Biossíntese e deposição de lignina nas paredes celulares

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Outline

- Revisando sobre a estrutura da lignina

- Biossíntese de monolignóis

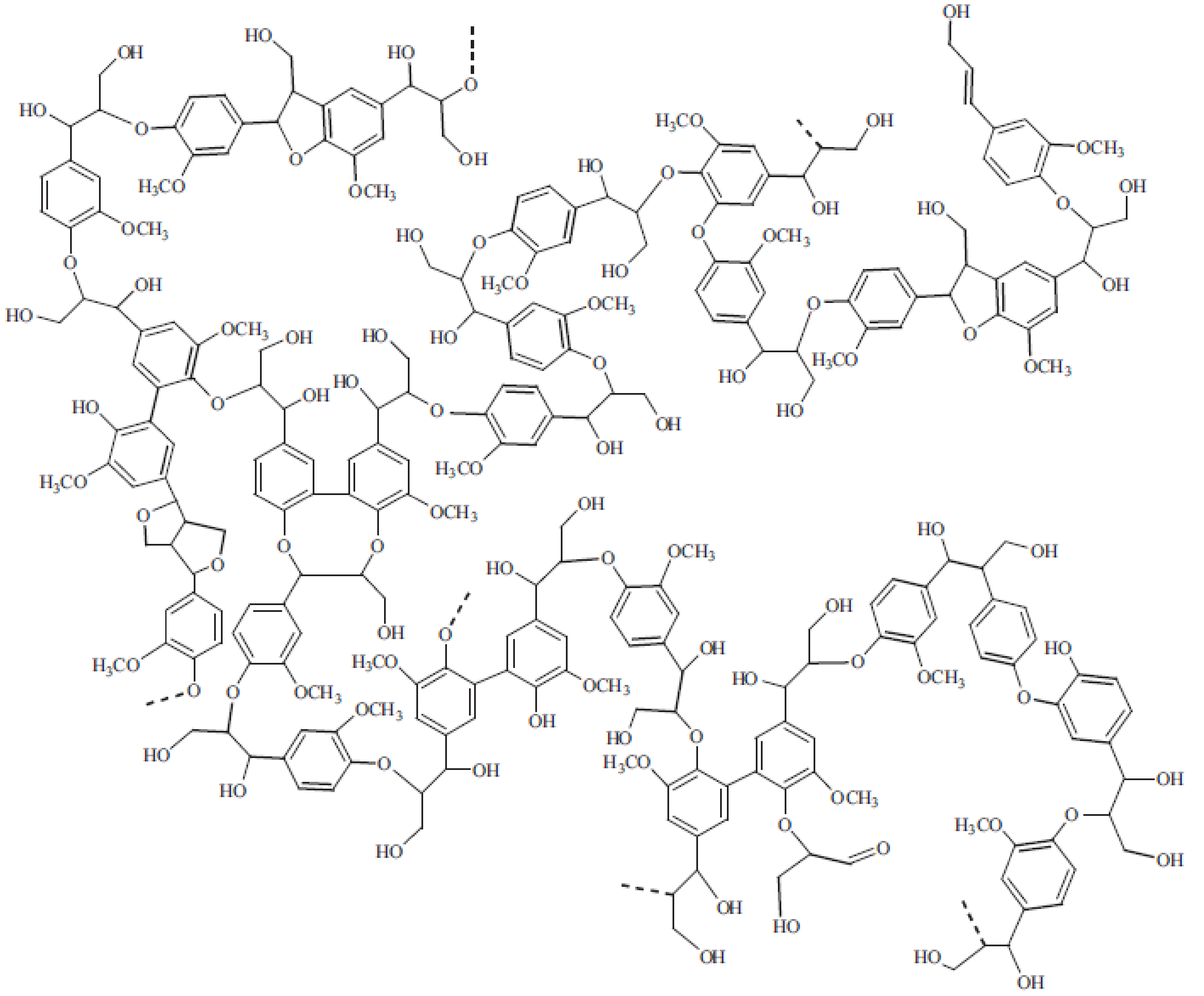
- Polimerização e deposição de lignina

Ref. básicas:

1. Gunnar Henriksson 2009. Lignin, In: Pulp and Paper Chemistry and Technology Vol 1: Wood Chemistry and Wood Biotechnology. Edited by Monica Ek, Göran Gellerstedt, Gunnar Henriksson, cap 6, pp. 121-147

