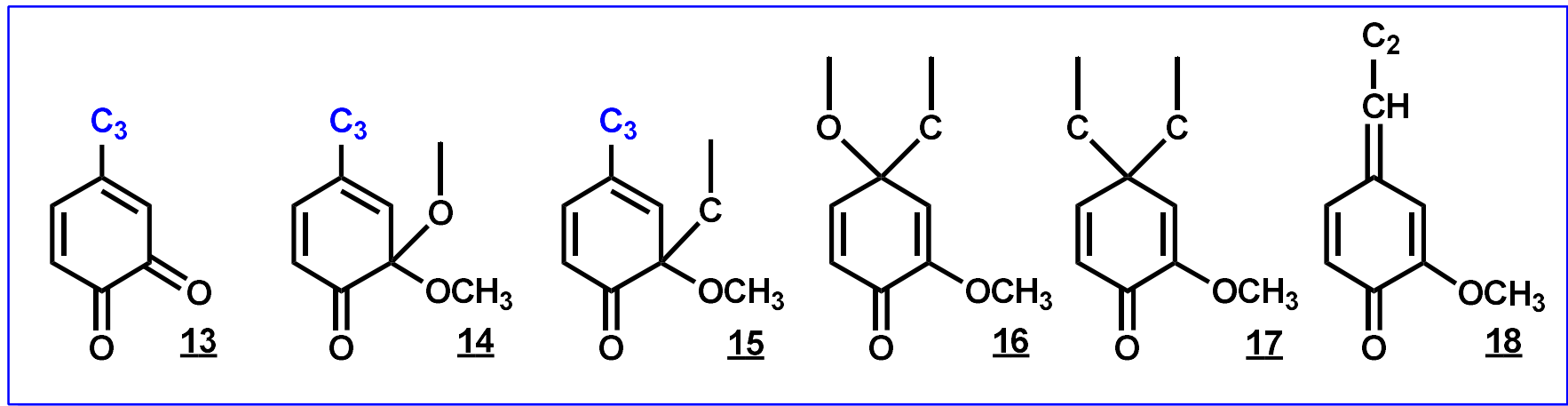
More frequent in hardwood lignin

Cyclohexadienone units

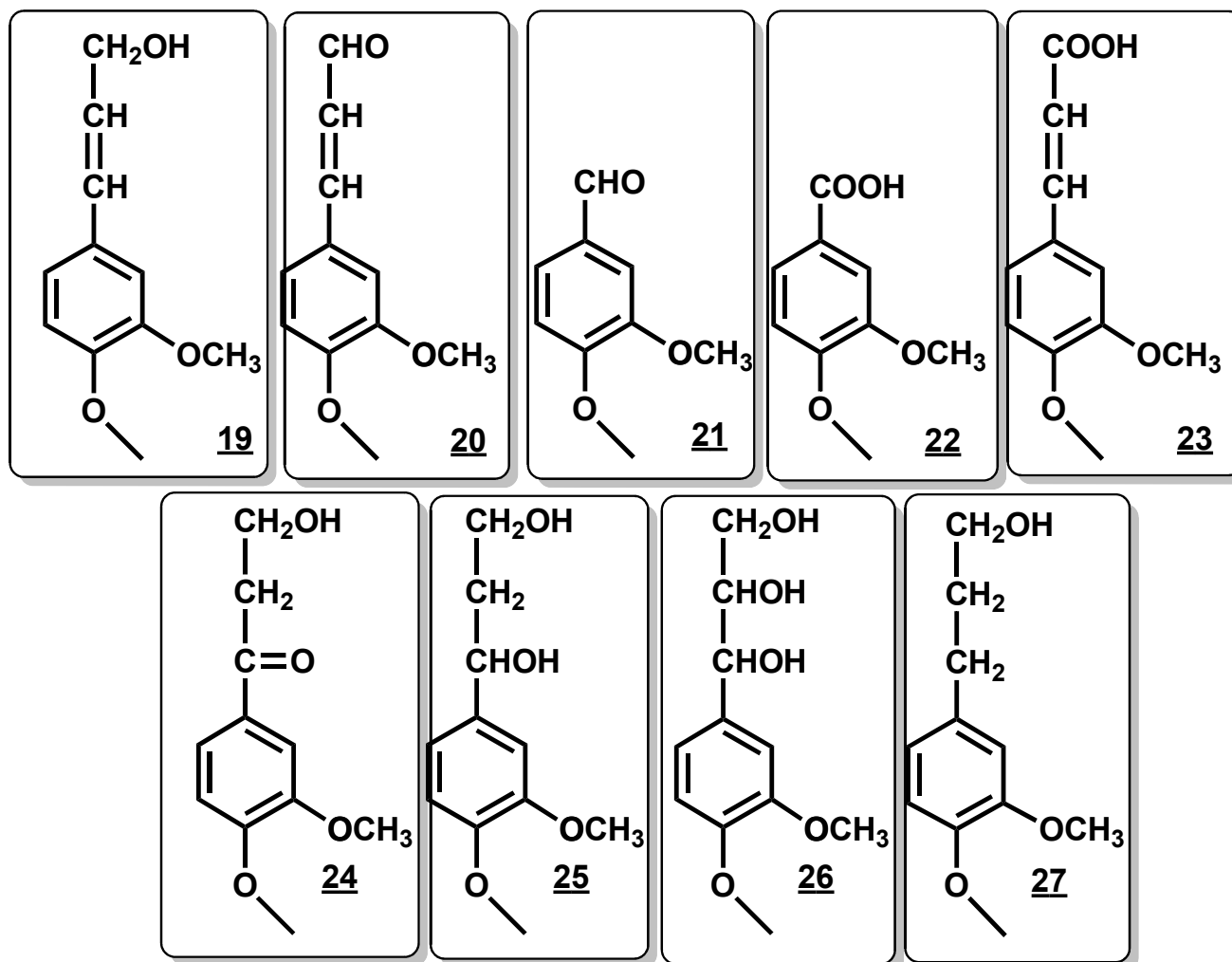
Phenol oxidation often results in the formation of cyclohexadienones and quinones.

Most of them carry a methoxyl group (14-18).

1-2% quinoid units in softwood lignin



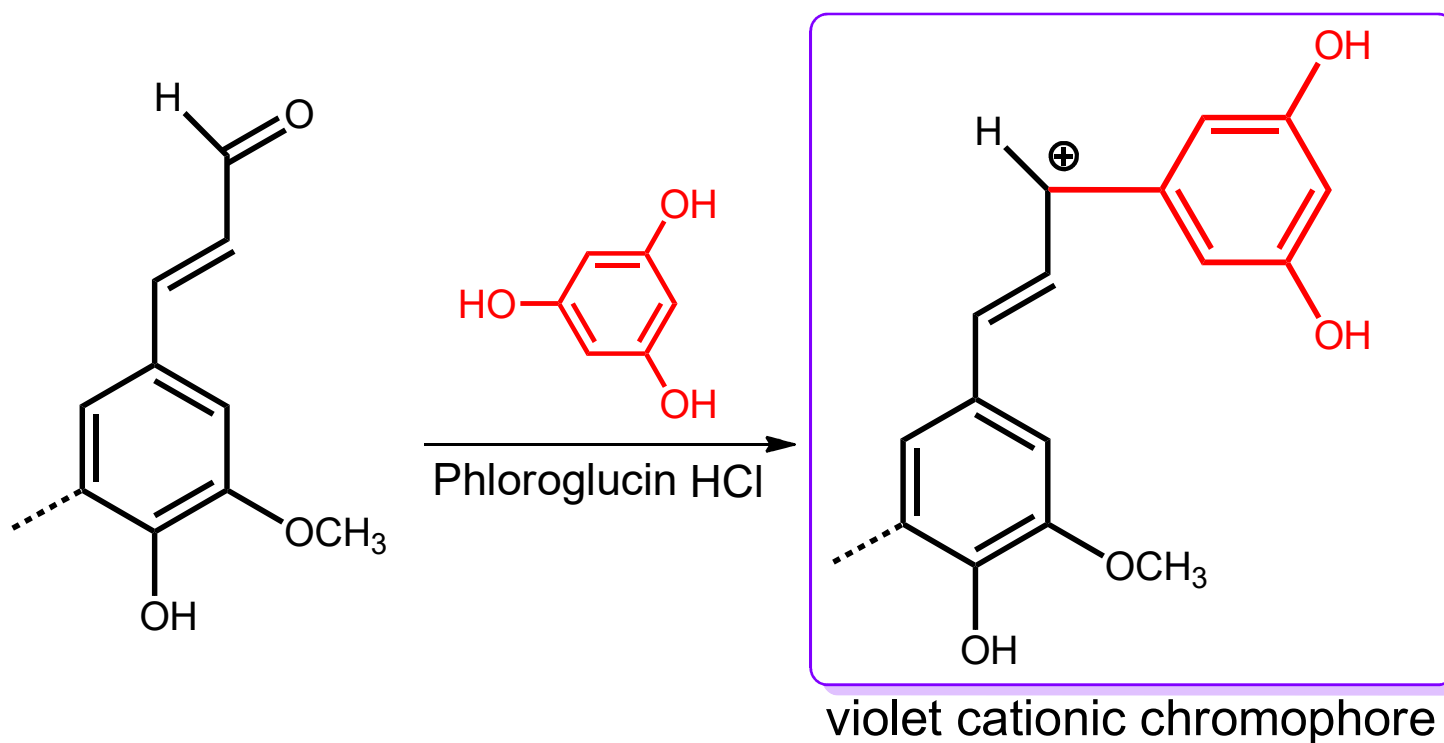
End-groups in lignin



- Cinnamyl alcohol (19)
- Cinnamaldehyde (20)
- Vanillin (21)
- 3-(4-hydroxy-3-methoxyphenyl)acrylic acid (23)
- Arylglycerol (26)
- Dihydroconiferyl-alcohol (27)

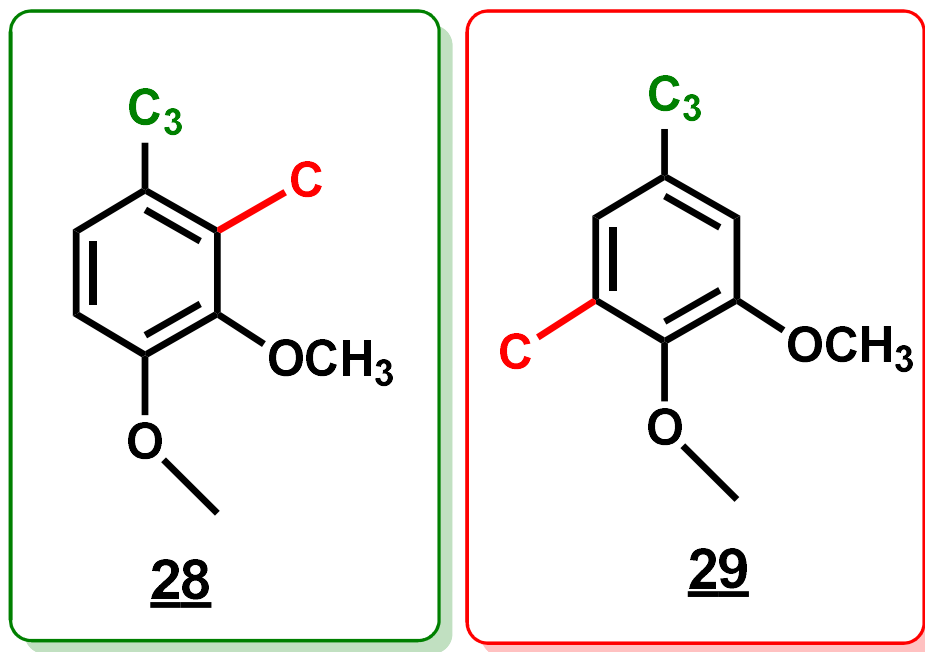
Color reaction of coniferyl aldehyde end groups

Wiesner reaction



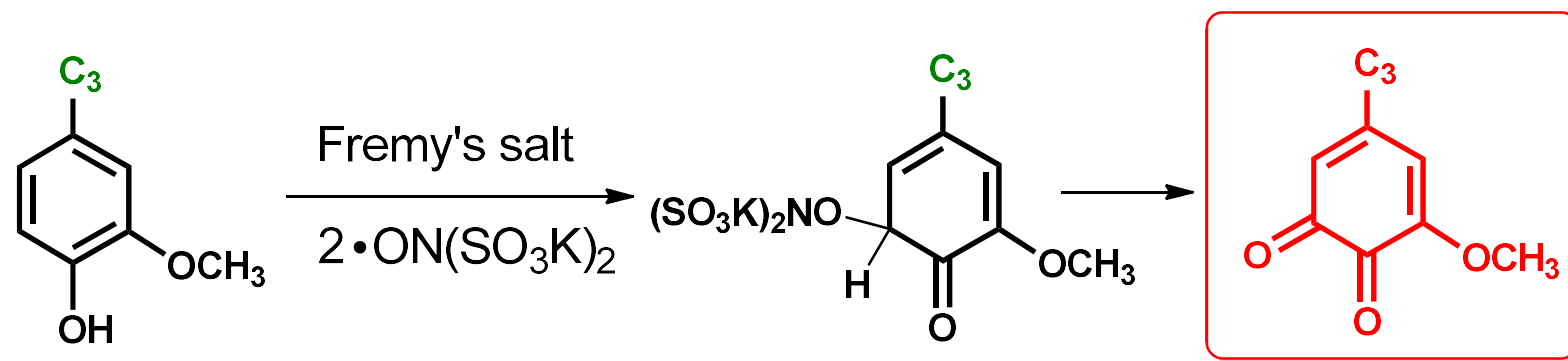
2.0-2.5 % coniferyl aldehyde end groups in spruce lignin,
and 3-4% in Western hemlock

Alternative condensed structures



Lignin units in which the aromatic ring is attached to an adjacent condensed unit (C-atom)

Determination of condensed units



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Oxidation of non-condensed guaiacyl unit at C-5 to o-chinones with **nitroso-disulphonate**.

The **ortho-chinone** can be quantitatively determined by means of spectrophotometry. Typically 50-60% of the phenolic units are uncondensed.

Distribution of condensed lignin units

Type of unit	Structures	SW Lignin	HW lignin
Biphenyl units, dibenzodioxocin	<u>3, 4</u>	23	9
Diarylether	<u>5</u>	4	7
Phenylcoumaran structure	<u>6</u>	10	4
β -6, α -6 units	<u>10,11</u>	3	2
Additional types of lignin	<u>28,29</u>	5	1
TOTAL	-	45	23

Functional groups (summary)

- Carbonyl groups: present in the structures 9, 12, 20, 21, 24
- Carboxyl groups: in 22, 23 (might be produced by isolation)
- Ester groups: units esterified with p-hydroxybenzoic acid (grass lignin).
- Olefinic groups: in 19, 20, 23; 0.05 – 0.1 ethylenic groups per phenylpropane unit in SW lignin.
- Hydroxyl groups: total number of OH in MWLs is 1.3 -1.5 (1.5) per phenylpropane units in SW (HW).
- Methoxyl groups: present in guaiacyl- and syringyl units (Zeisel procedure, ^{13}C NMR)

Percentage distribution of lignin units

Type of Unit	Structure	SW Lignin	HW Lignin
β -O-4 (erythro and threo)	<u>1</u>	35	45
Noncyclic benzyl aryl ether	<u>2</u>	2	4
Dibenzodioxocin	<u>4</u>	6	1
Phenylcoumaran	<u>6</u>	10	4
Pino- syringaresinol	<u>7</u>	4	8
1-aryl-2-aryloxy-1-propanone	<u>12</u>	1	2
β -1	<u>8</u>	3	5
β -6, α -6	<u>10, 11</u>	3	2
Units attached to α -6	<u>11</u>	2	1
Quinoid structures	<u>13</u>	2	2
Cyclohexadienone units	<u>14 - 18</u>	3	4
Cinnamyl alcohol	<u>19</u>	2	2
Cinnamyl aldehyde	<u>20</u>	4	2
Vanillin type structures	<u>21</u>	3	1
COOH containing units	<u>22,23</u>	4	4
Propane-1-one, propan-1,3-diol	<u>24,25</u>	1	2
Arylglycerol units	<u>26</u>	2	3
Dihydroconiferyl alcohol	<u>27</u>	1	0
Glyceraldehyde 2-aryl ether	<u>9</u>	2	2
Units attached to carbohydrates	-	1	2
Additional lignin units (by difference)	-	9	4
TOTAL	-	100	100

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Lignin-Carbohydrate Complexes (LCC)

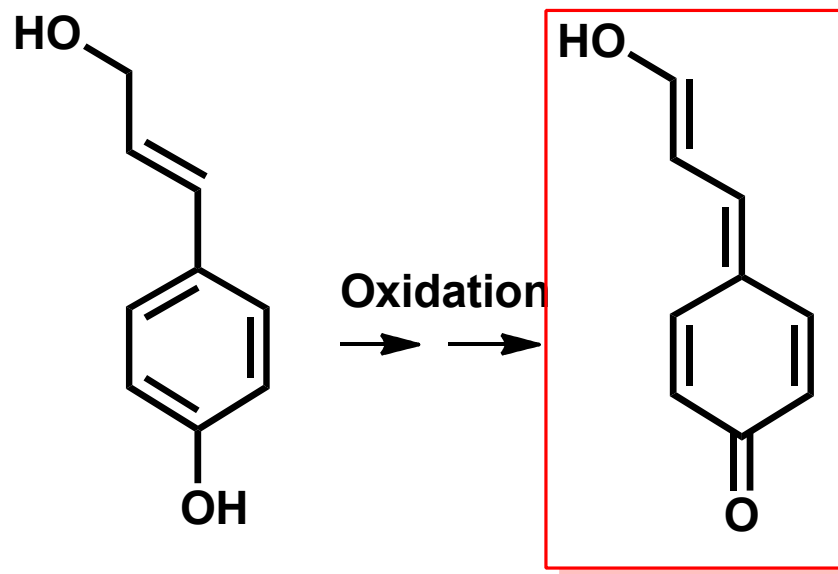
MWL preparation results in the isolation of lignin.

They contain lignin and carbohydrates which cannot be separated by solvent extraction or by chromatography (Björkman)

No final scientific proof of the existence of covalently bonded LCCs (their formation during the preparation cannot be excluded).

Biosynthesis of LCCs

Addition of nucleophiles to quinone methids, formed as intermediates in the oxidation of p-hydroxycinnamyl alcohols



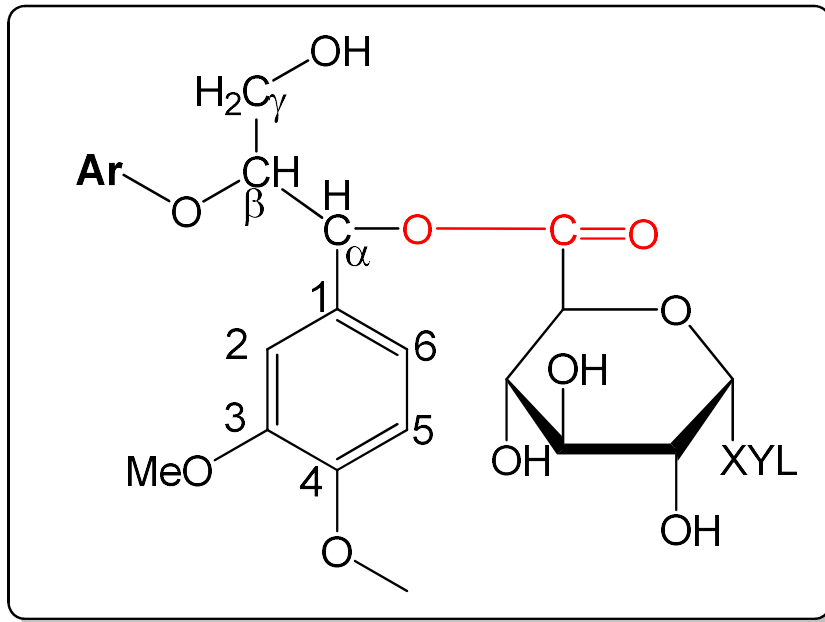
Isolation of LCCs

1. Milled wood lignin preparation: extraction of the **ball-milled wood with dioxane:water = 96:4**
2. Liquid-liquid extraction (pyridine-acetic acid-water and chloroform) instead of precipitation of the lignin in water from an acetic acid solution.
3. LCCs without "free" carbohydrates.

Types of LCC bonds

1. Ester bonds

Predominant in hardwood: birch released the major part of xylan: 4OMeGlcA units esterified with lignin, while from spruce lignin only 50% of carbohydrates (mainly xylan)

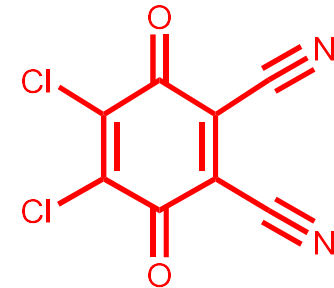
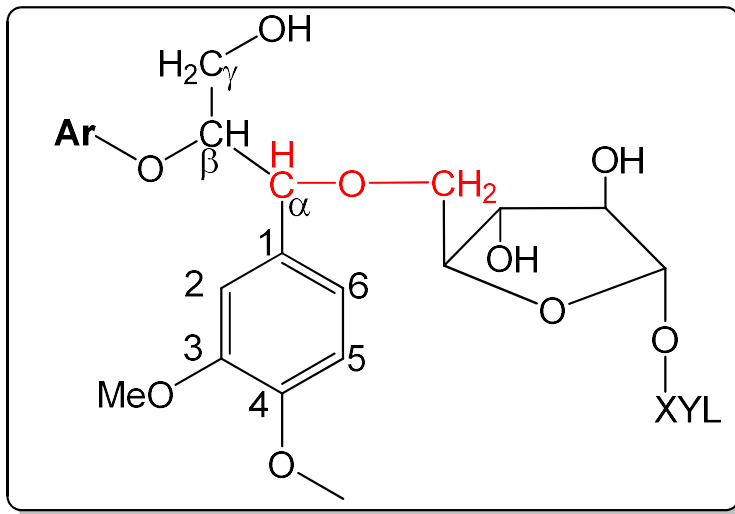


Types of LCC bonds

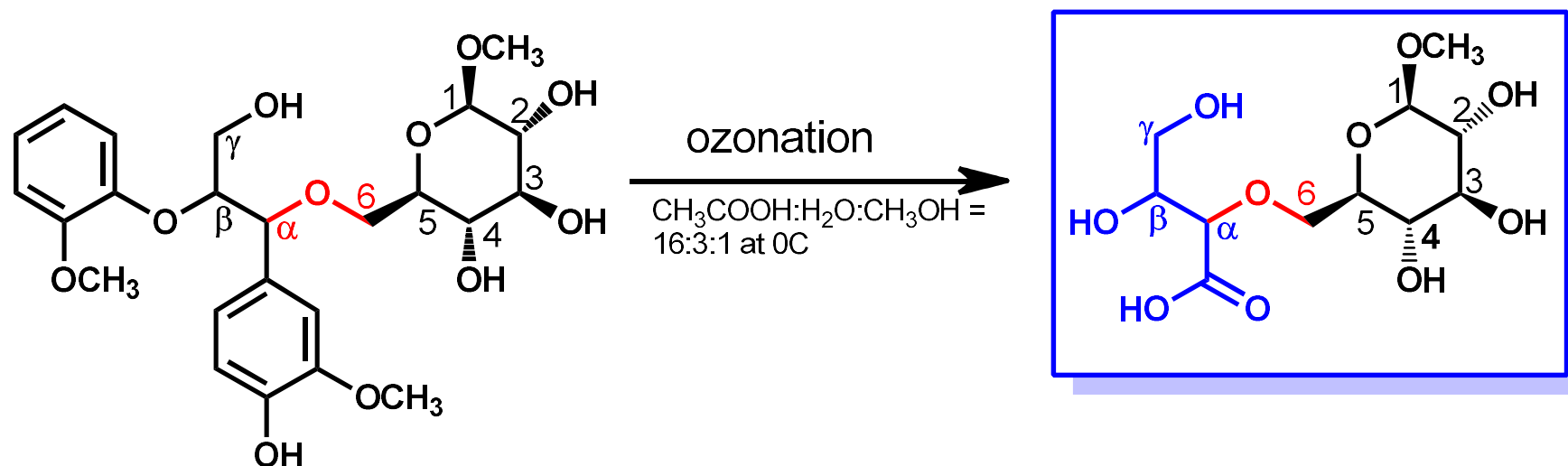
2. Alkali-stable bonds:

Benzyl ether bonds: Can be selectively cleaved by DDQ (dichlorodicyano-benzochinone). Not quantitative.

Quinone methide react with the OH groups of the carbohydrates.
Spruce lignin contains a large portion of alkali stable linkages-> ether bonds to arabinose units.



Ozonization of benzyl ether type model compound



Presence of benzylic ether type LCCs proven by ozonation treatment.

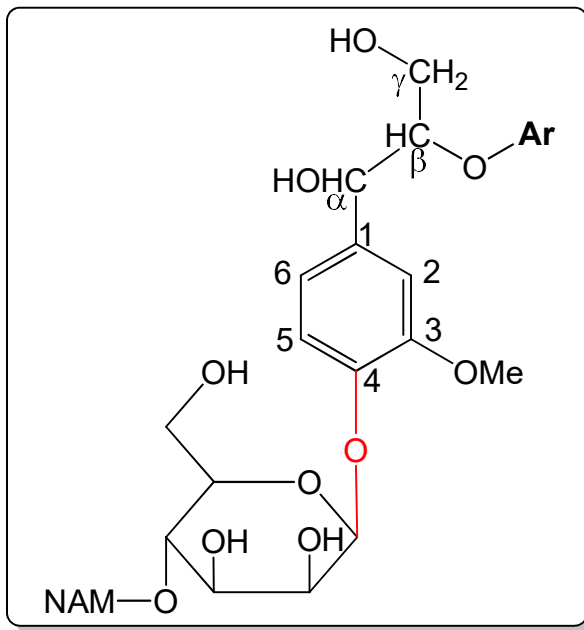
The α -benzylic ether bond was rather stable during ozonation.

The ether linkage between C_α and C_6 confirmed by HMBC, HSQC NMR

Types of LCC bonds

3. Phenyl glycosidic linkage:

Glycosidic bonds between lignin and carbohydrate moieties are discussed



LCCs

- 90% of the residual lignin in unbleached pulp and ~100% of the residual lignin in O-delignified pulp are covalently bound to carbohydrates.
- Wood LCCs rich in (G)GM have higher content in β -O-4, G-L fraction shows lowest β -O-4 content.
- After kraft cooking only three different LCCs, after O only one LCC could be isolated

Lawoko et al. **Holzforschung**. **2004**, 58, 603-610

Lawoko M., G. Henriksson, G.Gellersted. 2005, **Biomacromolecules**, 5, 3467-3473

Henriksson, G. et al. **Holzforschung**, 2007, 61,668-674.

Choi,J-W., Choi, D-H., O.Faix. **J.Wood.Sci.** 2007, 53, 309-313.

Gellerstedt,G et al.. **Biologies**. **2004**, 327, 817-826: occurrence of condensation between fibre lignin and dissolved lignin fragments in a one-electron transfer reaction.

Fractionation Scheme of *Spruce*-LCCs

Wood

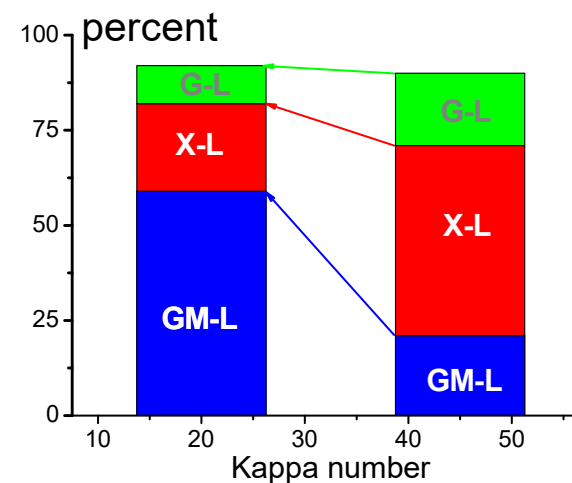
GGM-L-P _p	°8
GGM-L-X _p	20
X-L-GM _s	15
G-L _p	4
GM-L-X _p	28
X-L-GM _s	25

Unbl Pulp

G-L _p	12
GM-L-X _p	45
X-L-GM _s	27

O-Delignified

GM-L-X _p	80
---------------------	----



GGM=Galactoglucomannan, X=xylan, G=glucan; P=pectin, p=precipitate, s=soluble. °lignin yield, %

Learning outcome

1. Introduction
2. Occurrence
3. Biosynthesis
4. Isolation
5. Chemistry of lignin units
6. Lignin-carbohydrate complexes (LCCs)
- 7. Determining lignin structure by chemical degradation**
8. Spectroscopy of lignins

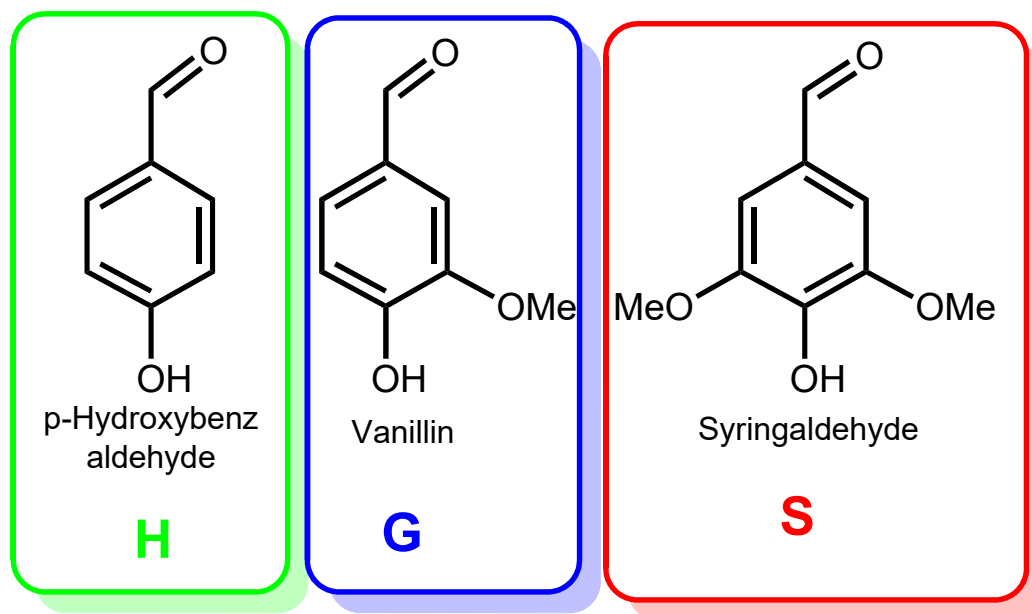
Chemical Degradation Methods

- Oxidative
 - Nitrobenzene oxidation
 - Permanganate oxidation
 - Ozonation
- Thioacidolysis
- Derivatization followed by reductive cleavage (DFRC)

Nitrobenzene oxidation (NBO)

Nitrobenzene, 2 M NaOH, 160-180°C, 2-3h.

- Degradation of C_6C_3 units into C_6C_1 monomers and dimers.
- Both $2e^-$ and $1e^-$ transfer mechanisms coexist.



Introduced by Freudenberg more than 50 a ago.

Nitrobenzene oxidation (NBO)

- **NBO provides S/G ratios** (syringaldehyde-to-vanillin). S/G higher than the actual proportions because S-units are less involved in condensed interunit bonds.
- NBO yields small amounts of **5-carboxyvanillin**, **5-formylvanillin** and dehydrovanillin from C-5 condensed guaiacyl units.

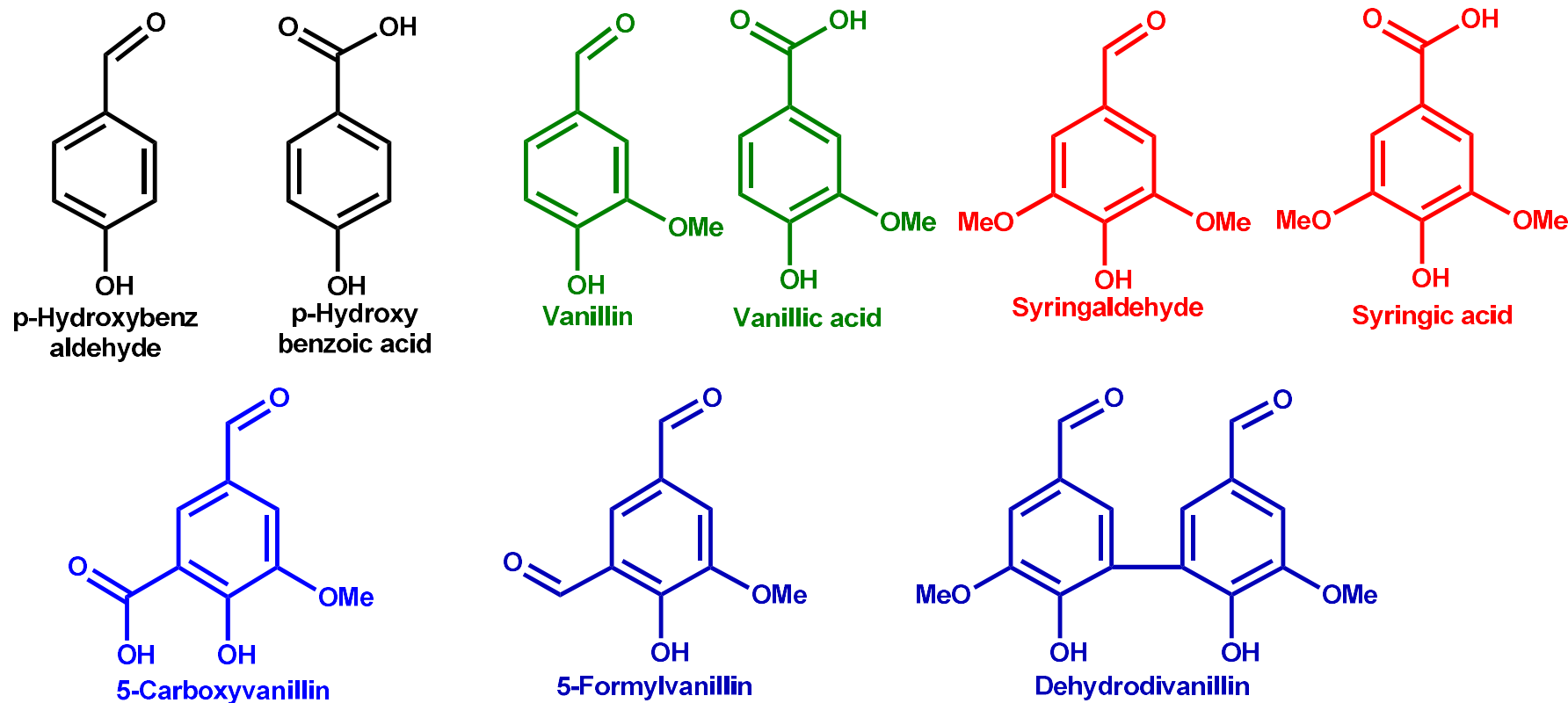
Introduced by Freudenberg more than 50 years ago.

Nitrobenzene oxidation (NBO)

- Lack of specificity: Nonlignin phenolics capable of generating the same benzaldehydes as lignin units.
- Grass lignin: most of the released hydroxybenzaldehyde (H) originates from p-coumaric esters.

Introduced by Freudenberg more than 50 years ago.

Main products recovered from NBO of lignin structures

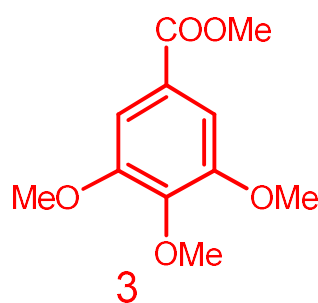
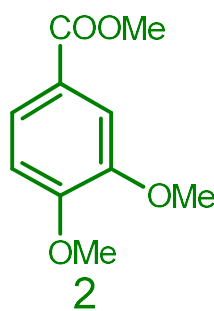
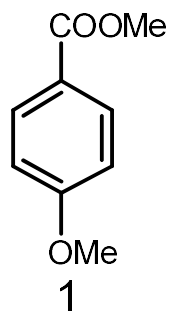


TMS derivatives of oxidation products are analyzed by GCD.
Quantification using dimethylphthalate as internal standard.

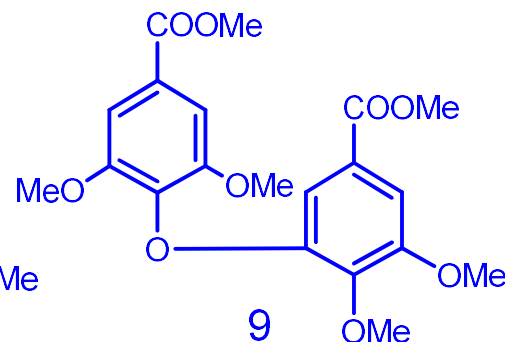
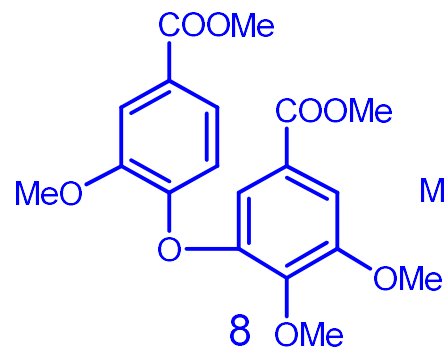
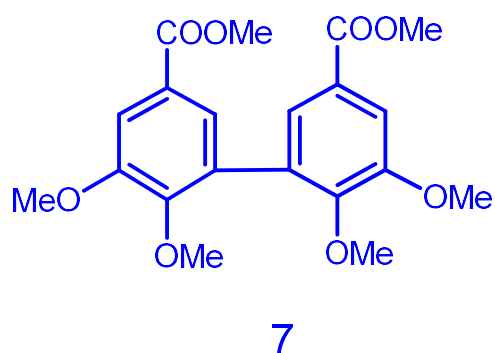
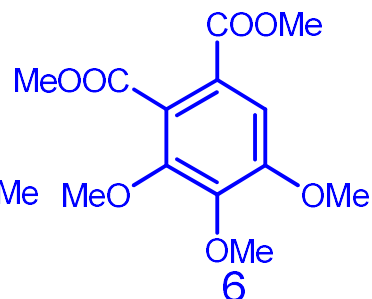
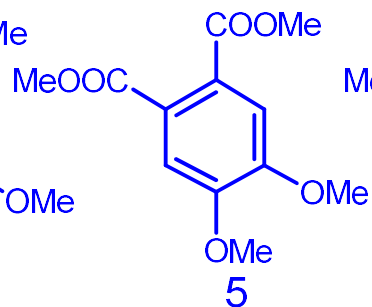
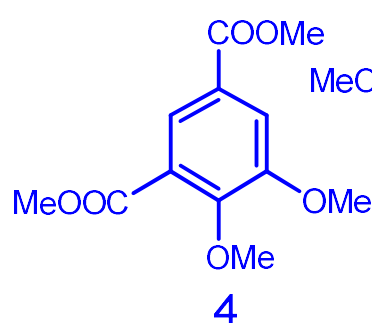
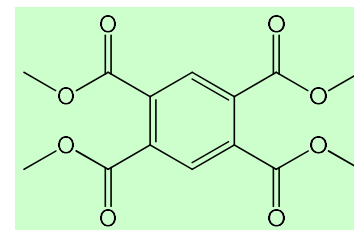
Permanganate Oxidation

- **Phenolic phenylpropane units only** (25-35% of total units) determined as acid methyl esters: G, S, α -5, condensed G and S, biphenyl, diralyether.
- **Initial permethylation** of the phenol-OH (dimethylsulfate), followed by 2 sequential oxidation steps with **KMnO₄** and **H₂O₂**, to yield mono- and dicarboxylic acids.
- These acids are methylated with diazomethane to obtain the methyl esters for quantification by GC.

Carboxylic acid methyl esters formed by permanganate oxidation



Analyzed by GC.
internal standard for
quantification is
pyromellitic acid
tetramethylester.

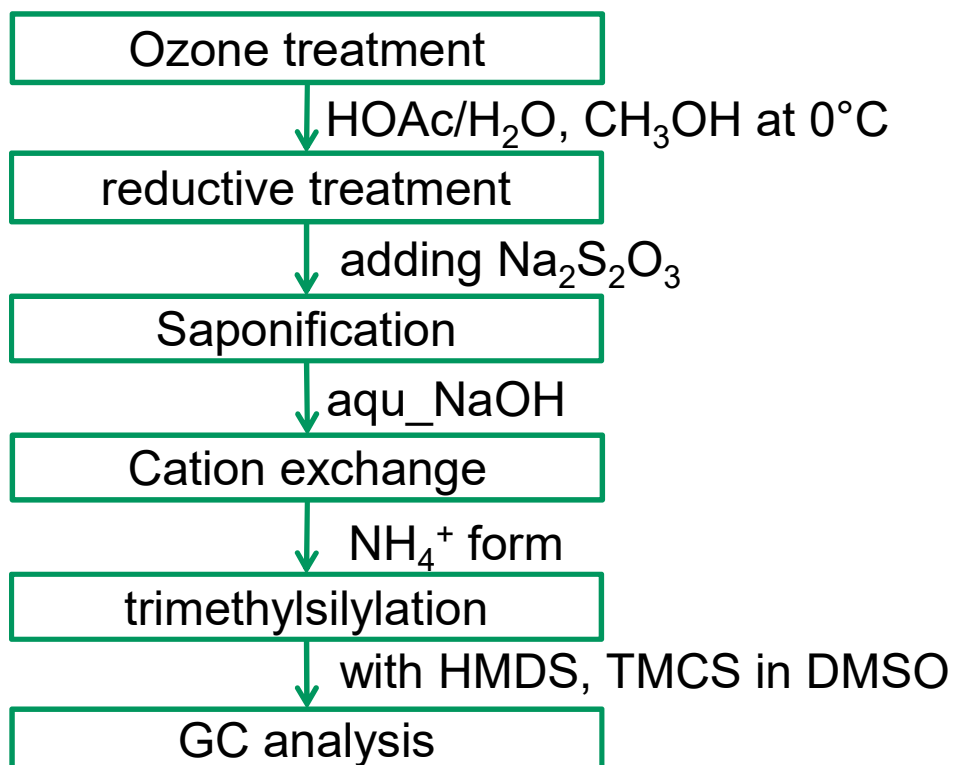


Permanganate Oxidation

- Non condensed units: monocarboxylic esters
- **Condensed units: dicarboxylic esters derive from C-5 or C-6 condensed units → Degree of condensation**
- Yield in aromatic carboxylic acids has been used to evaluate the frequency of free phenolic groups in lignins.

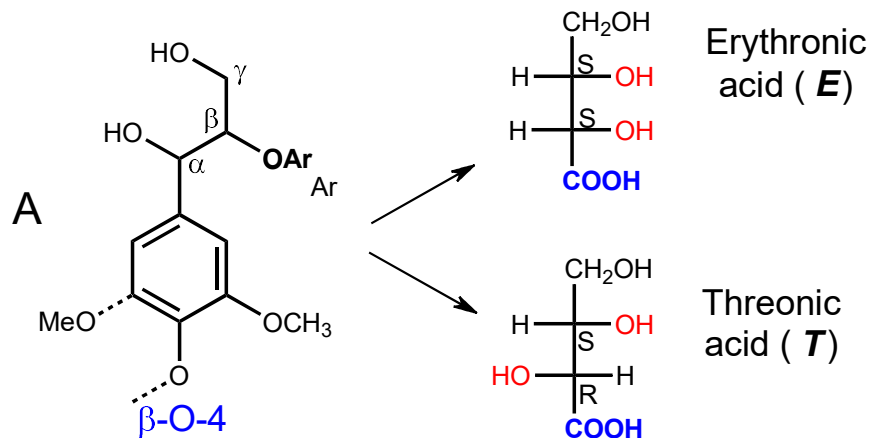
Ozonation

- Degradative method exclusively targeting at side chains: side chains preserved, **aromatic rings appear as COOH groups**.
- Side chain stereochemistry preserved: *erythro* (E)/*threo* (T)

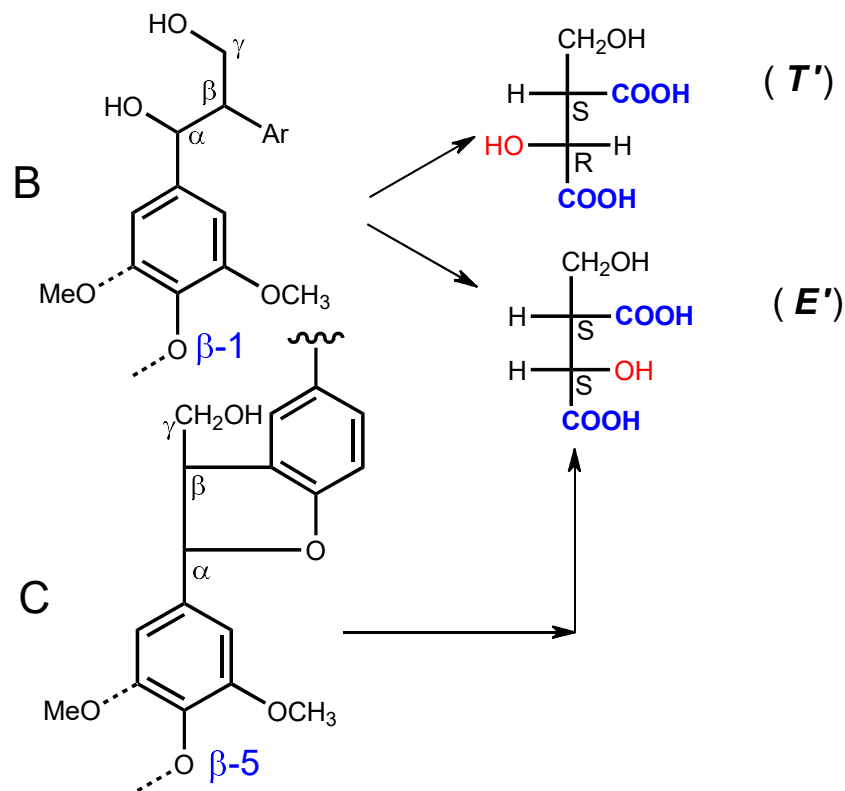


T. Akiyama et al. *J. Wood Sci* 48:210-215, **2002**

Ozonation



- *Erythro* and *threo* diastereoisomers of **arylglycerol- β -aryl** ether give rise to erythronic and threonic acids (*E* and *T*).