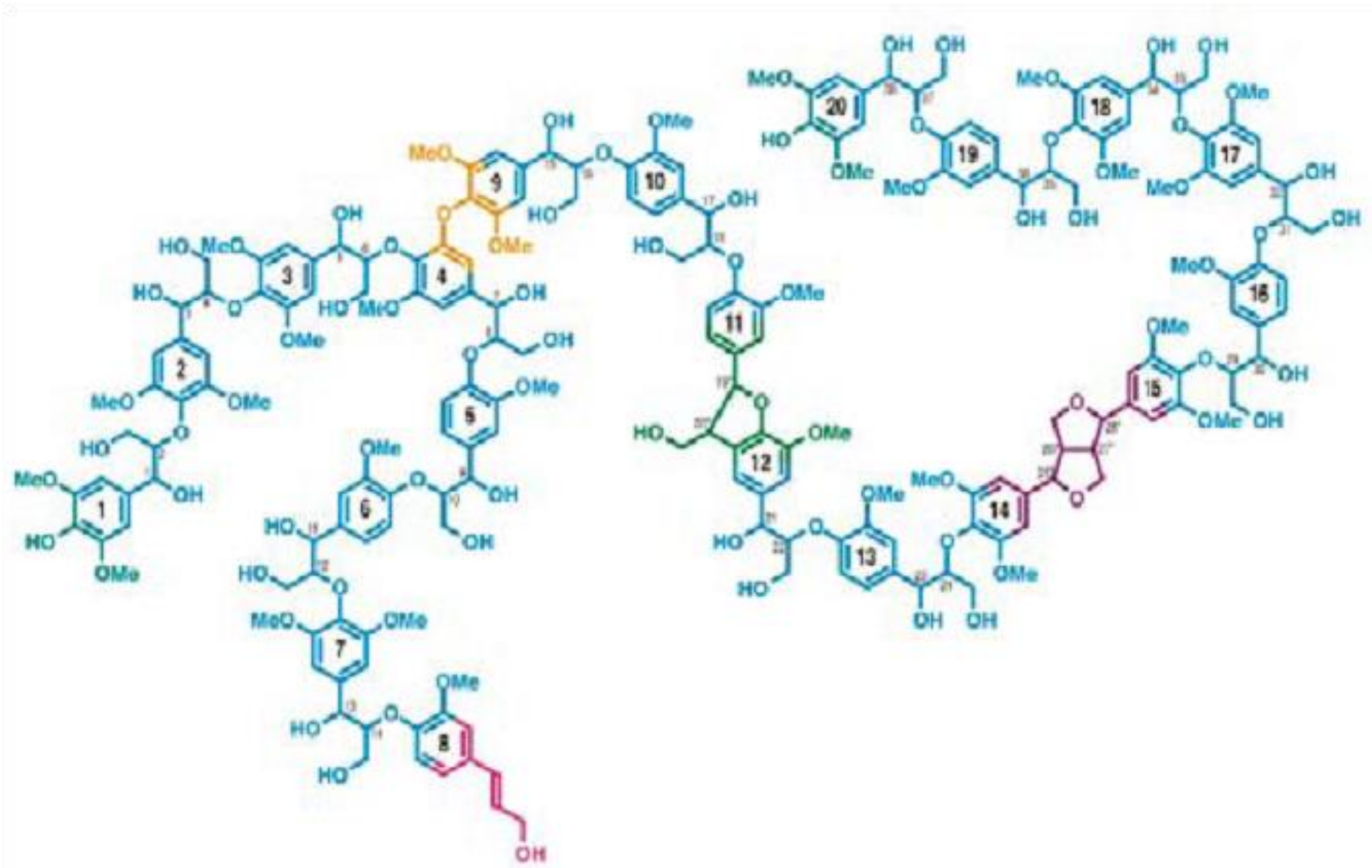
2. Wout Boerjan, John Ralph and Marie Baucher 2003. Lignin Biosynthesis. Annu. Rev. Plant Biol. 54:519-546.