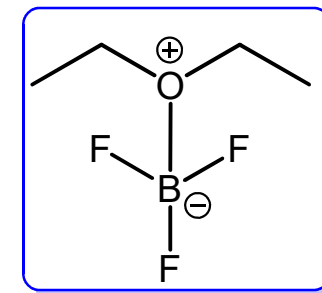
- **β -1** diarylpropane structures $\rightarrow$ *erythro* (*E'*) and *threo* (*T'*) isomers of 3-hydroxy-2-hydroxymethyl butanedioic acids.
- **β -5** phenylcoumaran, which occurs in the *erythro* configuration, only provides the *E'* isomer.

T. Akiyama et al. *J. Wood Sci* 48:210-215, **2002**

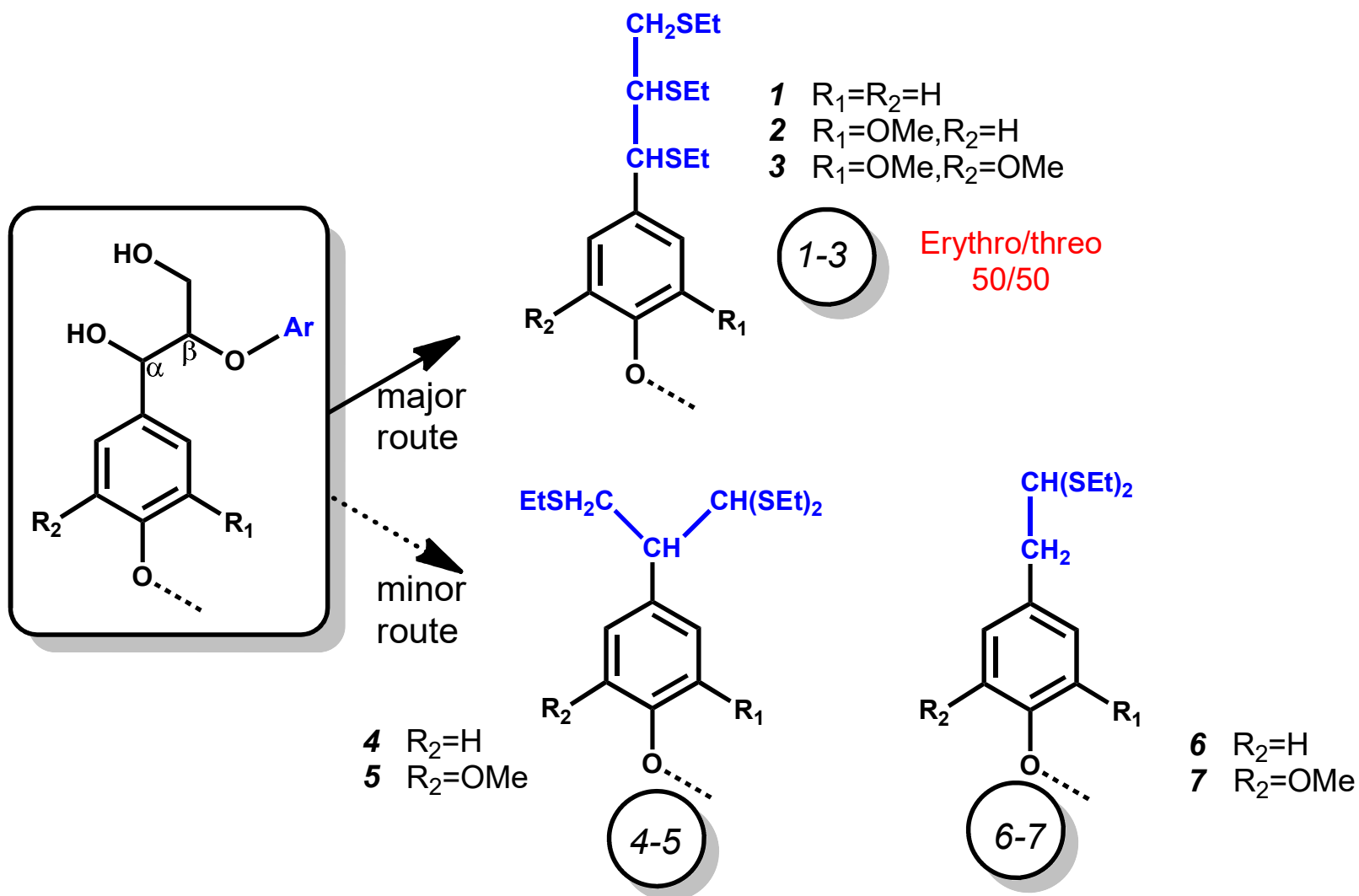
H. Aimi et al. *J. Wood Sci* 51:252-255, **2005**

Thioacidolysis

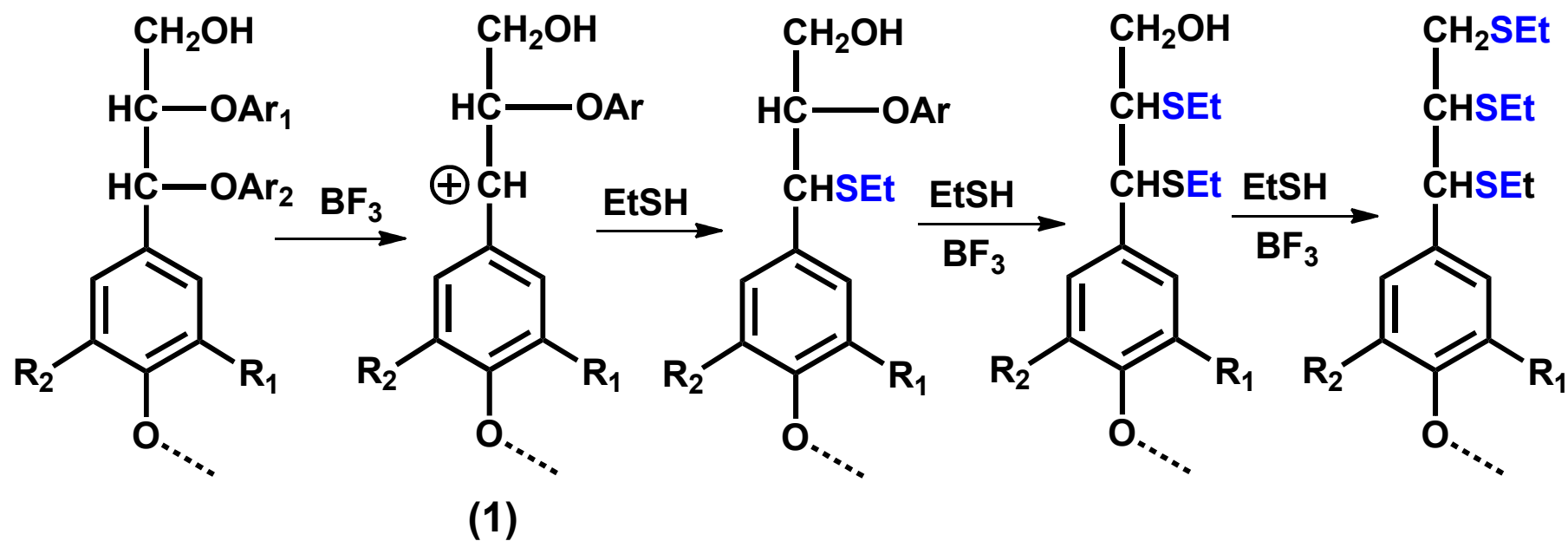
- Acid-catalysed reaction that results in β -O-4 cleavage:
4 h, 100°C, dioxane/EtSH (ethanethiol) 9/1 mixture with
0.2 M BF_3 etherate (Lewis acid).
- Gives rise to C_6C_3 products → information about side chains and interunit linkages.
- Free phenolic-OH in β -O-4 units evaluated if permethylation step prior to thioacidolysis.
- Post-desulfurization obtains lignin-derived dimers, representative of lignin resistant bonding patterns.



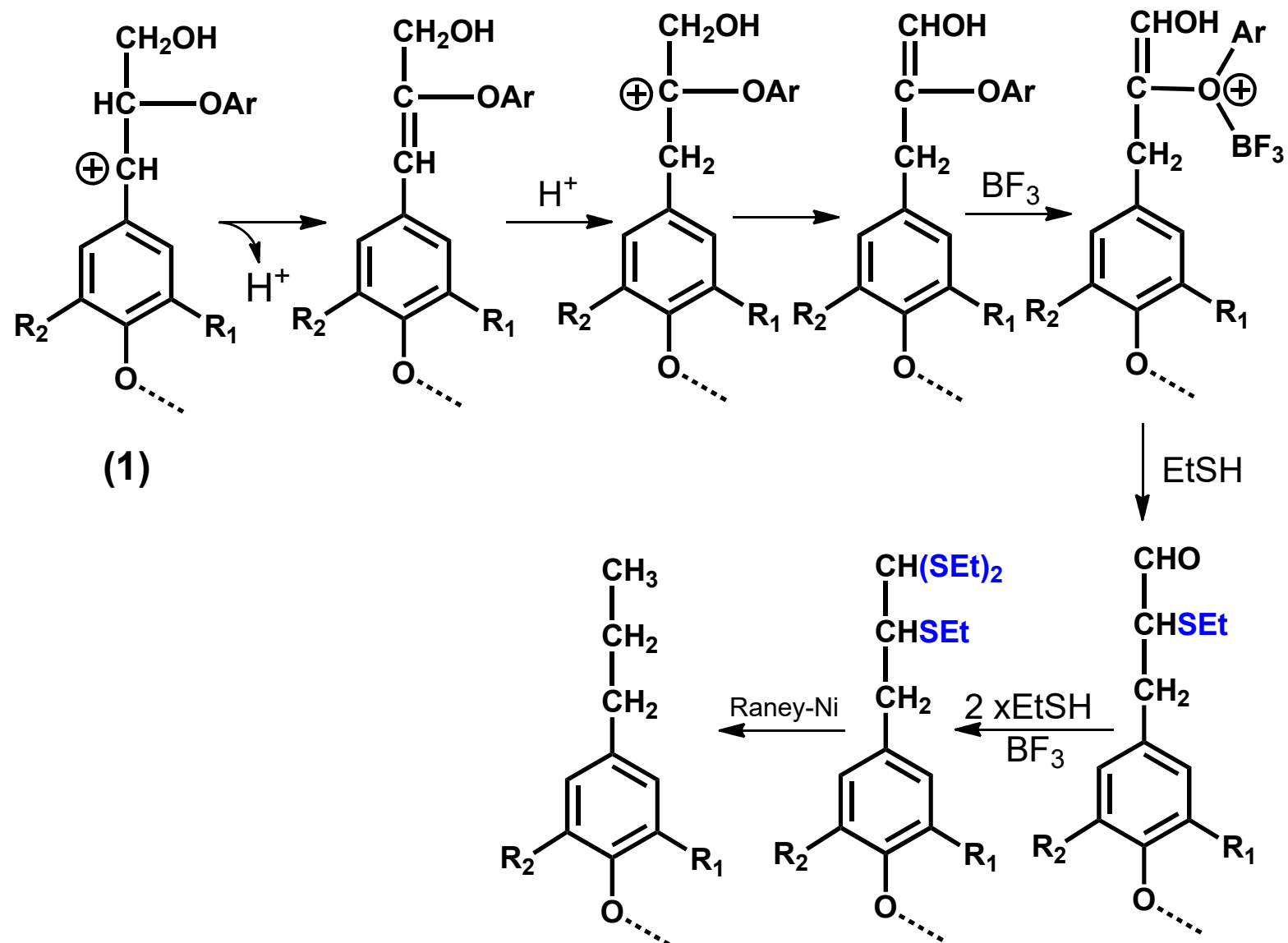
Thioethylated monomers recovered from thioacidolysis



Major Route



Minor Route



Lignin-derived monomers

- Efficiency of β -O-4 cleavage under standard conditions about 75-85% yield (model compounds)
- Cleavage of β -O-4 bonds, combining the hard Lewis acid, **boron trifluoride etherate**, with a soft nucleophile **ethanethiol**.
- **1-3**: lignin units involved in arylglycerol- β -aryl ether
- **6-7**: originate from vinyl ether structures (Ar-CH=CH-OAr)

Yield of thioacidolysis monomers

Monomer [μmol/g L] [R=SEt]	Poplar Wood Lignin	Spruce Wood Lignin
Ar-CHR-CHR-CH ₂ R H/G/S	2310 -/41/59	1260 2/98/-
Ar-CH ₂ -CHR ₂	155	85
Ar-CH=CH-CH ₂ R + Ar- CHR-CH ₂ -CH ₂ R	167	78
G-CHR-CH ₂ -CHR ₂	8	18
TOTAL	2640	1441

In **spruce only 32%** of lignin units are involved in β-O-4 bonds, whereas in **poplar about 60%** of lignin units are involved in β-O-4 bonds*.

*thioacidolysis yield is 80%, average molar mass of the C₆C₃ unit is 180 for SW and 200 for HW

Lignin-derived monomers

Lignin Sample	Total Yield	H/G/S	Mol % of parent structures
	mmoles/g KL		
Poplar <i>Populus</i>	2310	37%G, 63%S	58
<i>Fagus silvatica</i>	2400	29%G, 71%S	60
<i>Eucalyptus urophylla</i>	2450	26%G, 74%S	61
<i>Betula verrucosa</i>	2490	22%G, 78%S	62
<i>Picea abies</i>	1246	0.4%H, 99.6% G	28
Wheat straw, <i>Triticum aestivum</i>	1190	4.6%H, 43.2%G, 52.2%S	30
Corn, <i>Zea mays</i>	900	2.1%H, 36.5%G, 61.4%S	23

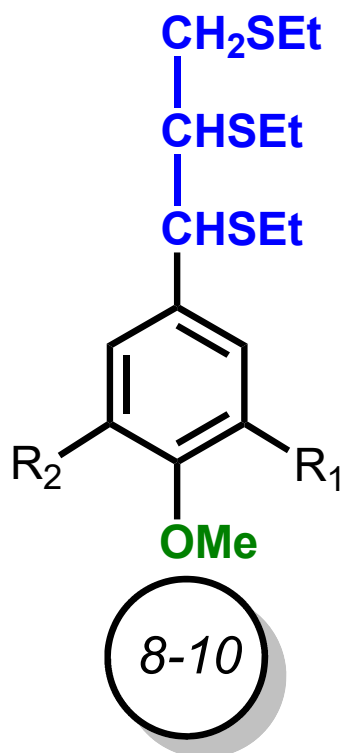
Grass lignins show low yield, indicative of high condensation degree.

Acylation of sinapyl alcohol in maize lignin alters its tendency to be involved in β -O-4 bonds: same frequency of S-units as HW lignin, but much lower thioacidolysis yield.

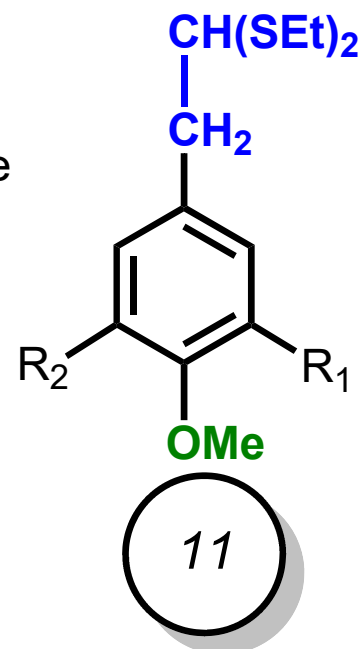
Free phenolic units in lignins

Thioacidolysis performed on CH_2N_2 -methylated samples.

The relative frequency of monomers **8–10** indicates the proportion of free phenolic groups within β -O-4 linked H, G, or S units.



- 8** R₁=R₂=H
- 9** R₁=OMe, R₂=H
- 10** R₁=OMe, R₂=OMe



Free phenolic units in lignins

Lignin sample	% Free Phenolic Groups in			
	H :100x 8/[8+1]	G :100x 9/[9+2]	S :100x 10/[10+3]	H:G:S
				mol%
Spruce, <i>Picea abies</i>	90	25	--	2:98:0
Pine, <i>Pinus maritima</i>	--	23	--	1:99:0
Poplar, <i>p.euramericana</i>	--	25	3,5	0:33:67
Wheat straw, <i>Triticum aestivum</i>	92	43	5	5:49:46
Corn stem, <i>Zea mays</i>	91	50	5	4:35:61

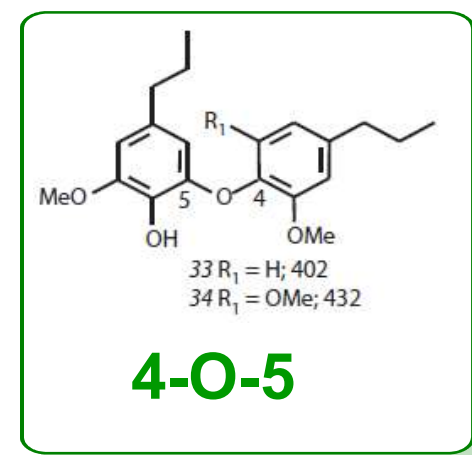
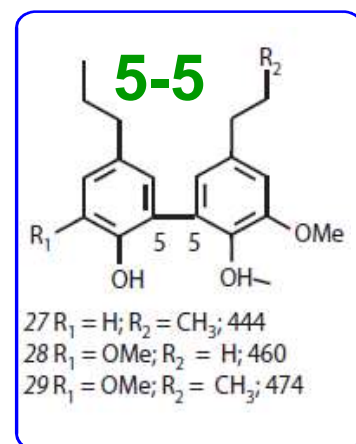
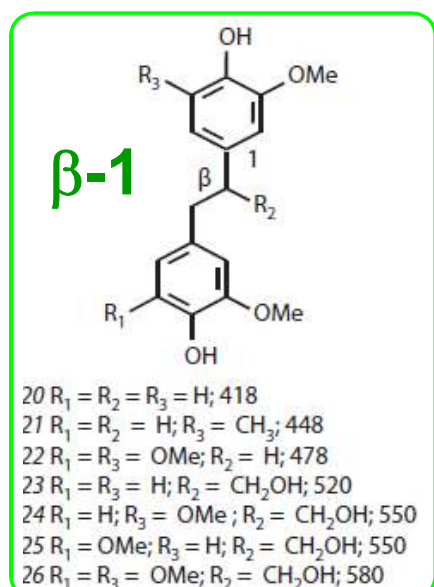
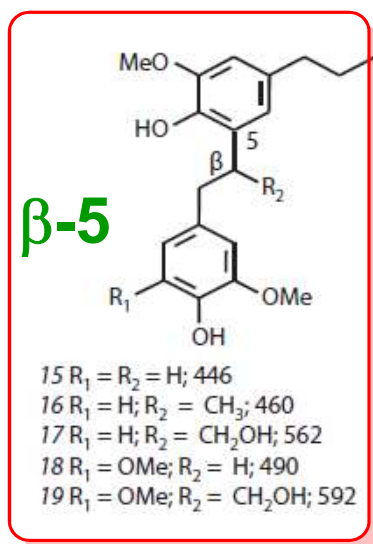
- H,G,S units not evenly distributed in native lignin.
- More than 90% of H units are terminal units with free phenolic groups.
- Intermediate behavior of G-units.
- S units are internal units with etherified phenolic groups.

Free phenolic units in lignins

- Thioacidolysis of permethylated samples revealed that **grass lignins** have a **twofold higher level of free phenolic G units** as compared with wood lignins .
- Reflects higher content in branching structures and/or smaller lignin domains easily extracted from the walls.
- **High frequency of free phenolic G units** in **grass lignins** is responsible for the **high alkaline solubility** (50-75% soluble in alkali at room temperature)

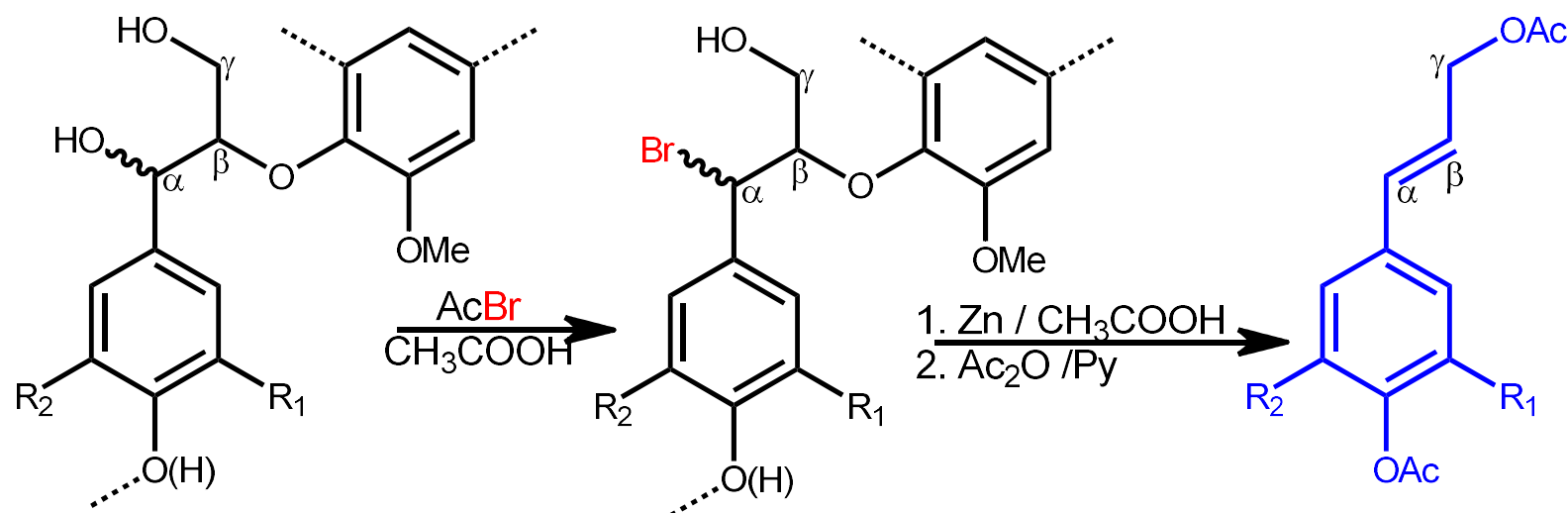
Thioacidolysis lignin-derived dimers

Analysis of lignin-derived dimers is possible after desulfurization of the thioacidolysis mixture over Raney nickel under mild conditions (in dioxane, 50°C, 4 h) to reduce their molar mass and isomeric forms.



GC/MS quantification of condensed structures

Derivatization followed by reductive cleavage (DFRC)



- **Selective cleavage of β -O-4 bonds** $\rightarrow$ coumaryl (H)-, ferulic (G)-, sinapylalcohols (S).
- Lower yield, quantitative determination difficult since several DFRC products.

DRFC provides new insights in lignin chemistry

1. **γ -ester remain intact.** Confirmation that p-coumarates exclusively attached to the γ -position of grass lignin.
2. Optically inactive β -5 and β - β DRFC dimers confirmed racemic nature of lignins.
3. Occurrence of **spirodienone** structures in lignin

Learning outcome

1. Introduction
2. Occurrence
3. Biosynthesis
4. Isolation
5. Chemistry of lignin units
6. Lignin-carbohydrate complexes (LCCs)
7. Determining lignin structure by chemical degradation
- 8. Spectroscopy of lignins**

Spectroscopy of Lignin

- UV/VIS spectroscopy
- Vibrational spectroscopy
- NMR of lignins

UV/VIS spectroscopy

- Refers to absorption spectroscopy. It uses light in the ultraviolet-visible spectral region.
- In this region of the electromagnetic spectrum, molecules undergo electronic transitions:
- Molecules with π - or non-bonding e^- can absorb energy to excite these electrons to higher anti-bonding MOs.
- The lower the energy gap between HOMO and LUMO, the longer the wavelength of light it can absorb.

UV/VIS spectroscopy

$$E = h\nu$$

Planck relation

$$A = -\ln\left(\frac{I_0}{I}\right) = \varepsilon \cdot l \cdot c$$

Beer-Lambert Law

$$D = \frac{A}{cl} [lg^{-1}cm^{-1}]$$

Absorptivity

Bathochromic shift: shift to a longer wavelength

Hypsochromic shift: shift to a shorter wavelength

ε extinction coefficient

L distance through the sample

Absorption to substituted benzenes, lignin model compounds

Lignin, with its aromatic structure, is responsible for almost all the UV-visible absorption of lignocellulosic materials.

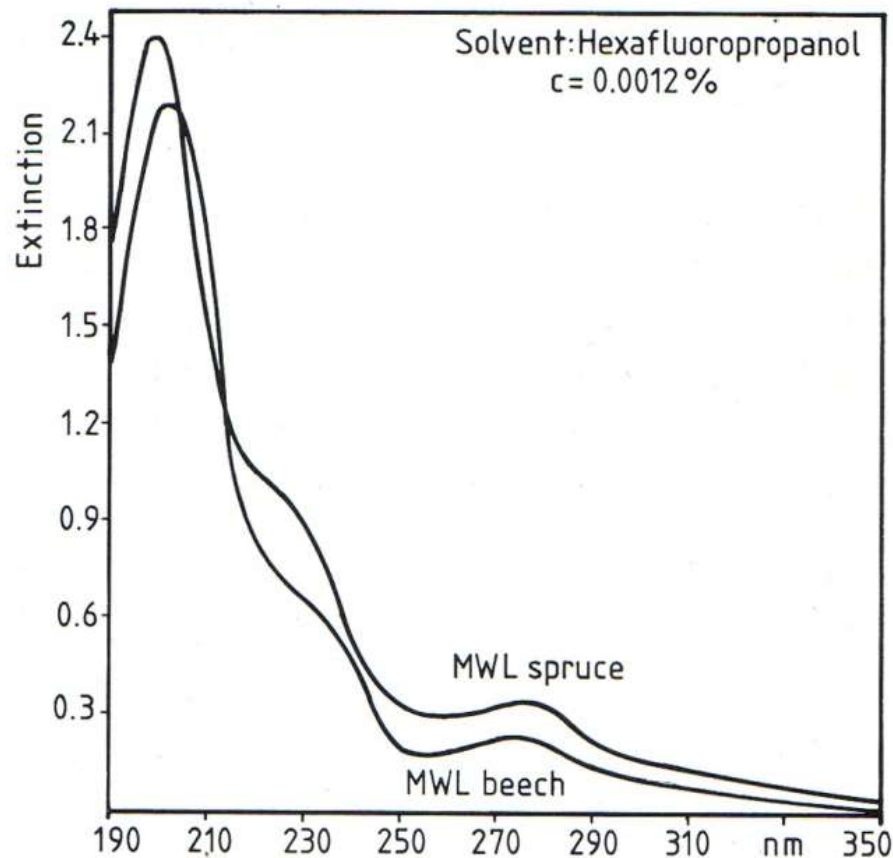
Benzene shows three bands, designated 1L_b , 1L_a , and 1B .

Red shifts through substitution on the benzene ring.

Notation	λ nm	ϵ L*mol ⁻¹ cm ⁻¹
1B	180	46 000
1L_a	203	7 400
1L_b	256	220

Substrates	1L_a		1L_b	
	$\lambda_{\max}$ nm	ϵ^*1E3 Lmol ⁻¹ cm ⁻¹	$\lambda_{\max}$ nm	ϵ^*1E3 Lmol ⁻¹ cm ⁻¹
Benzene	203	7,4	256	0,2
Benzaldehyde	249	1,1	279	
Phenol	210	6,2	270	1,5
p-Hydroxybenzaldehyde	283	16,0		
o-Hydroxybenzaldehyde	256	12,6	324	3,4
m-Hydroxybenzaldehyde	254	10,1	314	2,6

UV spectra of lignin



MWL and Kraft/Soda lignins typically determined at 280 nm (SW) and 275 nm (HW *):

ϵ (SW): 19.5 – 20.7 l.g⁻¹.cm⁻¹

ϵ (HW): 12.6 - 14.2 l.g⁻¹.cm⁻¹

Shoulder at 230 nm

Maximum at 205 nm

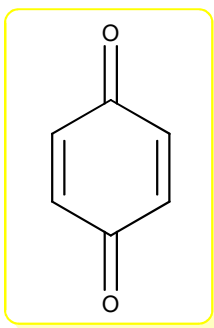
λ nm	ϵ L g ⁻¹ cm ⁻¹	Solvent
205	110	3% H ₂ SO ₄
233	35	H ₂ O
280	^o 13,3	H ₂ O, formamide

^ohardwood lignin

*higher symmetry of the lignin molecule caused by the higher amounts of syringyl units.

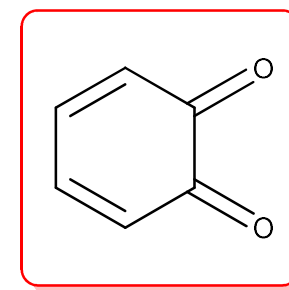
Quinones – strong chromophores

	$\lambda_{\max}$ (nm)	$\log \epsilon$	Assignment
<i>para</i> -Benzoquinones	240–300	3.8–4.5	$\pi \rightarrow \pi^*$
	285–485	2.4–3.5	$\pi \rightarrow \pi^*$
	420–460	1.2–2.3	$n \rightarrow \pi^*$
<i>ortho</i> -Benzoquinones	250–300	2.6–4.2	$\pi \rightarrow \pi^*$
	370–470	2.8–3.5	$\pi \rightarrow \pi^*$
	500–580	1.4–1.8	$n \rightarrow \pi^*$



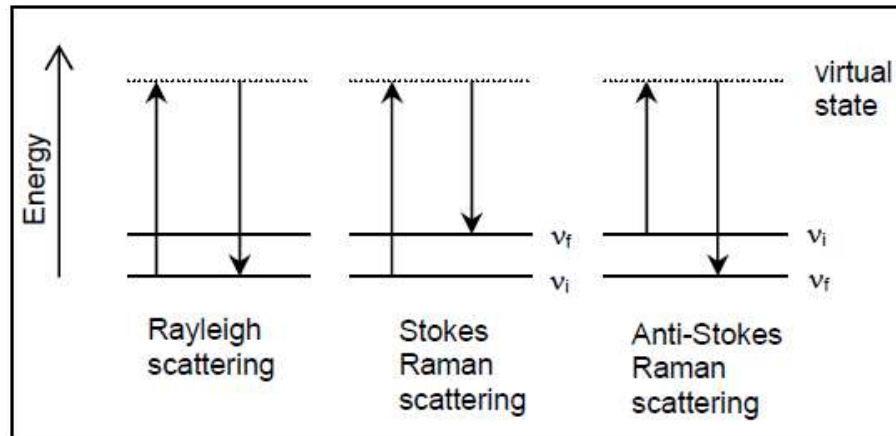
p-quinones contain a cross-conjugated π system, while *o*-quinones are linearly conjugated.

p-quinones are yellow, *o*-quinones are dark red.



Berger S. et al. *The chemistry of quinonoid compounds*, II. John Wiley and Sons, 1988

Raman spectroscopy



Energy-level diagrams of Rayleigh scattering, **Stokes Raman scattering**, and Anti-Stokes Raman scattering.

Inelastic scattering of monochromatic light (laser) in the visible or near infrared range (NIR): Only 1-to-1E7 of the scattered radiation is shifted to a different wavelength.

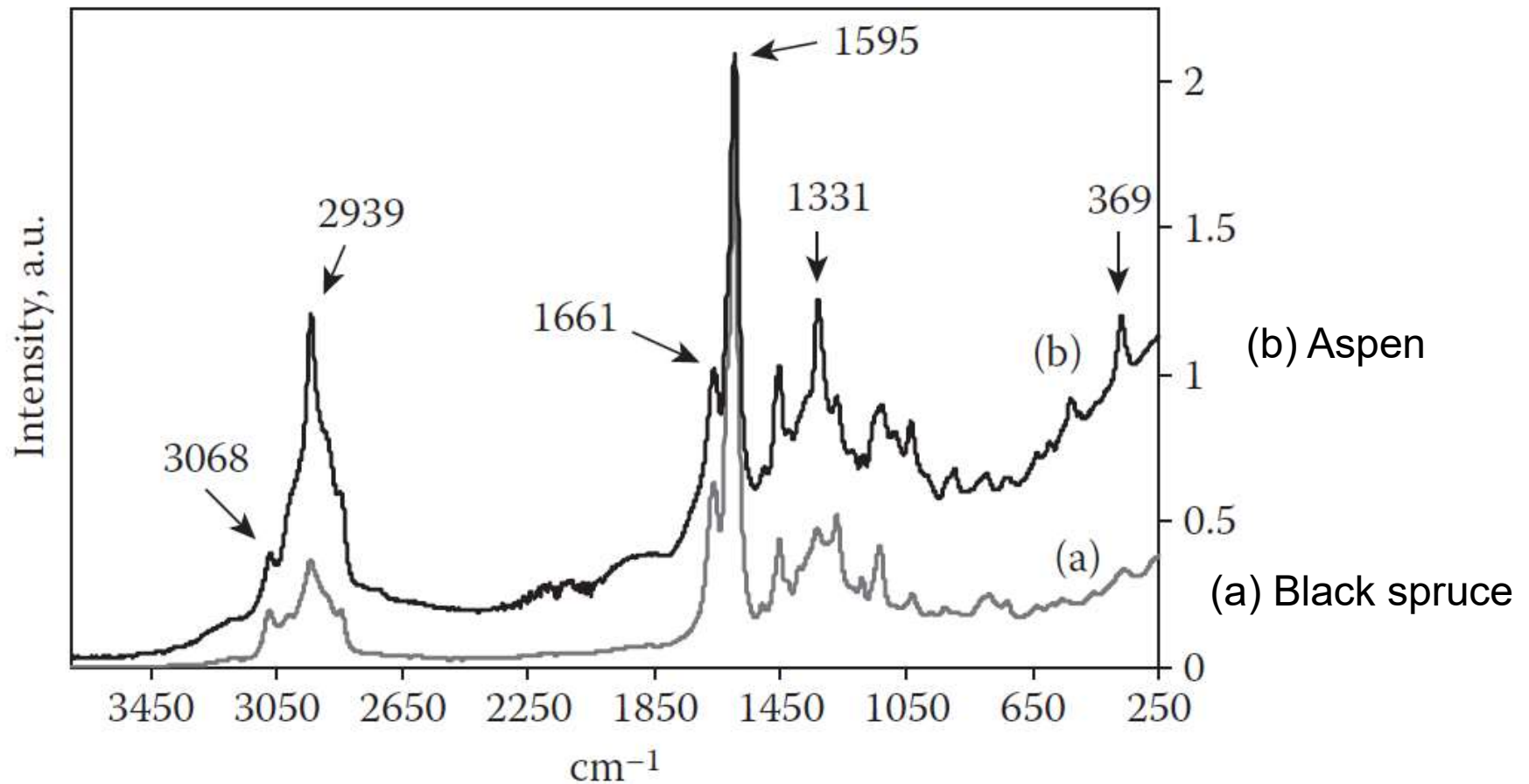
Chromophoric samples require NIR lasers (785 nm, 830 nm, 1064 nm) to avoid fluorescence.

Change in the molecular polarization potential is required for a molecule to exhibit a Raman effect.

Raman spectroscopy

- Most of the Raman scattered photons are shifted to longer wavelengths (*Stokes shift*), but a small portion to shorter wavelengths (*anti-Stokes shift*).
- **Typically, the Stokes region is used** (higher probability, thus more intense).
- Raman spectrum is a plot of the intensity of Raman scattered radiation as a function of its frequency difference from the incident radiation (cm^{-1}) → **Raman shift** independent of the frequency of the incident radiation.

Raman spectroscopy of MWLs

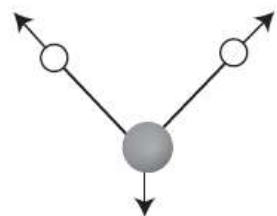


Assignment of bands in FT-Raman spectra of MWLs

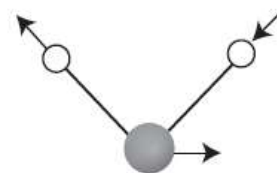
Guaiacyl (G)	G+S	Assignment
cm ⁻¹	cm ⁻¹	
3071	3068	aromatic C-H stretch
2940	2939	C-H stretch in O-CH ₃ , asymmetric
2845	2847	C-H stretch in O-CH ₃ , symmetric
1662	1661	ring conj C=C stretch of coniferyl/sinapyl alcohols C=O stretch of coniferyl-, sinapylaldehydes
1597	1595	aryl ring stretching, symmetric
1453	1455	O-CH ₃ deformation; CH ₂ scissoring; G/S ring vibration
1392	1395	phenolic OH bend
1334	1331	aliphatic OH bend
1272	1272	aryl-O of aryl-OH, aryl-O-CH ₃ ; G/S ring mode
1192	1190	a phenol mode
1136	1130	a mode of coniferylaldehyde/sinapylaldehyde
361	369	skeletal deformation of aromatic rings

Infrared spectroscopy

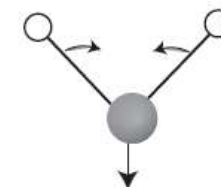
$$\bar{\nu} = \frac{1}{\lambda}$$



symmetrical stretching



asymmetrical stretching



scissoring (bending)

When molecules absorb IR radiation, transitions occur from a ground vibrational state to an excited vibrational state.

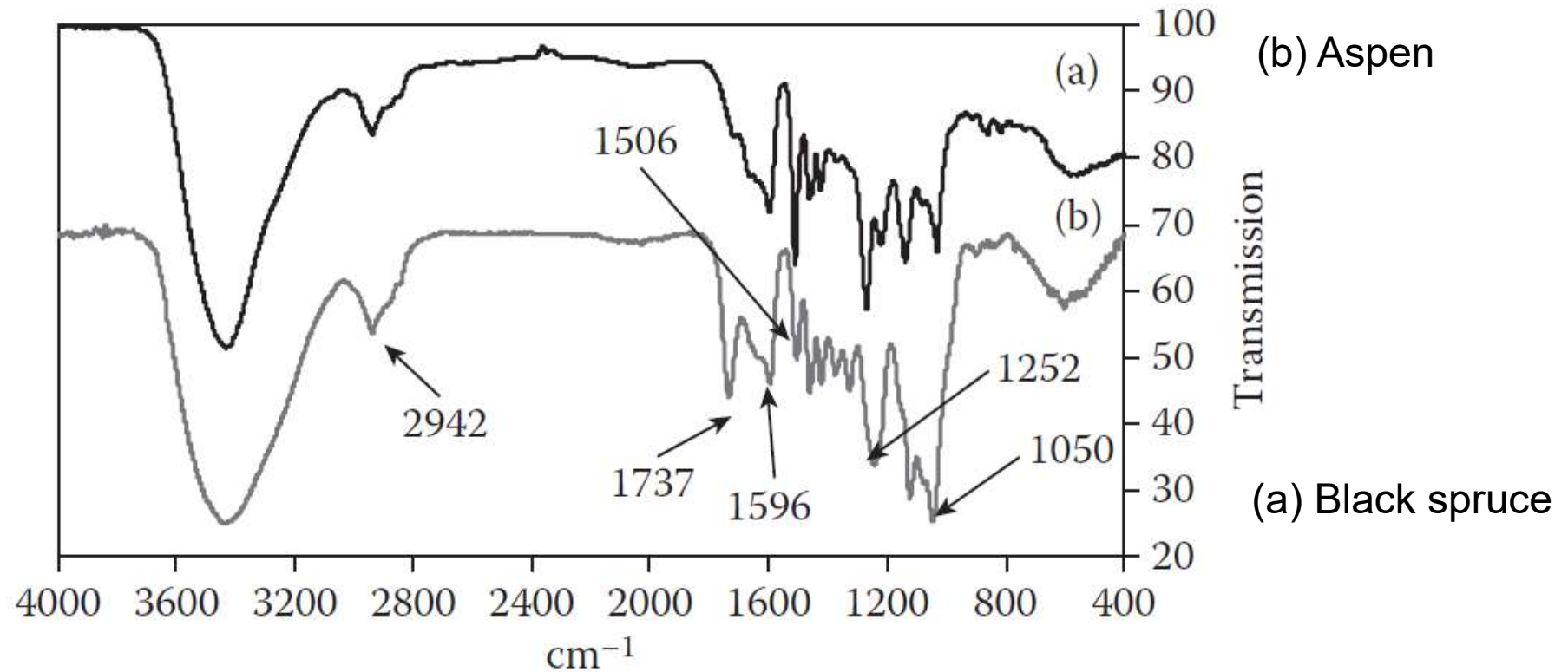
The frequency of the vibrations can be associated with a particular bond type

"IR active" molecules associated with changes in the dipole.

linear molecules: $3N - 5$ degrees of vibrational modes.

nonlinear molecules: $3N - 6$ degrees of vibrational modes.

Infrared spectroscopy of MWLs



Infrared spectroscopy of MWLs

Beech		Spruce	
KL	MWL	MWL	Assignment
[cm-1]	[cm-1]	[cm-1]	
3386	3435	3412	OH-stretch, H-bonded
2937	2939	2920	CH-stretch asymmetric
	2880	2879	CH-stretch
2839	2840	2820	CH-stretch symmetric
1712	1737	1722	C=O-stretch unconjugated ester, C=O stretch
	1665	1663	ring conjugated C=O stretch of coniferyl-, sinapylaldehyde
1592	1591	1596	aryl ring stretching, symmetric, S>G
1497	1503	1510	aryl ring stretching, asymmetric, G>S
		1495	Coumaran ring
1453	1460	1464	C-H deformation, asymmetric in-CH ₃ , and -CH ₂ -
1420	1420	1423	O-CH ₃ , C-H deformation, asymmetric
1311			OH-deform vibration
	1367	1367	Aliphatic symmetric C-H stretching in CH ₃ , phenolic OH
	1327	1326	S plus G condensed, G substituted in pos. 5. C=O stretching
1267	1265	1269	G ring plus C=O stretching; C-O-C stretch
1213	1223	1221	C-C plus C-O plus C=O stretch, G condensed > G etherified; C-O stretch
	1122		C-O-C - stretch, arom C-H-in-plane deform (S), sec-OH, C=stretch
1094		1086	C-O-C-stretching, secondary alcohol, aliphatic ether
1024	1031	1032	aromatic C-H in-plane deformation , G>S
912	921	919	C-H out-of-plane, G-units substitution in pos. 2,5, 6
845	835	858	C-H-out-of-plane, S-units substitution in pos. 2, 6
	705		ring-bending
663			out of plane ring bend
657			out of plane ring bend

Basics of NMR measurement

Exploits the magnetic properties of certain atomic nuclei to determine the chemical and physical properties of atoms.



Active nuclei in NMR need to possess a **spin, I**:

- even mass nuclei + even number of neutrons, **$I = 0$**
- Even mass nuclei + odd number of neutrons, **$I = 1, 2, \dots$** ^2D
- Odd mass nuclei + odd number of neutrons, **$I = \frac{1}{2}$** ^1H , ^{13}C

Basics of NMR measurement

1. Sample (dissolved in a suitable solvent) is placed in a very **strong magnetic field**.
Any atomic nuclei with a nuclear spin have different energy levels.
For ^1H and ^{13}C NMR, there are **2 energy levels**.
2. The **energy difference** is **very small** but increases with increasing strength of magnetic field.

Basics of NMR measurement

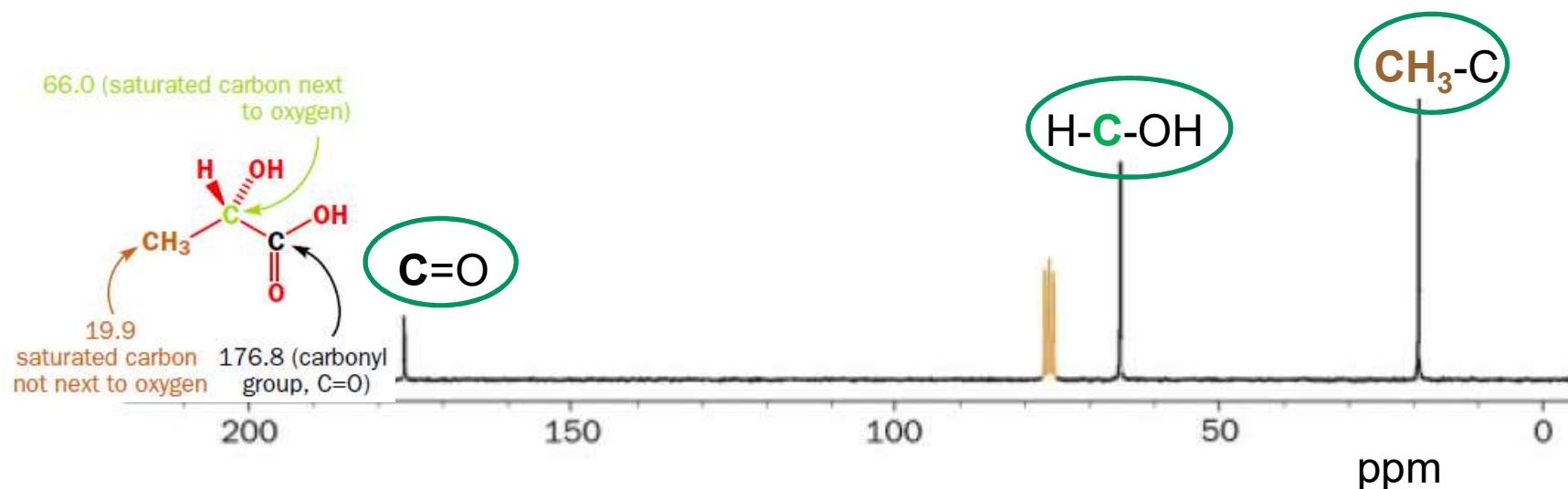
3. The **sample** is **irradiated** with a short pulse of **radiofrequency energy**.