A molécula de lignina

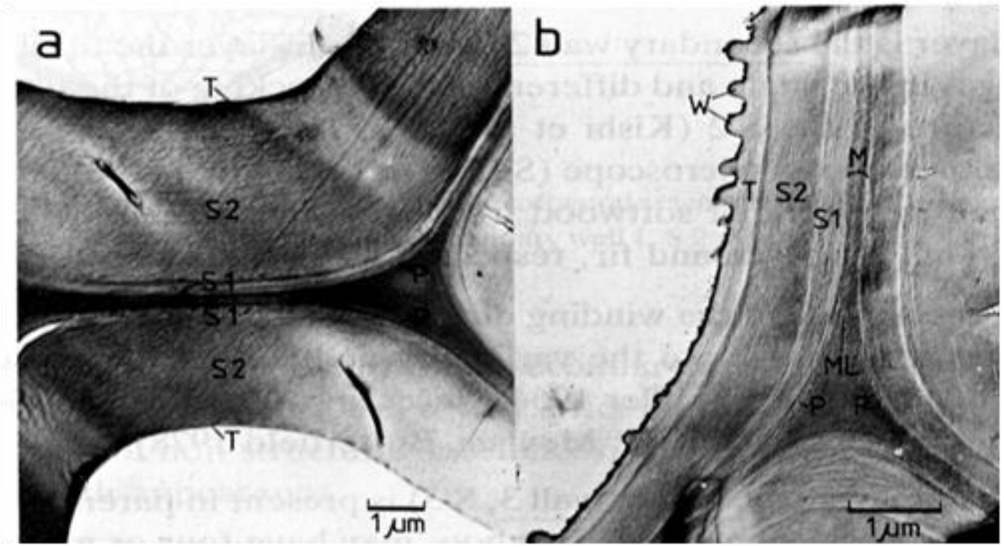


Henriksson 2009. Lignin, In: Pulp and Paper Chemistry and Technology - CONÍFERAS

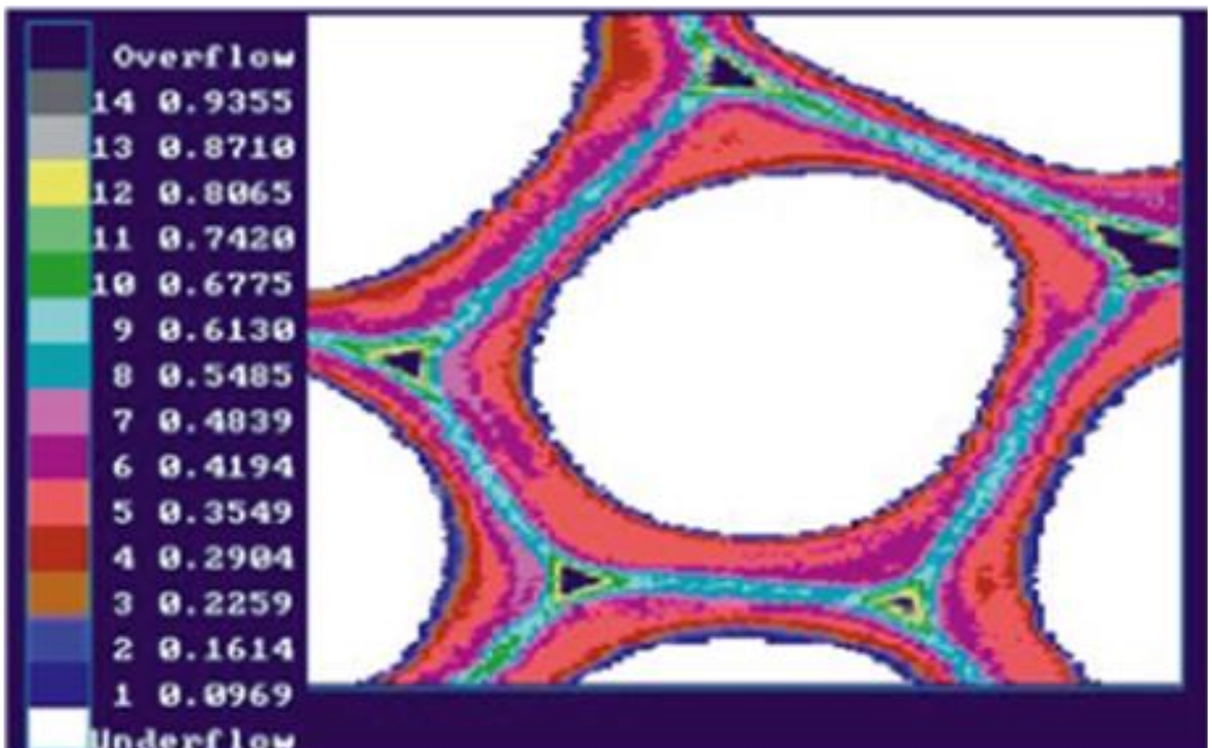
A molécula de lignina



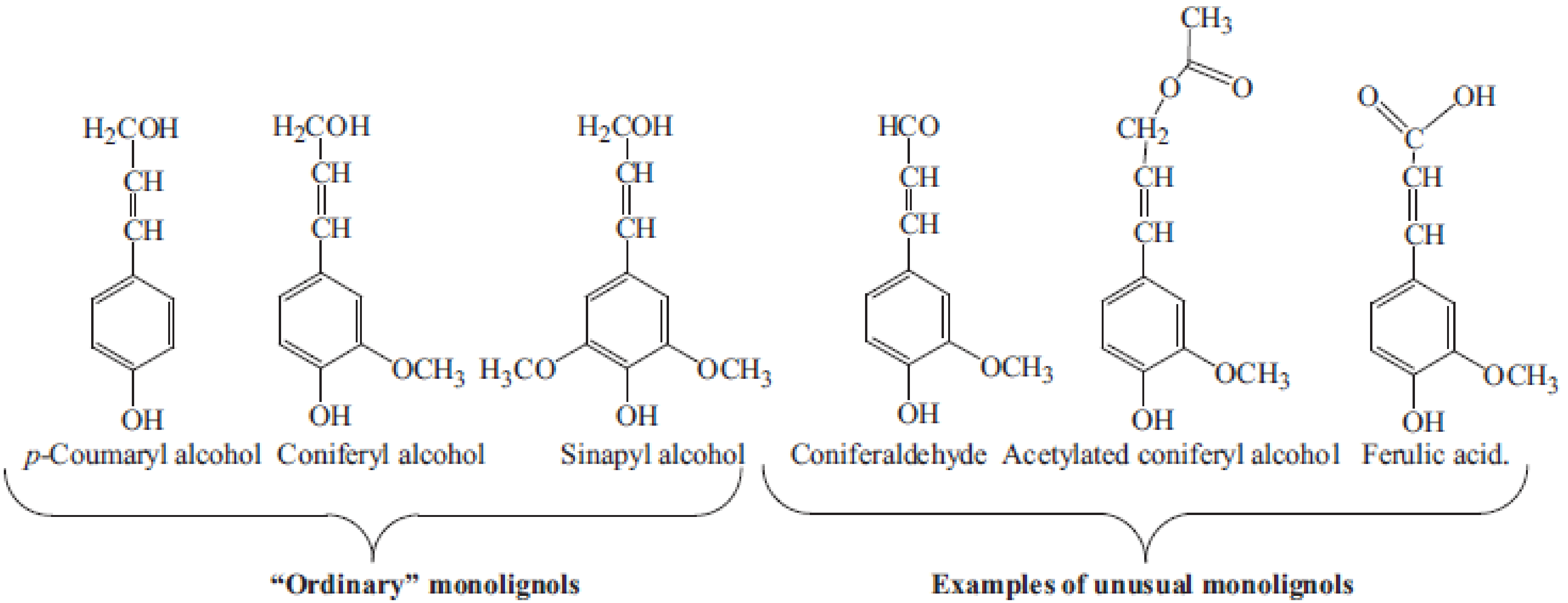
Ralph 2004. - FOLHOSAS



Lignina nas paredes celulares



Precursores da biossíntese de lignina



Proporção dos precursores em diferentes tipos de lignocelulósicos

Table 6.1. Composition of monolignols (lignin monomers) in different plants.

Plant	**p-Coumaryl alcohol (%)**	**Coniferyl alcohol (%)**	**Sinapyl alcohol (%)**
Coniferous¨ softwood	<5	>95	None or Trace
Eudocotyledonous hard-wood	0–8	25–50	46–75
Monocotyledonous grass	5–33	33–80	20–54