This provides the amount of energy needed to flip the nucleus.

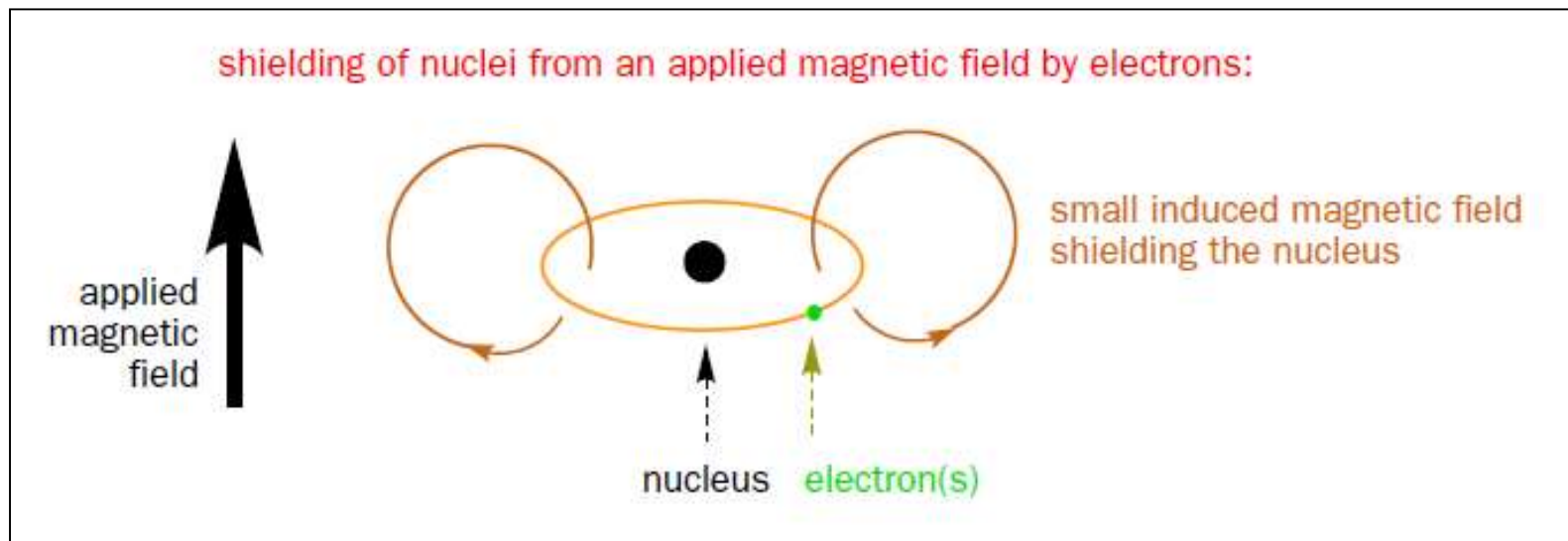
Some nuclei **absorb the energy** and are promoted to a higher energy level.

Basics of NMR measurement

4. The energy given out is detected with a radio receiver when the nuclei fall back down to the lower energy level.
5. The results are displayed in the form of intensity against frequency in ppm:



Why do chemically distinct nuclei absorb energy at different frequencies ?



- Electrons around the nucleus shield it from the applied field. Thus, the applied field strength must be increased for the nucleus to absorb at its transition frequency (**upfield**).
- Electronegative neighbours decrease the electron density around the protons. They resonate at lower field strength (**down field**)

Chemical shift

Changes in the **distribution of electrons** around a nucleus affect:

- The local **magnetic field** that the nucleus experiences
- The **frequency** at which the nucleus resonates
- The **chemistry** of the molecule at that atom

This variation in frequency is known as the chemical shift, δ

Chemical shift is defined as:

$$\frac{\text{*nuclear shielding*}}{\text{*applied magnetic field*}}$$

The chemical shift scale

- **Exact frequency** at which the nucleus resonates **depends** on the **external applied magnetic field**->
- Sample run with a different magnetic field resonates at a different frequency.

The chemical shift scale

- To avoid the dependency on the magnetic field, we say how far it is from some **reference sample**, as a fraction of the operating frequency of the machine.
- **TMS (tetramethylsilane)** is the reference sample.
The 4 C-atoms are all equivalent and are electron-rich (**shielded**) -> resonate at a frequency less than that of most organic compounds

The chemical shift scale

$$\delta = \frac{\text{frequency (Hz)} - \text{frequency TMS (Hz)}}{\text{frequency TMS (MHz)}}$$

Example:

NMR signal absorbs 400 Hz higher than does TMS at an applied frequency of 400 MHz has a δ of:

$$\delta = \frac{400 \text{ Hz}}{400 \times 10^6 \text{ Hz}} = 1 \times 10^{-6} = 1 \text{ ppm}$$

Chemical shift is independent on applied magnetic field

The chemical shift scale

Operating frequency ω_0 is calculated from the Lamor equation*.

The proton operating frequency for a 9.4 T magnet is:

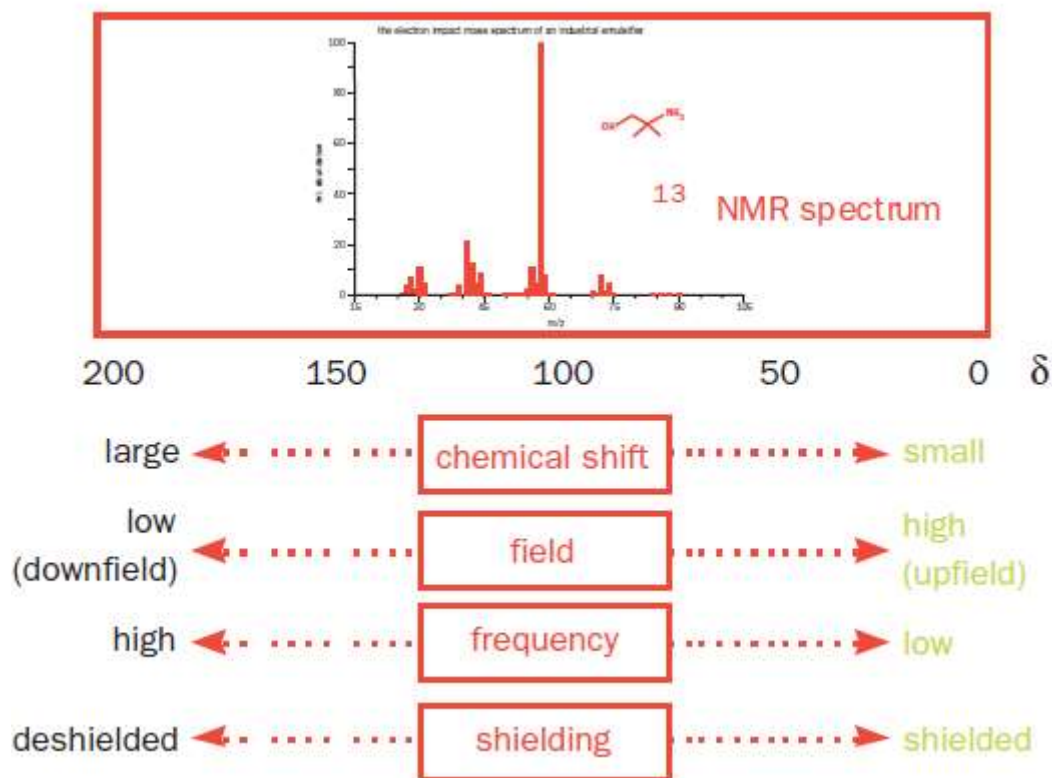
$$\omega_0 = \gamma * B_0 \quad \gamma = \frac{\mu * \mu_N}{h * I}$$

$$\omega_0 = \frac{2.79 * 5.05 * 10^{-27} J/T}{6.62 * 10^{-34} J_s * 1/2} * 9.4 T = 400 MHz$$

γ *gyromagnetic ratio*
 μ *magnetic moment*
 μ_N *nuclear magneton*
 h *Planck constant*

* Lamor precession of the magnetic moments of atomic nuclei, ..

Describing the chemical shift



- Chemical shift scale runs from zero where TMS resonates.
- **Shielding:**
 - Simple saturated C nuclei are the most shielded.
 - Electronegative O atom moves the chemical shift downfield $\rightarrow$ nucleus becomes deshielded.
 - Unsaturated carbons experience even less shielding

^1H NMR vs. ^{13}C NMR

- ^1H is the major isotope of hydrogen (99.985% natural abundance), while ^{13}C is only a minor isotope (1.1%)
- **^1H NMR is quantitative**: the area under the peak tells us the number of hydrogen nuclei, while ^{13}C NMR may give strong or weak peaks from the same number of ^{13}C nuclei

^1H NMR vs. ^{13}C NMR

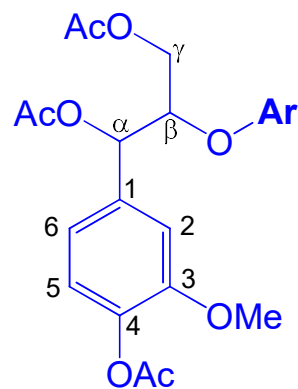
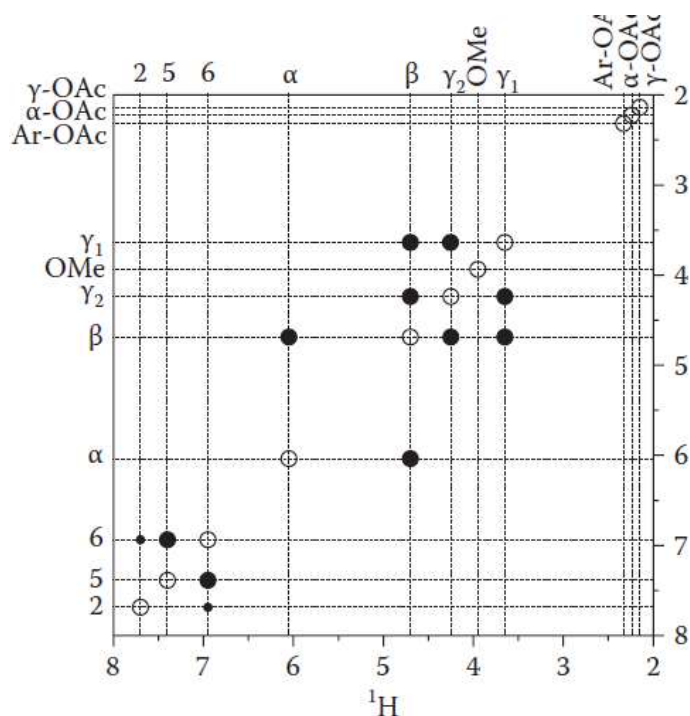
- Protons interact magnetically to reveal the connectivity of the structure (**coupling constant**), while ^{13}C is too rare for coupling between ^{13}C nuclei to be seen.
- ^1H NMR shifts give a more reliable indication of the local chemistry than that given by ^{13}C spectra

2D NMR

- Second dimension by acquiring a series of spectra in which a delay period in the pulse sequence is incrementally increased.
- Correlation of a chemical **shift of carbon** in **one dimension** with the **proton chemical shift** of its attached proton in **a second dimension**:
- Many of the protons in lignin from different units severely overlap, but their carbons are often distinct.

COSY

correlation spectroscopy



Hypothetical lignin model compound

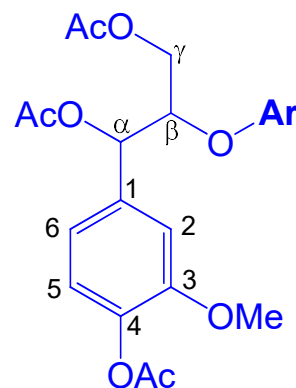
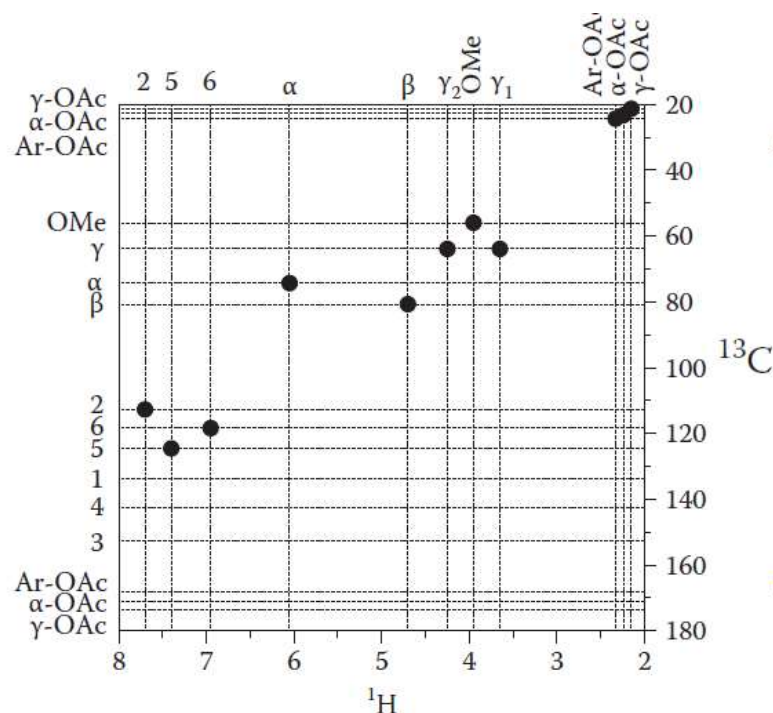
Correlates directly coupled protons:

H_{α} with H_{β} , which also correlates with the two H_{γ} protons.

Weak H_2 - H_6 correlation is also shown

HSQC

heteronuclear single-quantum correlation



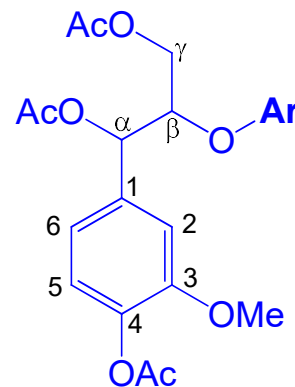
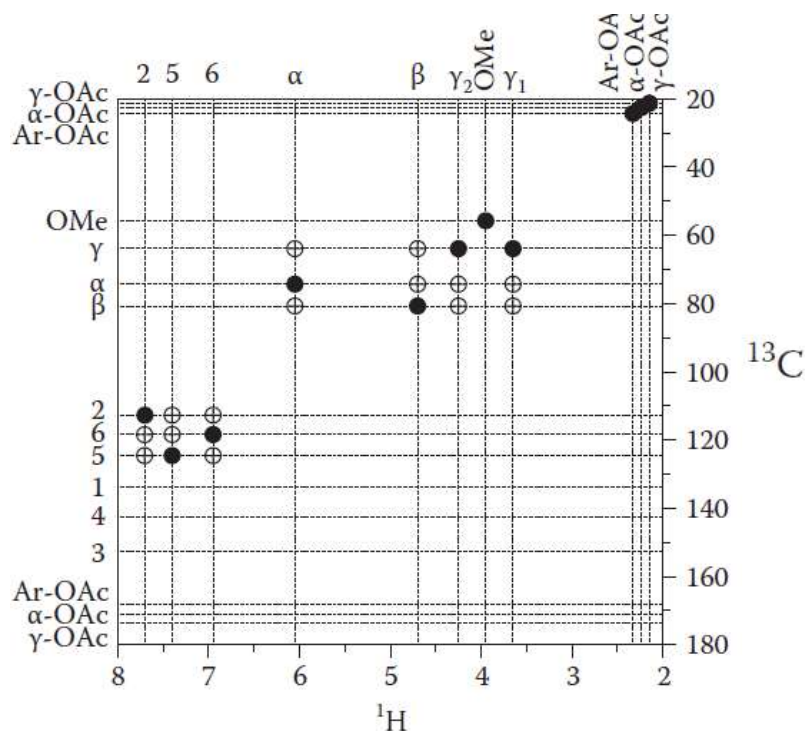
Correlates each carbon with its directly attached proton.

H_α correlates with C_α , etc.

An **HSQC** contour implies that a proton at the proton frequency of the x-axis is directly attached to a carbon at the carbon frequency of the y-axis.

HSQC-TOCSY

total correlation spectroscopy



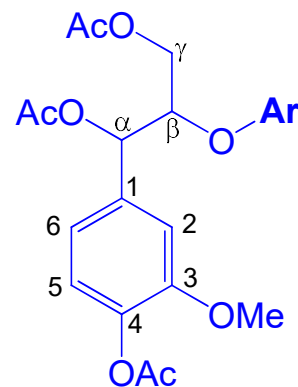
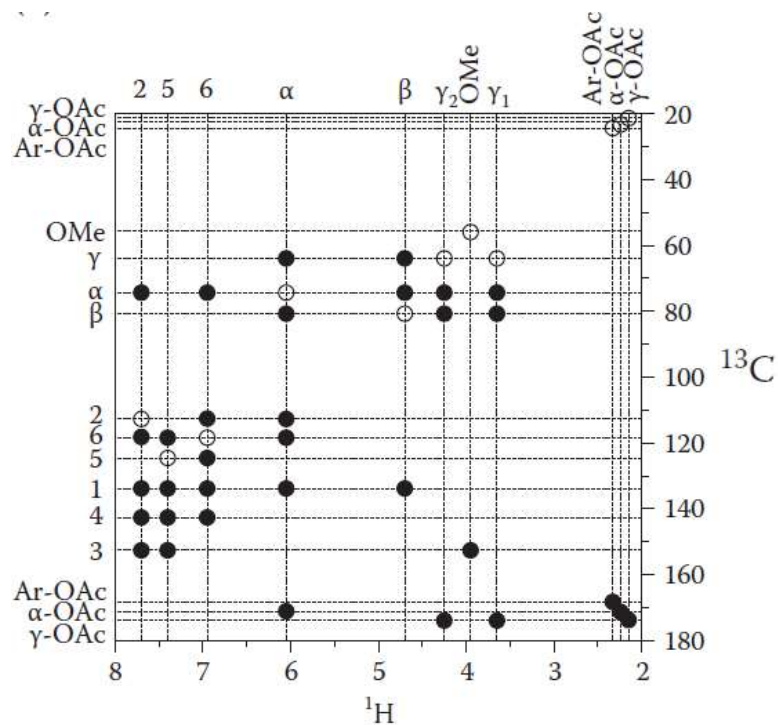
Extends the HSQC by correlating a carbon not only with its attached proton but all other protons in the same coupling network:

C_α correlates with $\text{H}_\alpha, \text{H}_\beta, \text{H}_\gamma$'s;

H_α correlates with the 3 side-chain carbons

HMBC

heteronuclear multiple-bond correlation



Correlates protons and carbons that are related by long-range coupling interactions over 2-3 bonds.

H_α correlates with all carbons within 3 bonds; C_β , C_γ , C_1 , C_2 , C_6 and the α -acetate carbonyl.

C_3 is the only carbon that correlates with the OCH_3 protons.

NMR-Analysis

Sample preparation (10 – 100 mg): dissolved in CDCl_3

1) Quantitative 1D NMR (220 mg/mL)

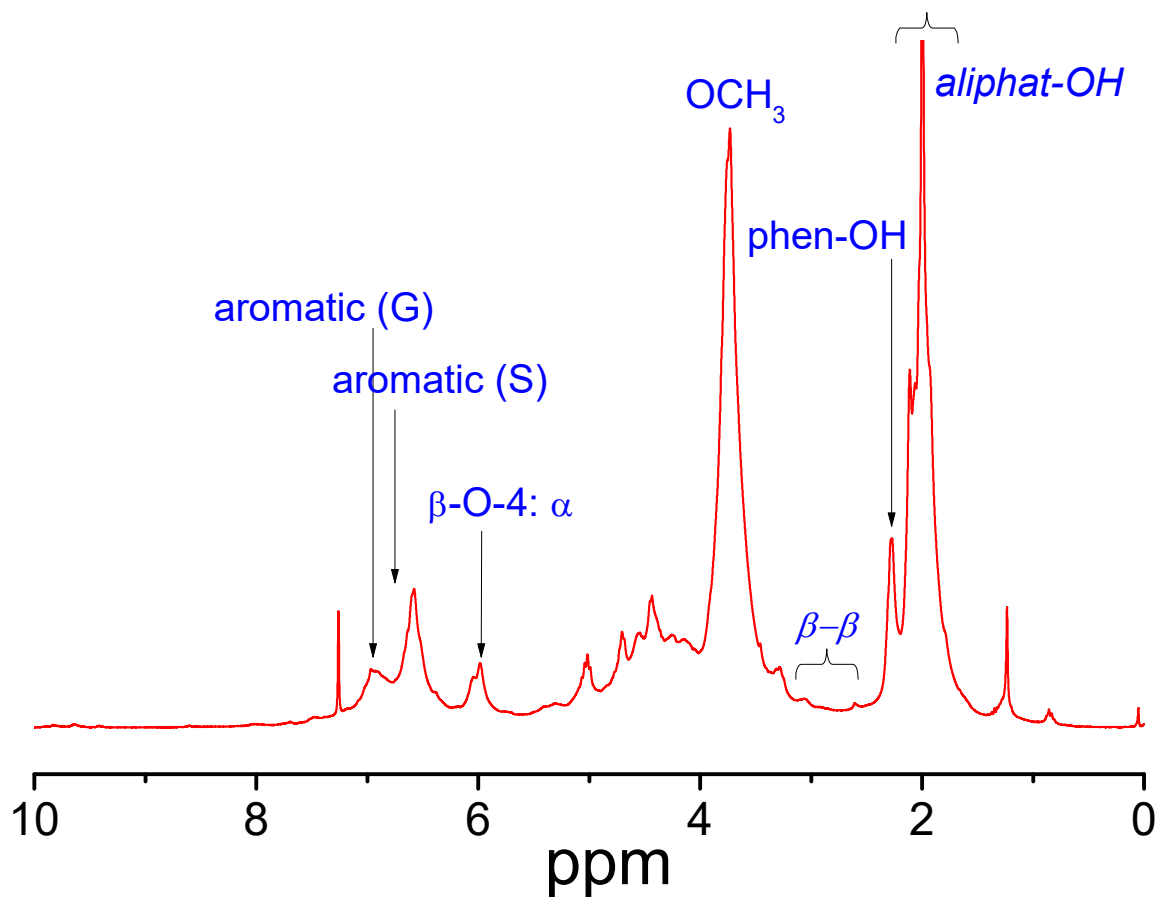
- **^1H -NMR**
- **^{13}C -NMR**
- ^{31}P -NMR

2) 2D NMR (conc 100 mg/mL)

- **COSY**
- **HSQC**
- **HMBC**
- **HSQC-TOCSY**

^1H -NMR-Spectroscopy of Lignin

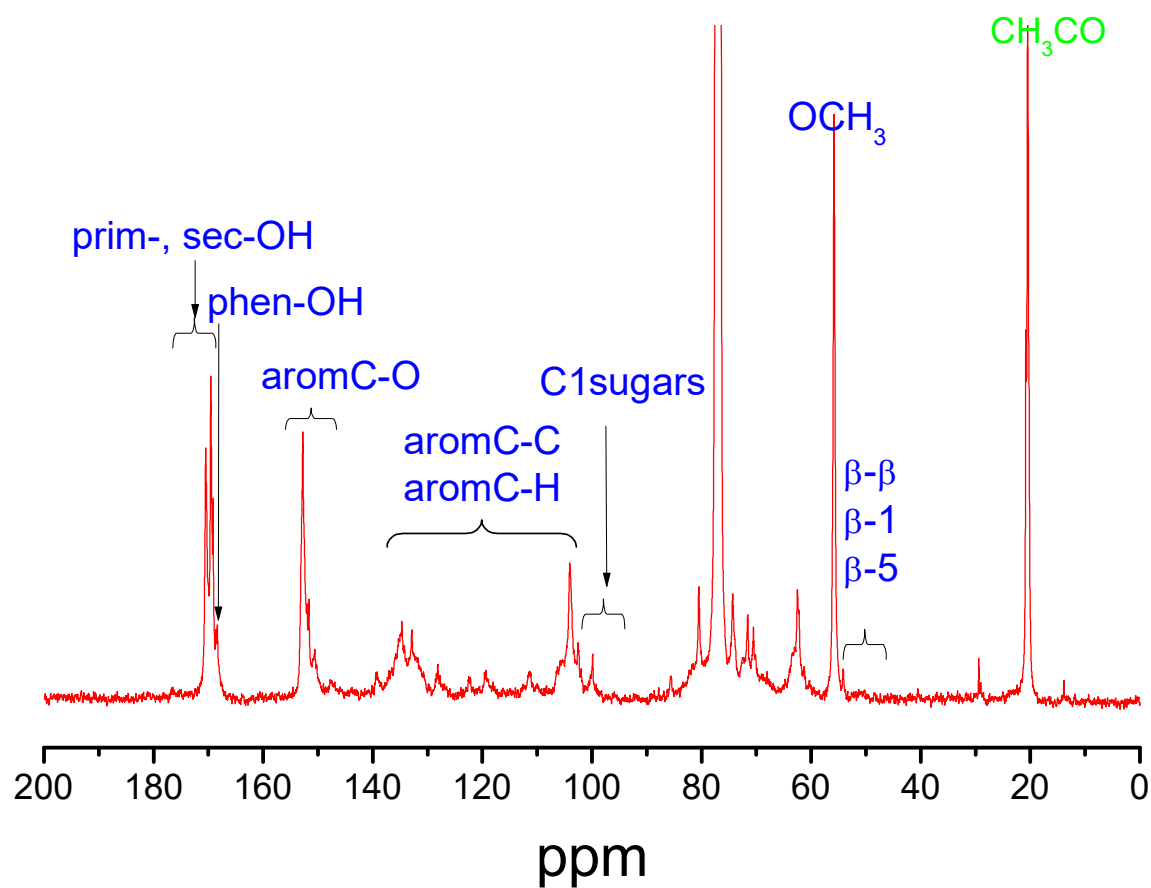
Substrate: acetylated *E. globulus* MWL



100 mg/mL in CDCl_3

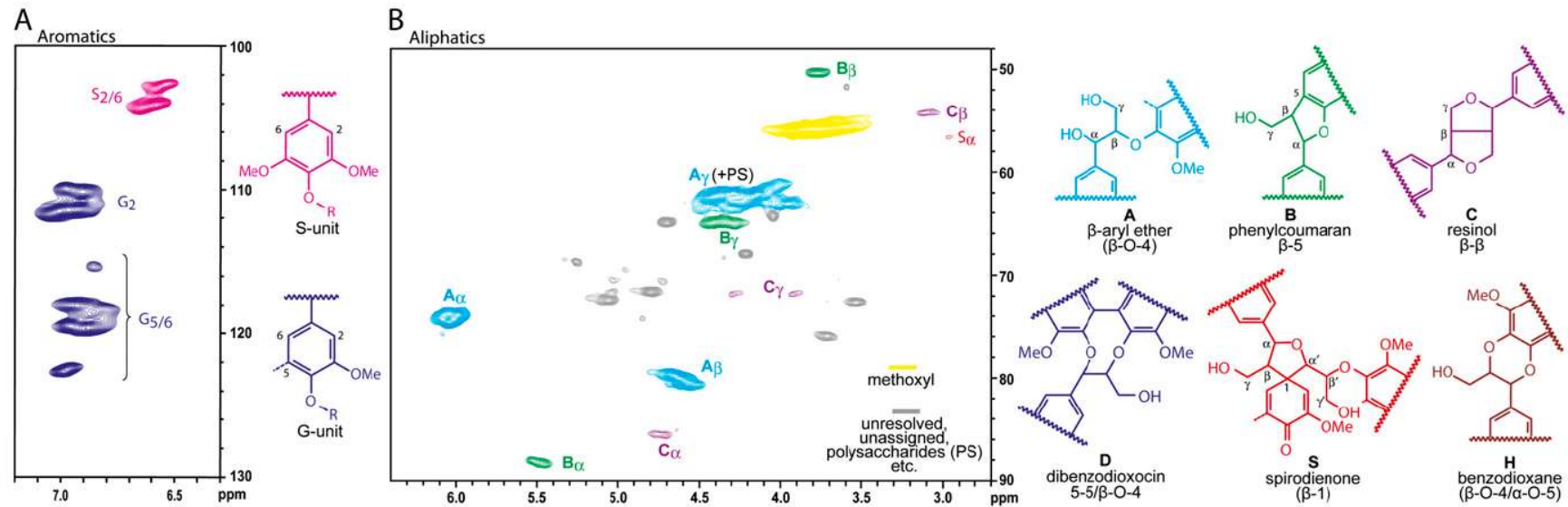
^{13}C -NMR-Spectroscopy of Lignin

Substrate: acetylated *E. globulus* MWL



200 mg/mL in CDCl_3 + 1.3 mg $\text{Cr}(\text{acac})_3$

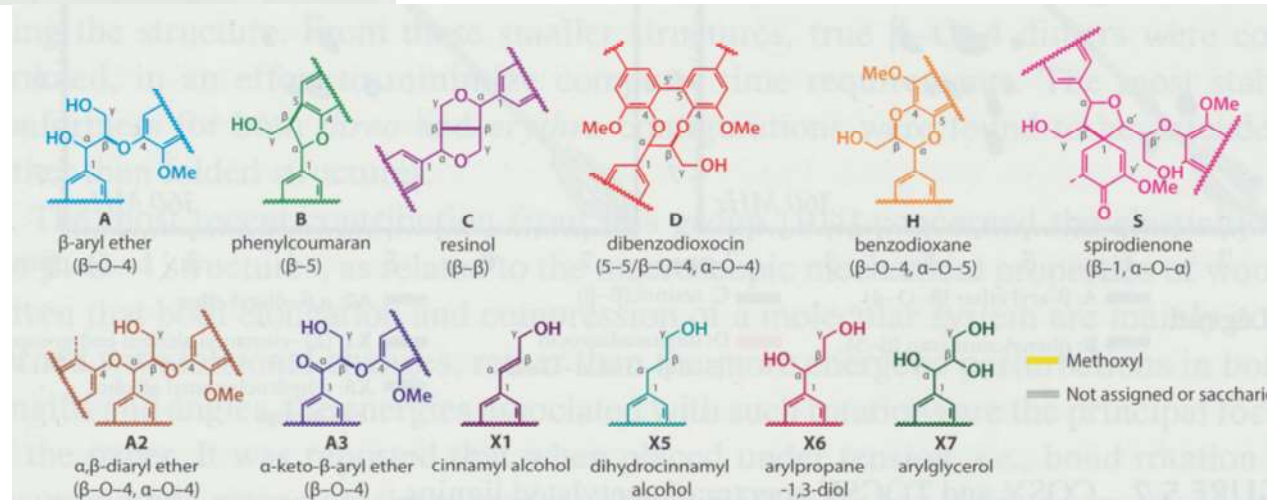
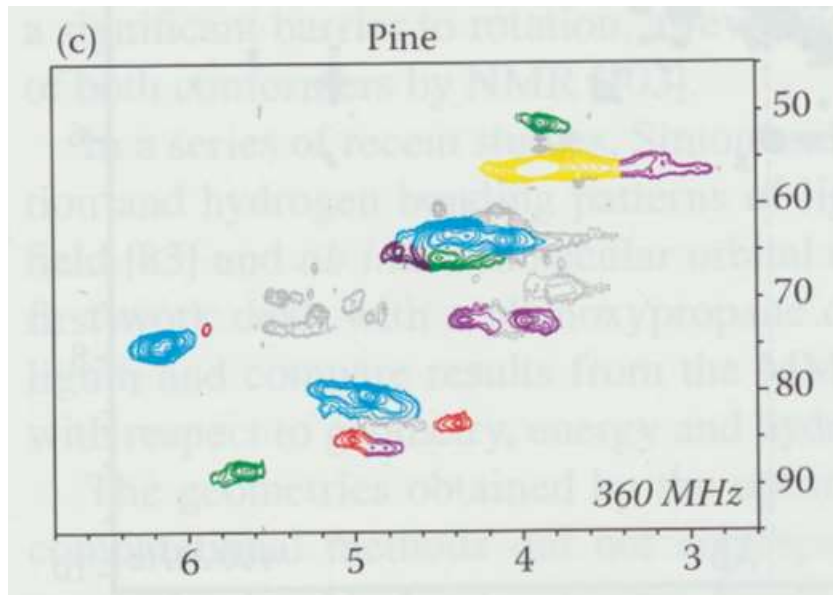
HSQC of acetylated lignin



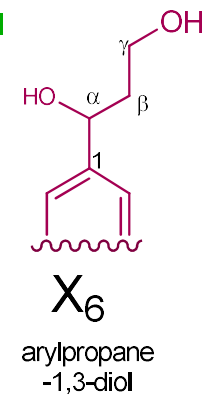
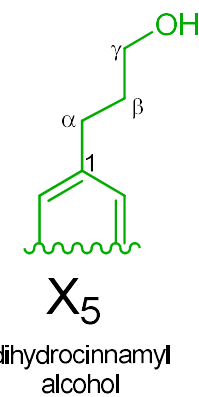
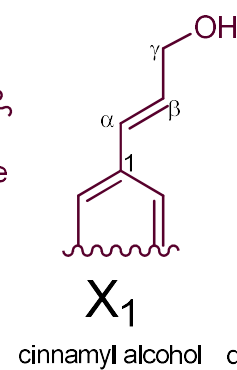
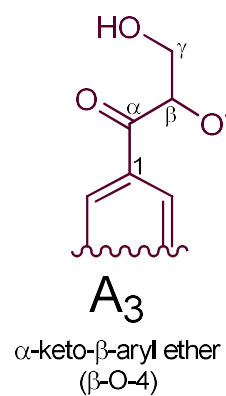
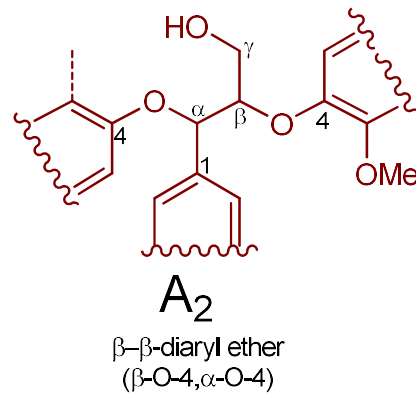
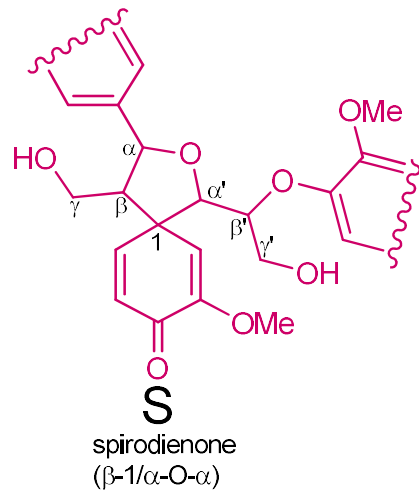
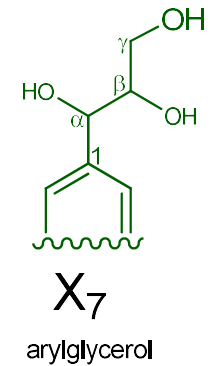
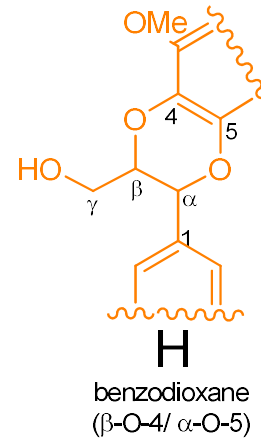
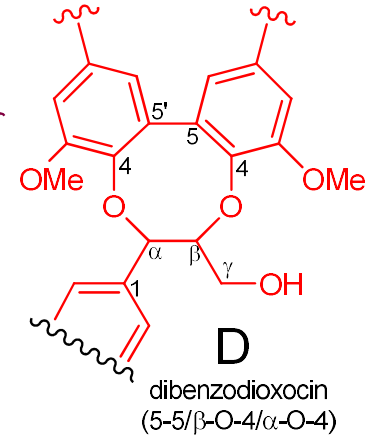
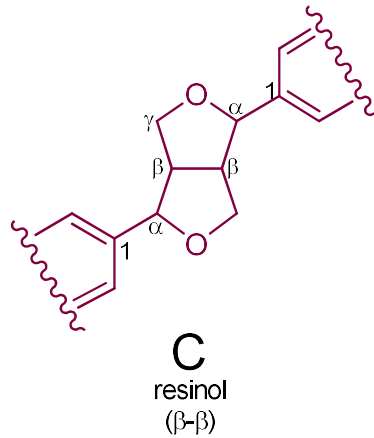
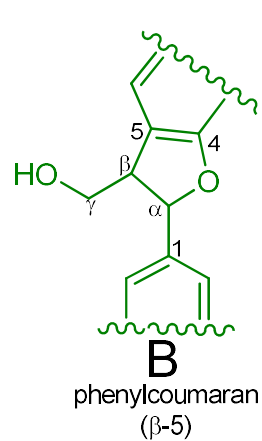
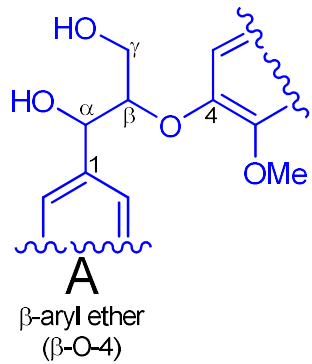
Acetylated **Arabidopsis cellulolytic enzyme lignin** that contains the whole lignin fraction from the cell wall but is depleted in polysaccharides after their removal via treatment with crude cellulases.

Ruben Vanholme et al. *Plant Physiology*, 895-(2010)

HSQC of acetylated pinewood lignin

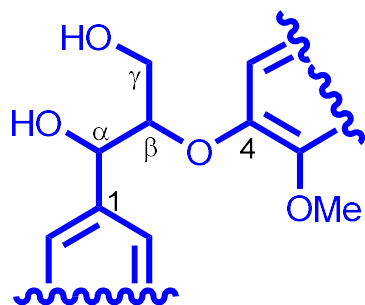


Lignin Structures



Elucidation of lignin substructures

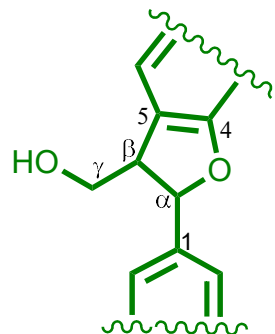
β -O-4-ethers: most studied lignin unit. β -G ether units are 50:50 *erythro:threo*, β -S units show 75:25 in favor for *erythro* (sterical/chemical reasons).



A

The *threo*-isomers always have a higher-field γ -proton.

β -5 units (Phenylcoumarans): second most abundant inter unit linkage type in lignin.

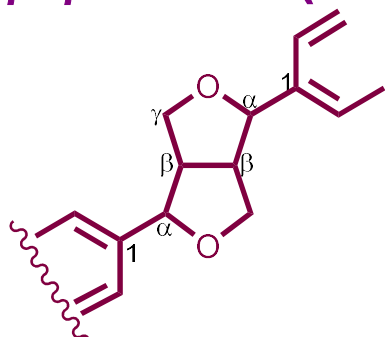


B

Phenylcoumaran structures in lignin primarily *trans*-configuration (^1H) NMR.

Elucidation of lignin substructures

β - β -Units (Resinols): In 2D NMR, the contours for the resinol

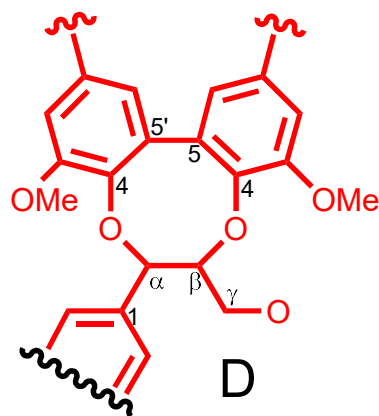


C

unit are twice as intense as for any other unit.

High β - β -coupling propensity of sinapyl alcohol in dimerization reactions explains lower amounts of unsaturated sidechains in S-rich lignins.

5-5 Units (Biphenyls, dibenzodioxocin): side-chains of



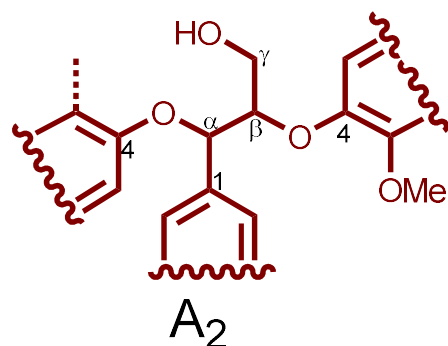
D

biphenyl units are difficult to assign, but units coupled to them may be highly diagnostic.

Dibenzodioxocins show easily in HSQC or HMBC, where the **C_{α} - H_{α}** and **C_{β} - H_{β}** correlations are unique and well-resolved (acetylated samples).

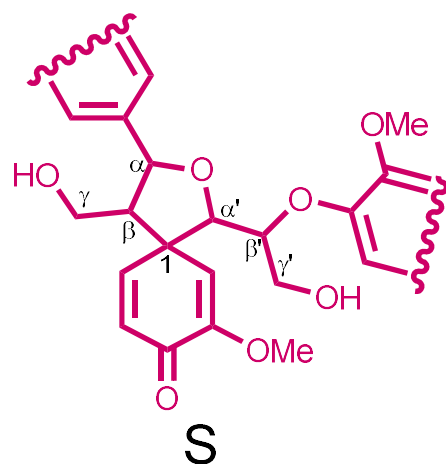
Elucidation of lignin substructures

α -Aryl Ethers (α -, β -Diaryl Ethers): difficult to detect.



A significant proportion can be attributed to D structures. Non-cyclic α -ethers are very rare in wood lignins (0.3% in pine lignin).

β -1 (Diarylpropane, spirodienone): Difficult to identify



because of overlapping signals in 1D and 2D experiments.

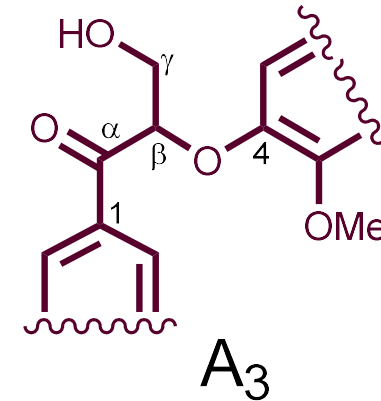
TOCSY authenticated β -1 units in acetylated poplar MWL. High-S lignins appear to contain elevated amounts of spirodienones.

Elucidation of lignin substructures

α -Keto- β -Ethers:

Mainly found in **high-S** isolated lignins.

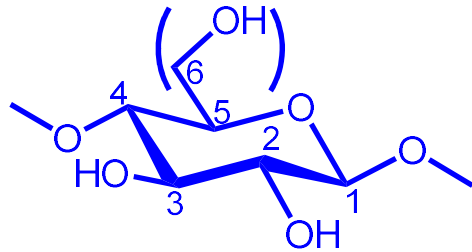
May arise during isolation steps,
e.g. during ball milling



Hydroxyl Groups: are characteristic in lignins. Various types of phenols are recognized in proton spectra run in DMSO (this solvent does not favor proton exchange).

In ^{13}C NMR, the acetate carbonyl resonances for phenolic, primary and secondary benzylic acetates are resolved.

Assignment of HSQC to Lignin Structures

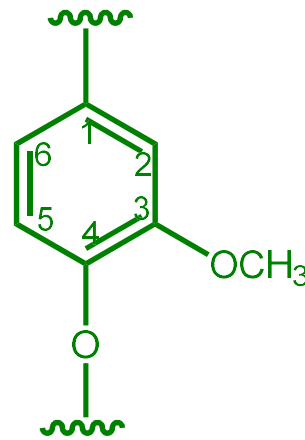


E₁: 99.6 / 4.4

E₂: 70.5 / 4.7

E₃: 71.5 / 5.0

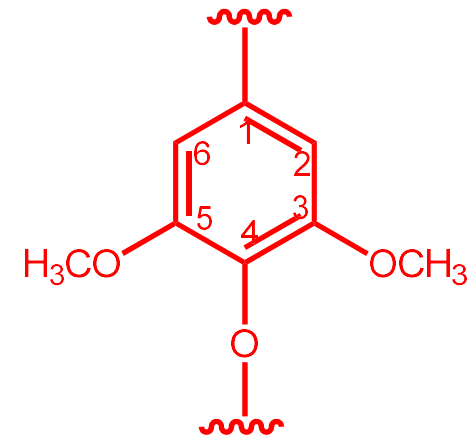
E₅: 62.2 / 3.3, 3.9



G₂: 110.9 / 6.9

G₆: 119.3 / 6.9

G₅: 122.3 / 6.9



S_{2,6}: 103.9 / 6.6

S_{2,6}: 105.8 / 7.1

2D Correlation shifts of acetylated lignins in acetone-d6

Structure	Type	H _α	H _β	H _{γ2}	H _{γ1}	C _α	C _β	C _γ
A	Ge	6,03	4,84	4,33	4,22	74,5	80,2	63,2
	Gt	6,07	4,82	4,23	4,01	75,3	80,4	63,3
	Se	6,03	4,73	4,39	4,15	75,4	81,4	63,4
	St	6,08	4,63	4,24	3,9	76,8	81,6	64,3
B	G	5,66	3,77	4,36		88,4	51,1	65,8
C	G/S	4,8	3,1	4,26	3,9	86,5	55,4	72,4
D	G/S-GG	4,94	4,3	4,45	4,07	85,1	83,3	64
H	S/G	4,98	4,39	4,28	4,02	76,8	75,9	63,4
S	SA	5,2	3,1	4,06		83,2	57,6	61,9
	SB	4,82	4,5	4,3	4,18	86,9	77,4	64,7
A2	G	5,61	4,89	4,56	4,45	80,9	81,5	63,5
A3	S		5,59	4,48		195	81,3	64,7
X1	G	6,63	6,23	4,65		134	122	65,3
X5a	G	2,62	1,9	4,04		32,1	30,9	64
X5b	G	2,73	1,89	4,11		32,2	30,9	64,1
X6	G	5,83	2,18	4,1		73,1	35,9	61,2
X6'	G		3,33	4,41		198	37,6	60,4
X7	G	5,91	5,4	4,1		74	72,9	61,2
	S	5,88	5,37			73,5	72,2	61,5

e erythro
t threo
A β-O-4,
B β-5
C β-β
D dibenzodioxocin
A2 α,β-di-ether,
A3 α-keto-β-ether,
H benzodioxane,
S spirodienone,
X1 cinnamyl alcohol,
X5a 5-β and 4-O-β
X5b 5-5
X6 arylpropanediol,
X6' benzylic-oxidized
 arylpropanediol,
X7 arylglycerol

Quantitative ^{13}C NMR of Lignins

Problem to be solved:

- Nuclear Overhauser enhancement (nOe)
- Difference in relaxation rates of carbon nuclei.

Nuclear Overhauser Enhancement

- Change in signal intensity of one nucleus when another nearby nucleus is perturbed in some way.
- Carbon signal enhancement **results upon irradiating the proton nuclei**, such as is done during decoupling.
- Enhancement is not equal for all types of C-signals.

Nuclear Overhauser Enhancement

- Elimination of **nOe** by the inverse-gated experiment in which the proton decoupling is gated off during the interpulse delay.
- Major disadvantage of eliminating this irradiation and consequent **nOe** is the lower resultant sensitivity.

Different relaxation rates of C-nuclei

- May be solved by **providing a relatively long delay between scans** so the C-nuclei are completely relaxed.
- The major relaxation process is the **spin-lattice relaxation**, defined by the time constant T_1 .
- Complete relaxation requires a delay of **at least $5 \times T_1$ seconds**.

Different relaxation rates of C-nuclei

- In lignin the carbon nuclei relax very rapidly. This is an advantage in quantification (disadvantage for resolving close peaks).
- Relaxation of quaternary Cs very slow (1-4 s)

Quantification

- Area of aryl region, 95-160 ppm, is set to 6.0 C atoms. Approach not valid if extensive aliphatic unsaturation in the side-chains, vinyl ethers, stilbens. For kraft lignin not appropriate.
- Use of internal standards: “spiking” method by adding e.g. a vinyl ether model compound can be useful. Low molar mass “standards” should be avoided due to very long relaxation times.
- In summary, true quantification in lignins is difficult due to signal overlap. The best quantification can be achieved by inverse-gated technique, along with internal standard substance.

Relative abundance of main inter-unit linkages in lignins of different origin

Linkage abundance, %	Eucalyp tMWL	Spruce MWL	Sisal MWL
β -O-4 (A)	76	65	85
β -5 (B)	2	18	2
β - β (C)	17	11	4
β -1 (S)	2	0	7
5-5' (D)	0	5	0
Cinnamyl alcohol (X1)	3	1	2
γ -acetylation	0	0	72
S/G	2.9	0	3.9

Based on semiquantitative analysis of the HSQC cross-signals.

ANNEX

Lignin properties

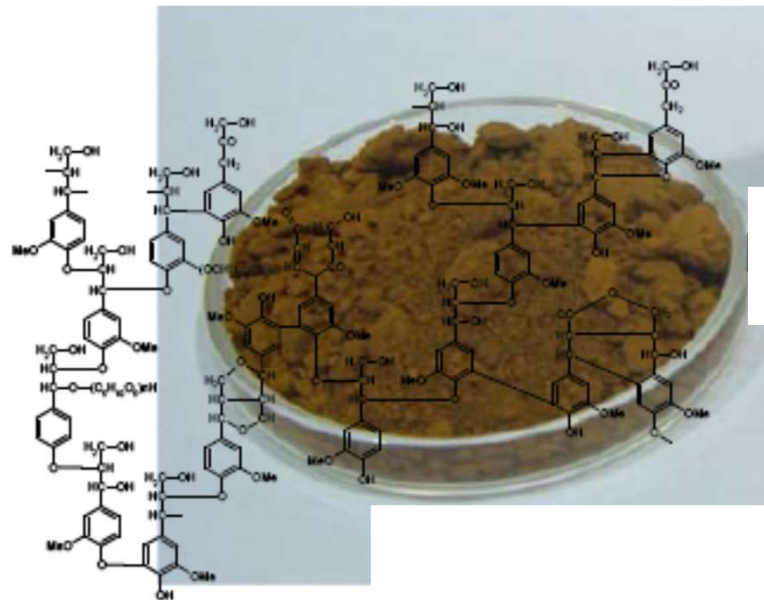
Lignin sources

Lignin applications

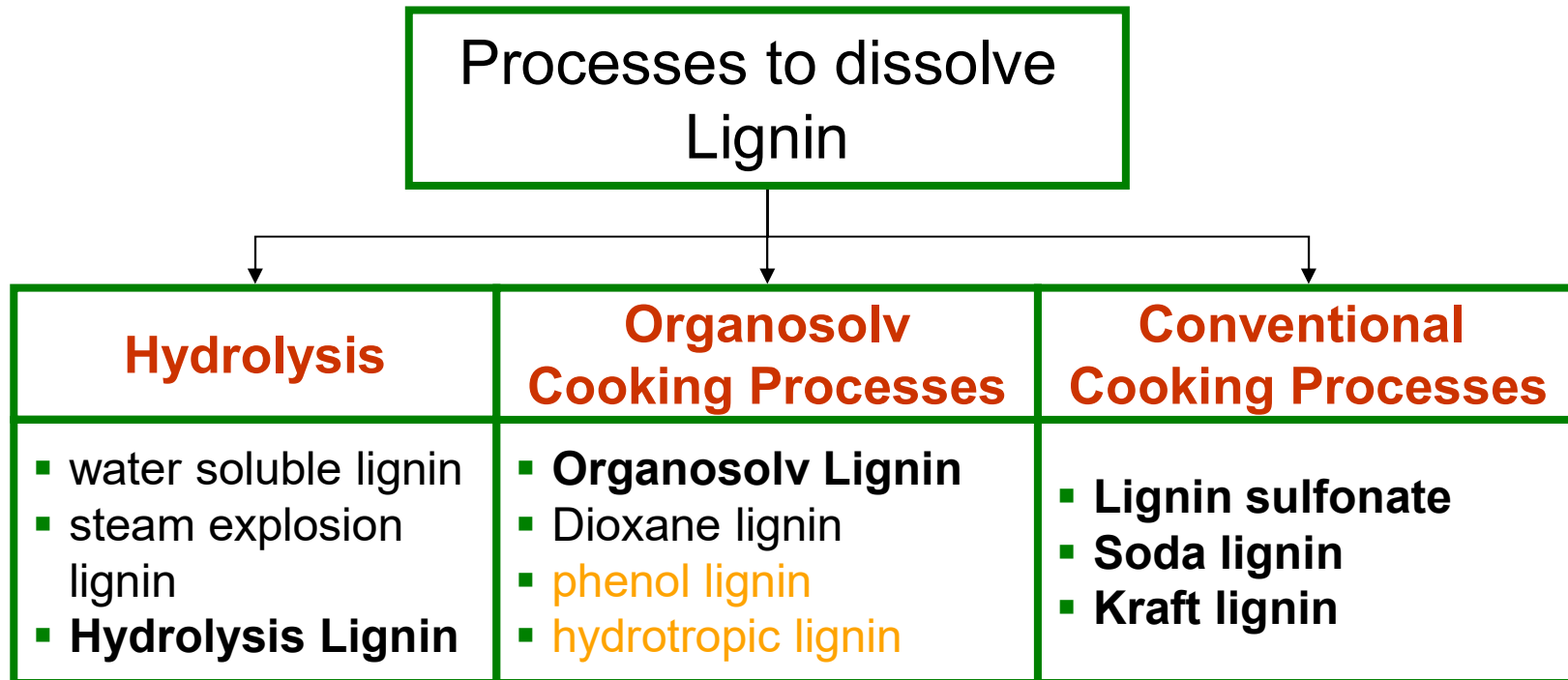
Physical properties

- Spheric particles of 10-100 nm size.
- Thermoplastic
- Binder; gluing wood cells together, stiffness forming.
- Soluble in dioxane, acetone, THF, DMF, DMSO and ethylacetate.

Lignin-based Biorefinery



Lignin Sources



Commercial Sources and Applications

- Lignosulfonates
- Kraft Lignin
- Soda Lignin
- Hydrolysis Lignin
- Organosolv Lignin

Lignosulfonates - LS

Majority of lignosulfonates produced from **acid sulfite pulping** of hard- and softwoods.

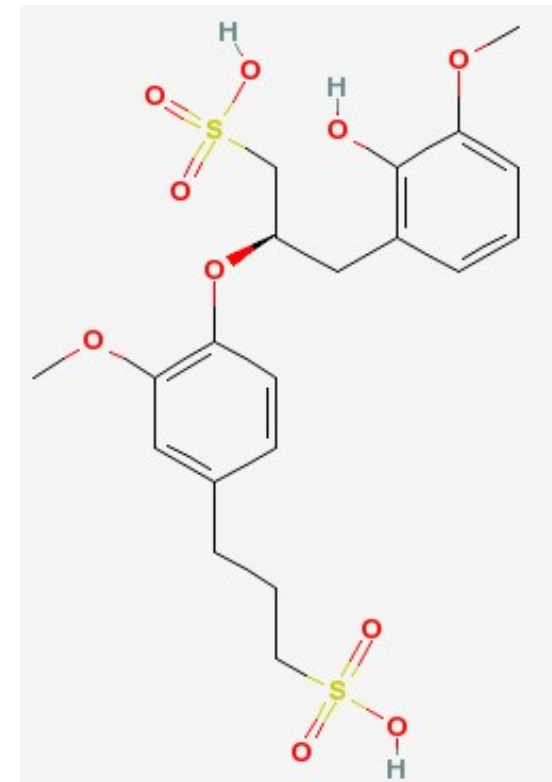
Small amounts result from NSSC process and from sulfonation of Kraft lignin (Mead Westvaco).



Fe/Cr



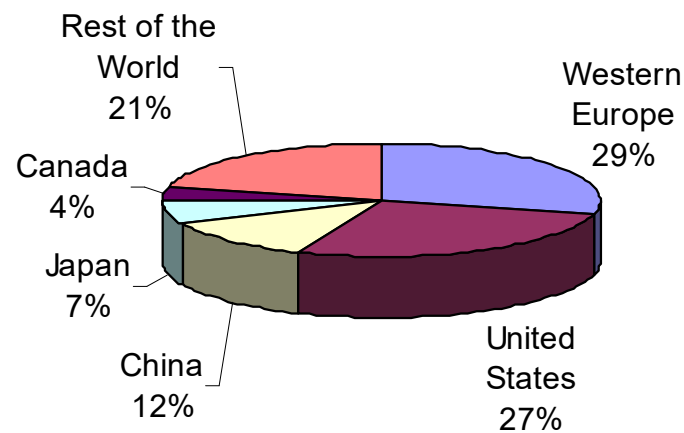
Ca



Lignosulfonate Producers

The world lignosulfonate industry is dominated by Borregaard **LignoTech** and **Tembec**.

They account for about 55% of the world's capacity of 1.8 Mio t LS (2005). Estimated consumption figures for 2009 are about 1.0 - 1.1 Mio t of LS



Lignosulfonate Products

Major Uses

- Concrete Admixtures: 400 – 510 kt dry LS
- Animal Feed Pellets: 124 kt dry LS
- Dust control: 75 kt dry LS
- Pesticide Dispersant: 46 kt dry LS
- Oil well drilling muds: 23 kt dry LS

Minor Uses

- Gypsum wallboard dispersant
- Dye dispersants
- Coal and carbon black binder (Germany)
- Leather tanning: 6 kt/a
- Vanillin production: 2-3 t/a
- Dye manufacturing: Na-LS to disperse textile dyestuffs

Kraft Lignin

Commercially, Kraft lignin is isolated through the acid precipitation of black liquor. The resulting product is water insoluble.

In this form there are only a few applications in which Lignin can be used economically:

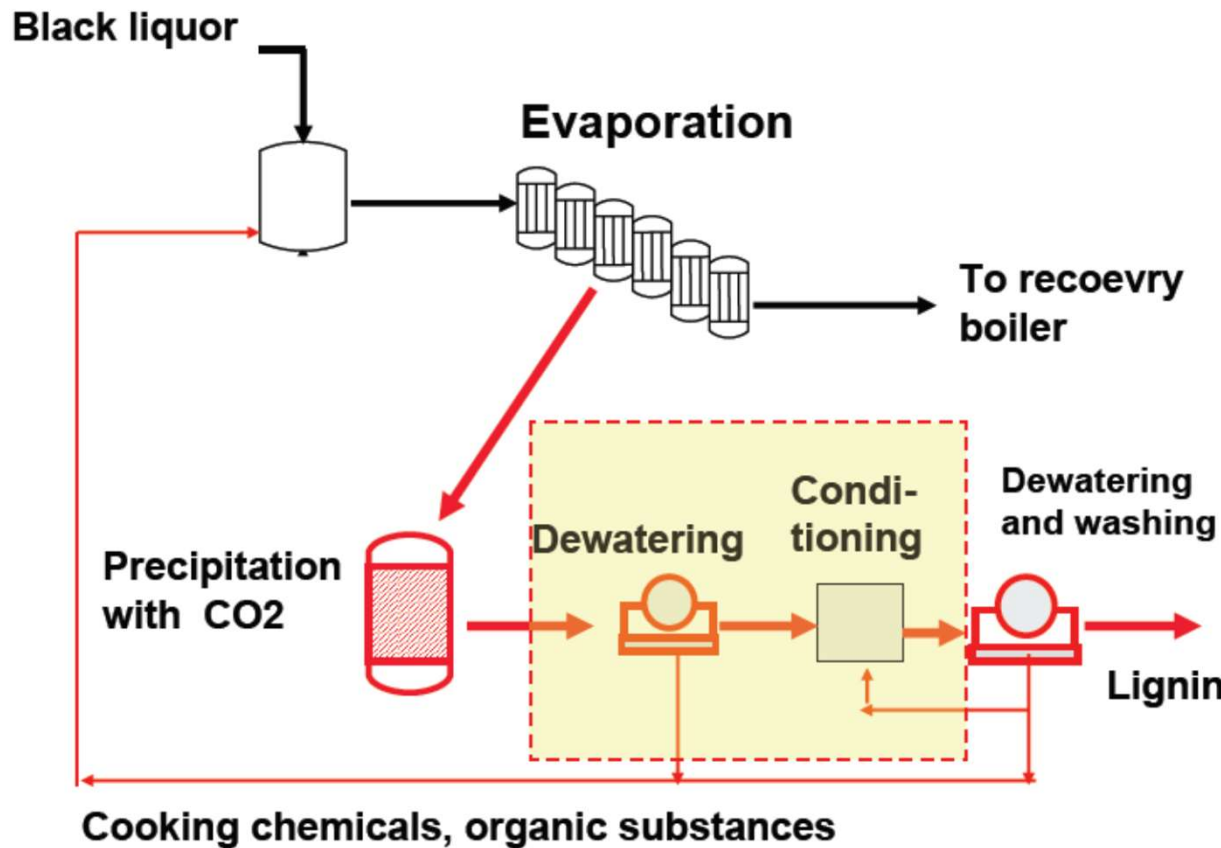
- **Solid fuel**
- Rubber reinforcers
- Activated carbon
- Phenolic resin components
- Raw material for the production of **Methylsulfonates** (via sulfomethylation) → Lignosulfonates

Kraft Lignin

- Annual „production“ > 70 Mio tons
- Utilized less than 100 kt/a
- Dominating company: MeadWestvaco
- Little reactivity left due to condensation and fragmentation reactions
- Sulfur content 2-3%

LignoBoost - Solid Kraft Lignin

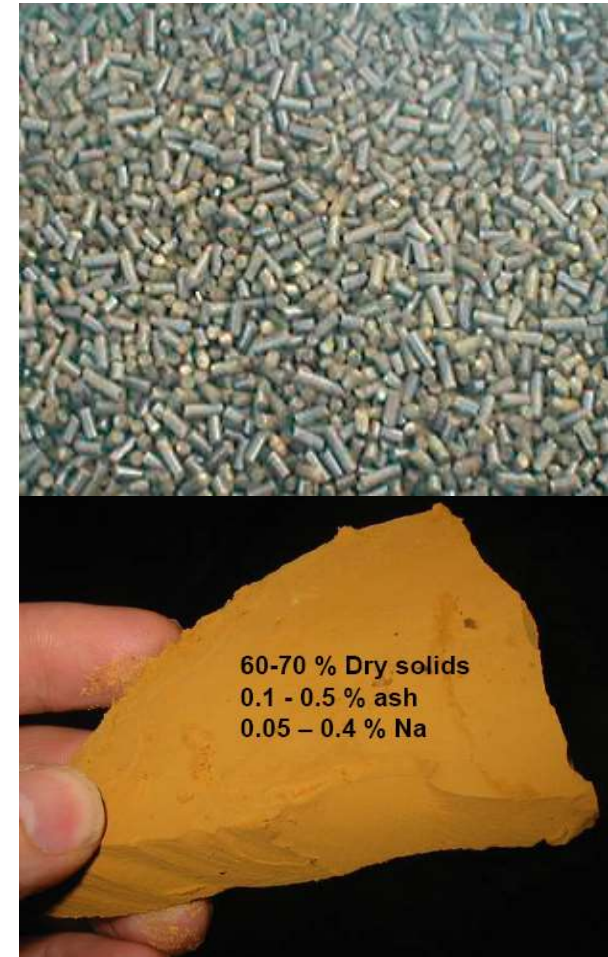
LignoBoost Process – Demonstration Plant



To ensure good filtration and washing, lignin dissolution at low ionic strength and high pH have to be avoided. Thus, pH and ionic strength profiles have to be evened out in a conditions stage prior to washing where the filter cake is re-dispersed once again with filtrate after washing .

LignoBoost Process

- 190-210 kg lignin/ton DS
- 260-280 kg CO₂/ton lignin
- 70% Dry solid content
- 0.2% ash content
- 25.4 MJ/kg DS HHV
- 17.1 MJ/kg 30% moisture content
- 64.0 % carbon
- 26.4% oxygen
- 5.7% hydrogen



Hydrolysis Lignin

- Russian speciality: several hydrolysis plants in operation such as Onega, Tulun, Khor, Lobva, Tavda, ...
- Lignin has undergone severe acid condensation reactions and therefore has little to no water solubility
- Feedstock for CS₂ production, lignin coal, carbon sorbent for sorption of heavy metals, fuel lignin, active carbon
- Soil additives

Areas of Lignin Utilization

- Power, fuel Syngas
- Macromolecules
 - adhesives for wood material
 - Cement additives for enhanced low-temperature durability
- Polymer component
 - Co-reactant for polymer and resins
- Low-molecular fraction, aromatics, miscellaneous monomers
 - Vanillin from Lignosulfonates
 - DMSO production from kraft lignin

Macromolecular Lignin

- **CARBON FIBERS**

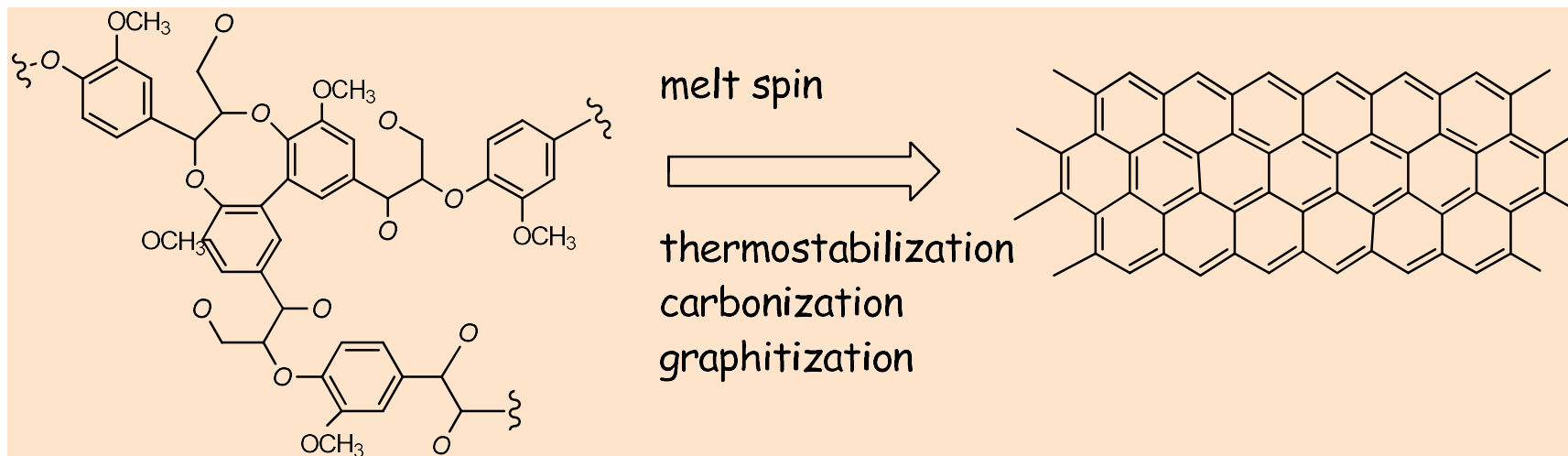
lignin

represents a potential low-cost source of carbon suitable for displacing polyacrylonitrile (PAN) in the production of carbon fiber.

Challenges:

- purification of commercial kraft lignin;
- adjustment of molecular weight polydispersity by removing low- and high molecular fractions.
- Derivatization of lignin to optimize thermal (T_g), melt flow and melting point properties.
- Uniform quality for high-rate melt spinning.
- Melt-spun lignin fiber is thermally converted to carbon fiber.

Carbon Fibers (CF)

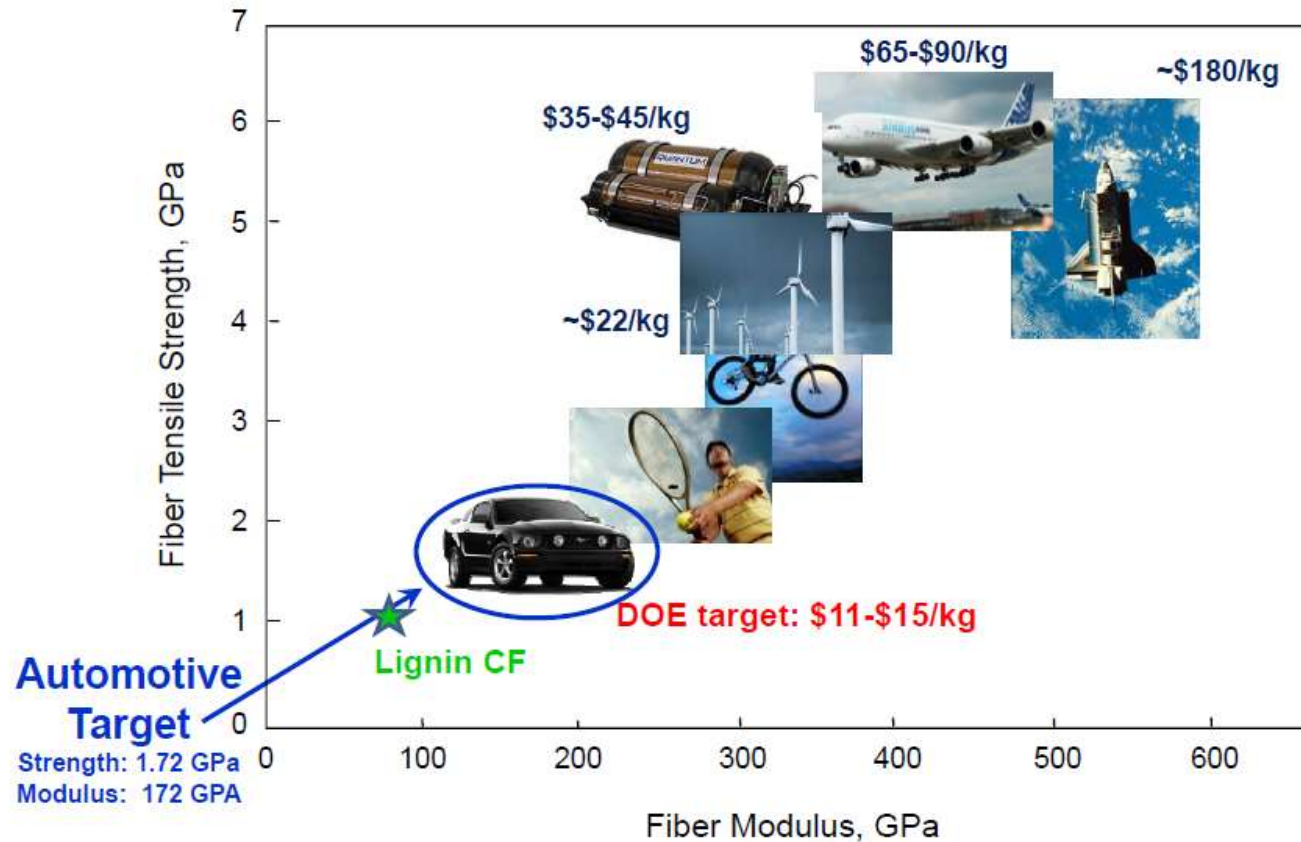


Scheme of Low-cost carbon fiber manufacture from lignin

Global Production of CF

- 63 000 t produced (2010)
- 84 000 t capacity (2010)
- Annual growth rate: 15 – 20%
- High demand on "low-cost" CF for the automotive sector to reduce the weight:
- 10% weight reduction means 5-7% fuel savings*.

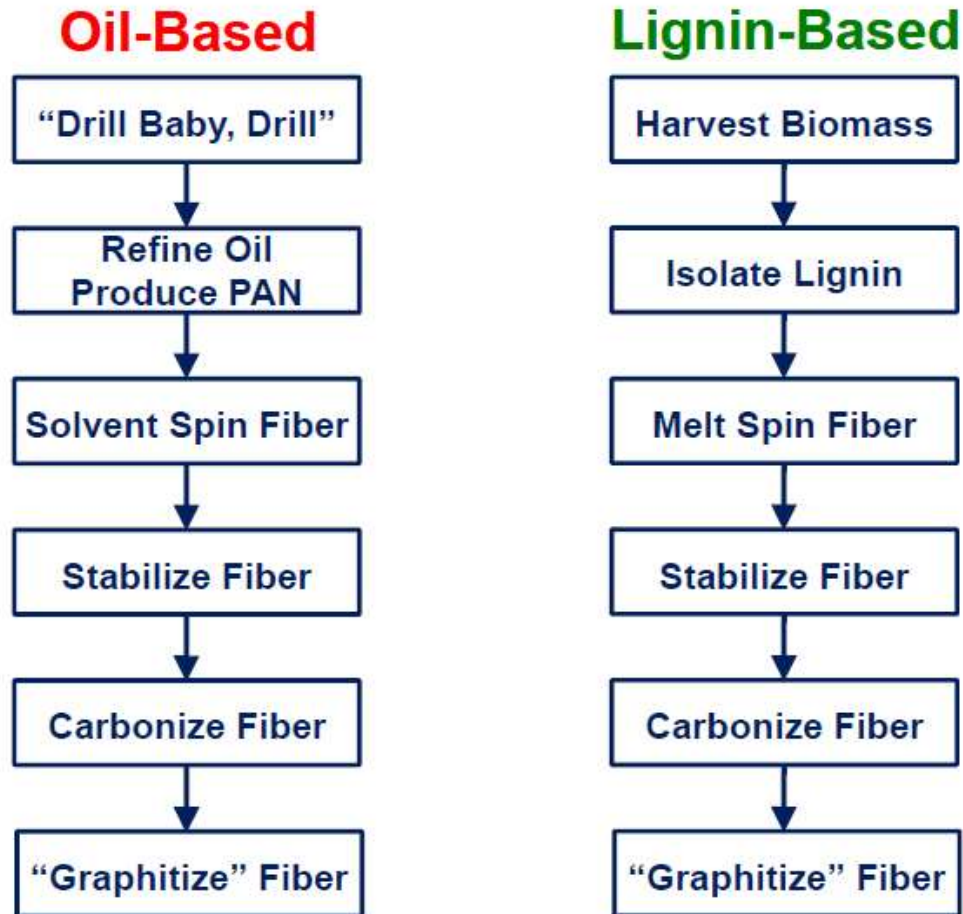
Structural Applications of Carbon Fibres



Properties and Cost of CFs

Type of CF	Modulus of Elasticity	Breaking Strength	Cost
	Gpa	Gpa	\$/kg
Industrial	230	0.38	18 - 24
Standard	220	3.5 - 3.8	36 - 44
Medium-modulus	300	3.5 - 6.2	65 - 73
High-modulus	350 - 450	3.5 - 5.5	100 - 150
Ultrahigh-modulus	500 - 950		250 - 1900
Stainless steel	190	0.86	

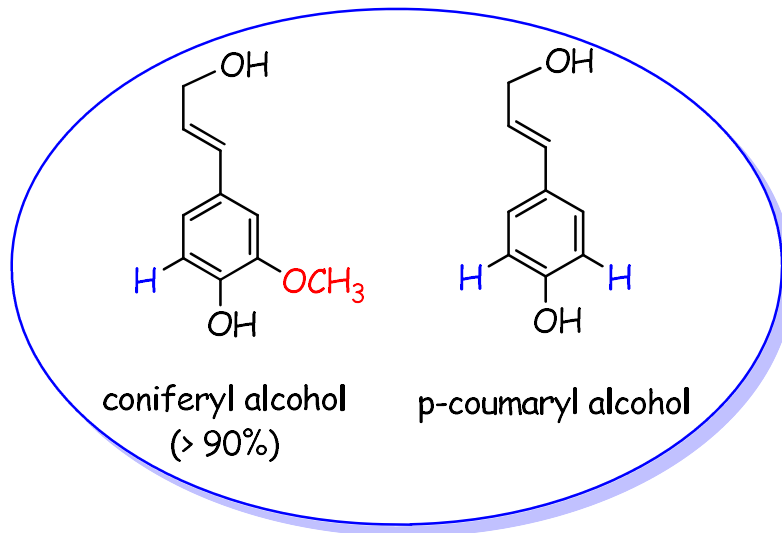
Manufacture of CF – Key Steps



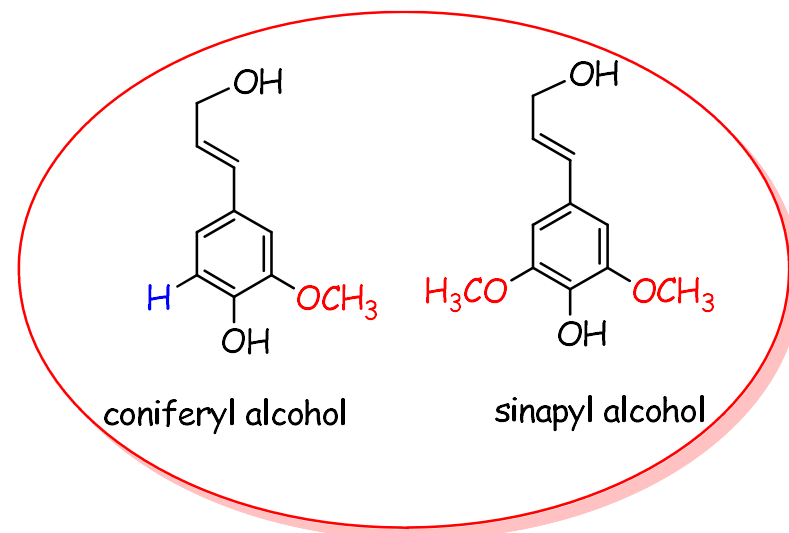
High Demand on Lignin Specification

- > 99% Lignin
- < 500 ppm residual carbohydrates
- < 1000 ppm ash content
- < 500 ppm non-melting particles
- Target Price: ≤ 1 € / kg ready for melt spinning

Hardwood vs Softwood



More difficult to melt spin
More amenable to X-linking



More amenable to melt spin
More difficult to X-linking

Low Cost Production of Carbon Fiber from Lignin

Melt Spinning and Thermal Processing
of Lignin into Carbon Fiber

At Oak Ridge National Lab, Tennessee, USA

Acc.V Spot Magn Det WD Exp | 50 μ m
2.00 kV 5.0 400x SE 10.9 67127



Aalto University
School of Chemical
Technology

F.S. Baker, lecture notes from the NWBC conference in Stockholm, Sweden, 2011

Carbon Fiber Processing



Filament fiber spun
From Kraft hardwood
lignin



Finished carbon fiber being spooled

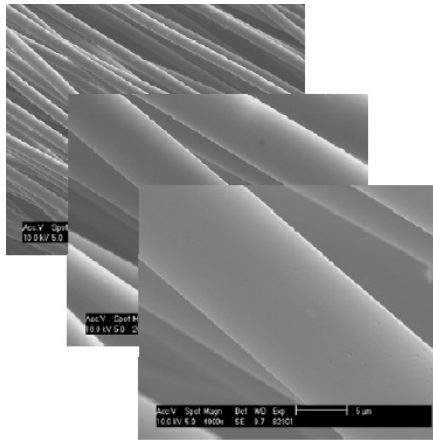


Carbon Fiber Processing

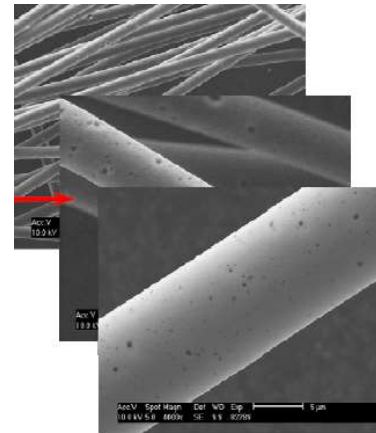
- Viscosity of the melt stays only constant when lignin is highly purified (by organic solvent).
- Melting point of lignin $\sim 170^{\circ}\text{C}$, $190\text{-}240^{\circ}\text{C}$ spinning temperature.
- Thermostabilization improved with higher MM of lignin by oxidative cross-linking.
- Carbon fiber yield (at 1000°C) from lignin feedstock 46-53%.

Carbonization

- 1200 - 1600°C, N₂ and drawing.
- Fast carbonization rate essential; otherwise introduction of pores:



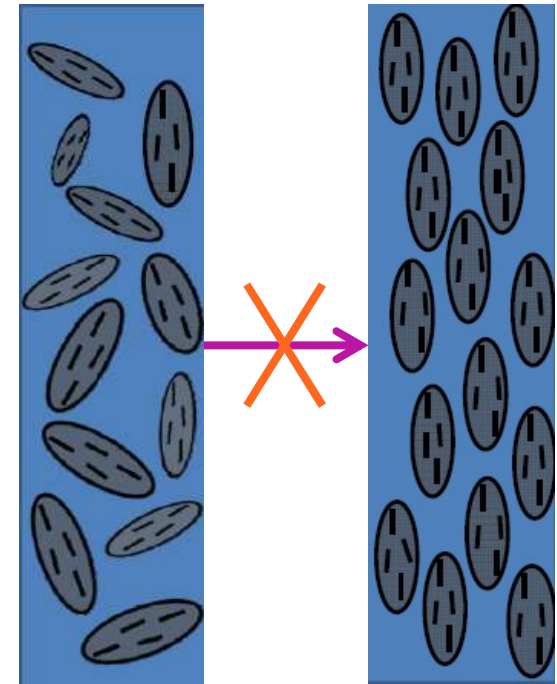
Good fiber structure



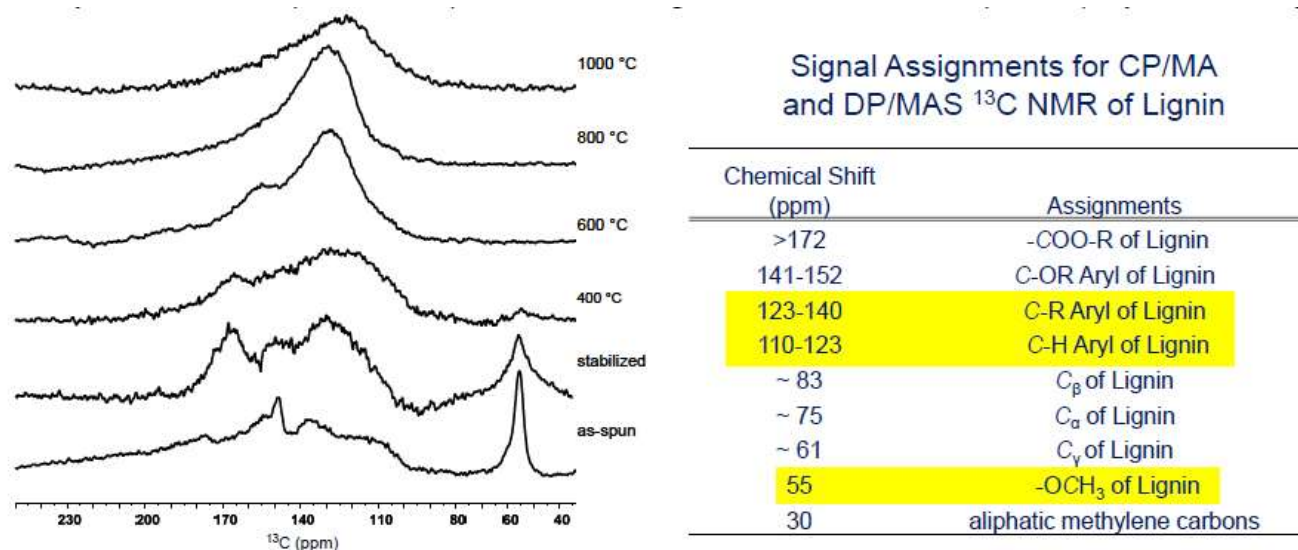
Poor fiber structure

Graphitization

- "Graphitic structure" can be created in lignin-based CF through high T treatment: 2100 - 2400°C.
- No increase in strength properties
- "Graphicity" of lignin-based CF heat-treated to 2100C comparable to that of T-300 (PAN):
- However, modulus not "tracking" degree of graphitic structure. Contrary to T-300, low degree of orientation of crystallites.



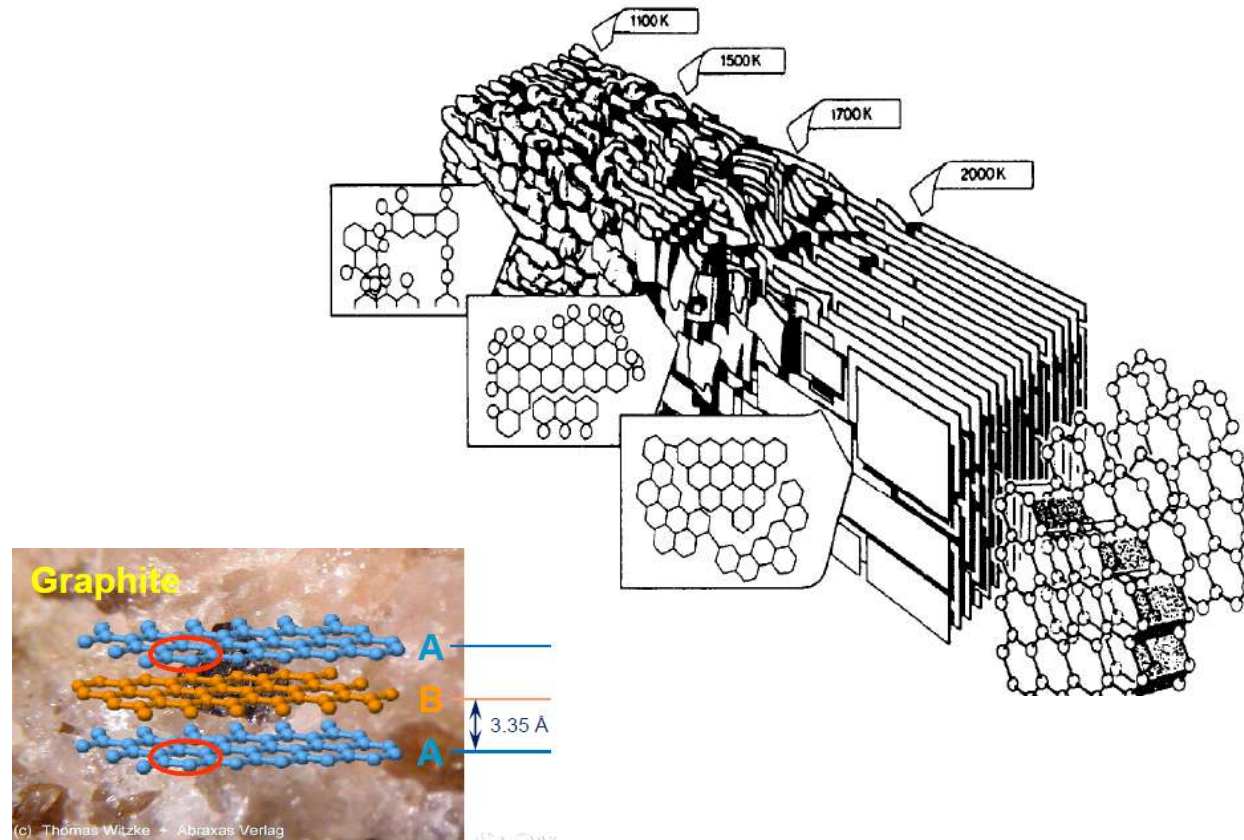
Chemistry of Conversion of Lignin into CF



Broad resonance centered near 123 ppm represents presence of aryl and condensed aryl carbon fiber. Apparent loss of these condensed structures at 1000°C.

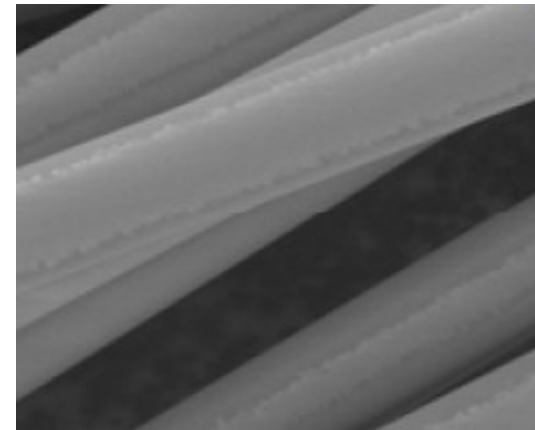
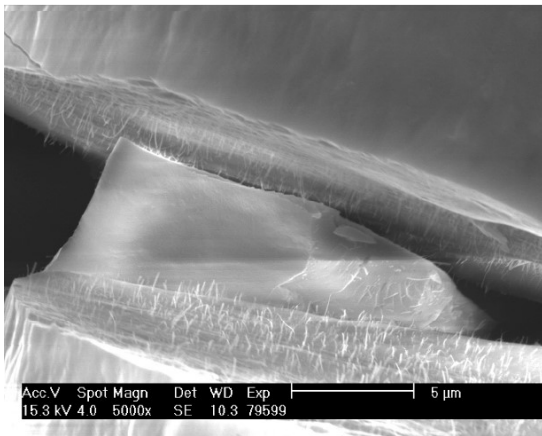


Model of Carbonization, Graphitization Process



Mechanical Properties Obtained

- Tensile Strength: 1.1 GPa (62% of target)
- Modulus: 70 – 80 GPa (50% of target)



Melt-spun fibers



Potential Use in Phenolic Resins

- Oil-based phenol (synthesized from benzene) can be replaced by pure, reactive and low-molecular weight organosolv lignin.
- Oriented strand board (OSB) manufacture with lignin with high phenolic content.
- Benefit to OSB manufacturers: up to 35% of phenol-formaldehyde resin with an equal weight of organosolv lignin will provide substantial improvements to board properties→reduced swelling, higher modulus of rupture.

Potential Use in Phenolic Resins

- Resin binders for exterior grade wood panel production is the largest market for PF resins.
- Lignin could replace up to 50% of the expensive MDI* on an equal weight basis in an isocyanate resin used in wood binder application.
- Thermoplastic processing of lignin through injection Molding (ARBOFORM®)



Dimethylsulfoxide (DMSO)

American Process

To make DMSO from black liquor