Revendo a nomenclatura na molécula de lignina

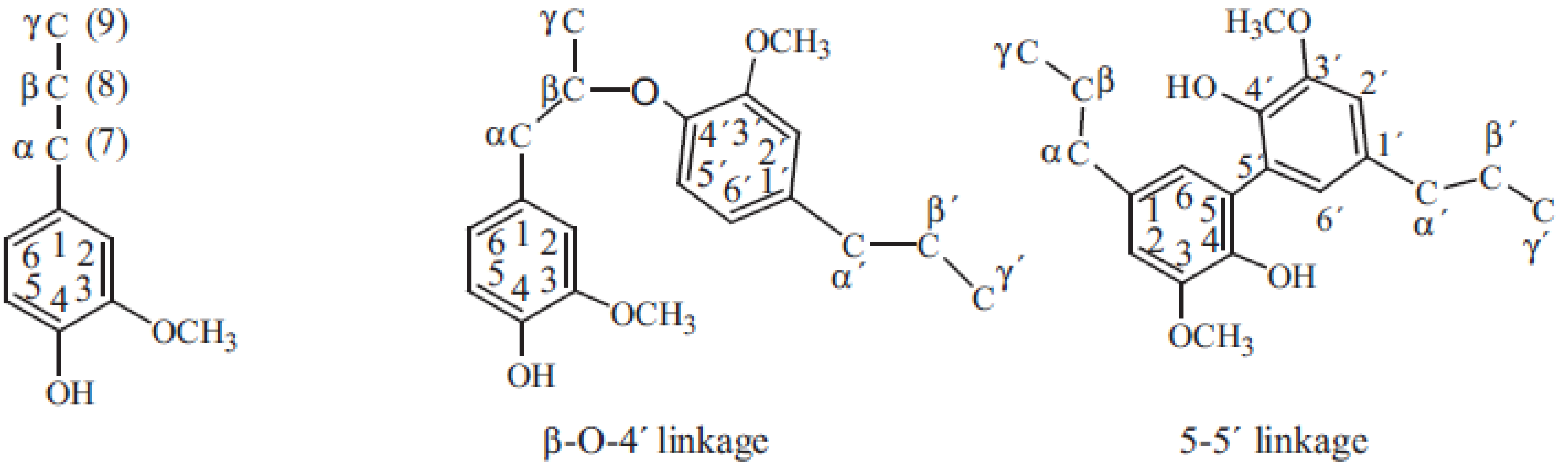
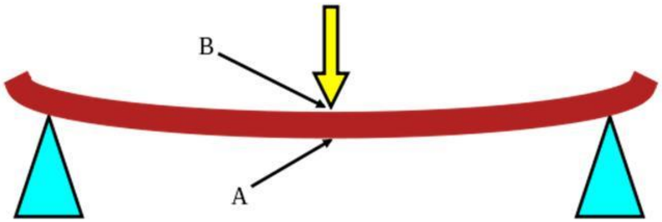


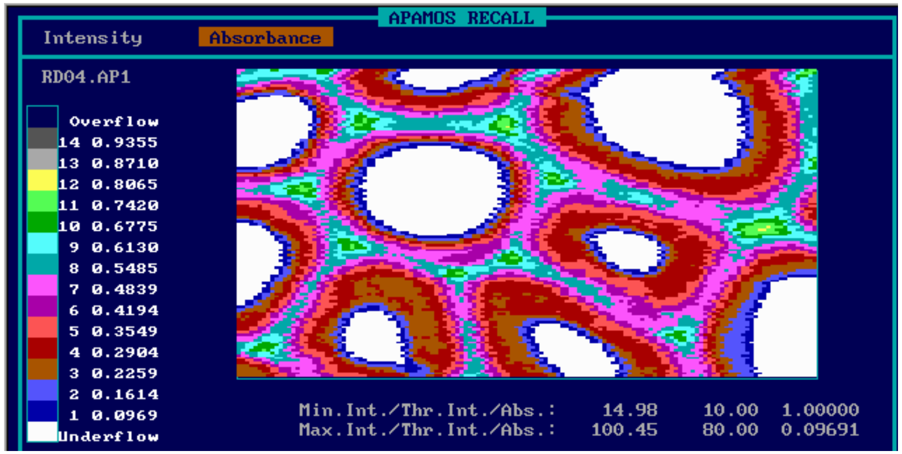
Figure 6.3. Nomenclature of lignin carbons.

Funções biológicas da lignina

- Lignin gives stiffness to the cell walls



- Lignin glues different cells together in woody tissues



- Lignin makes the cell wall hydrophobic

- Lignin is a protection against microbial degradation of wood

Iniciação da polimerização dos precursores por peroxidases e lacases - formação do radical fenoxila

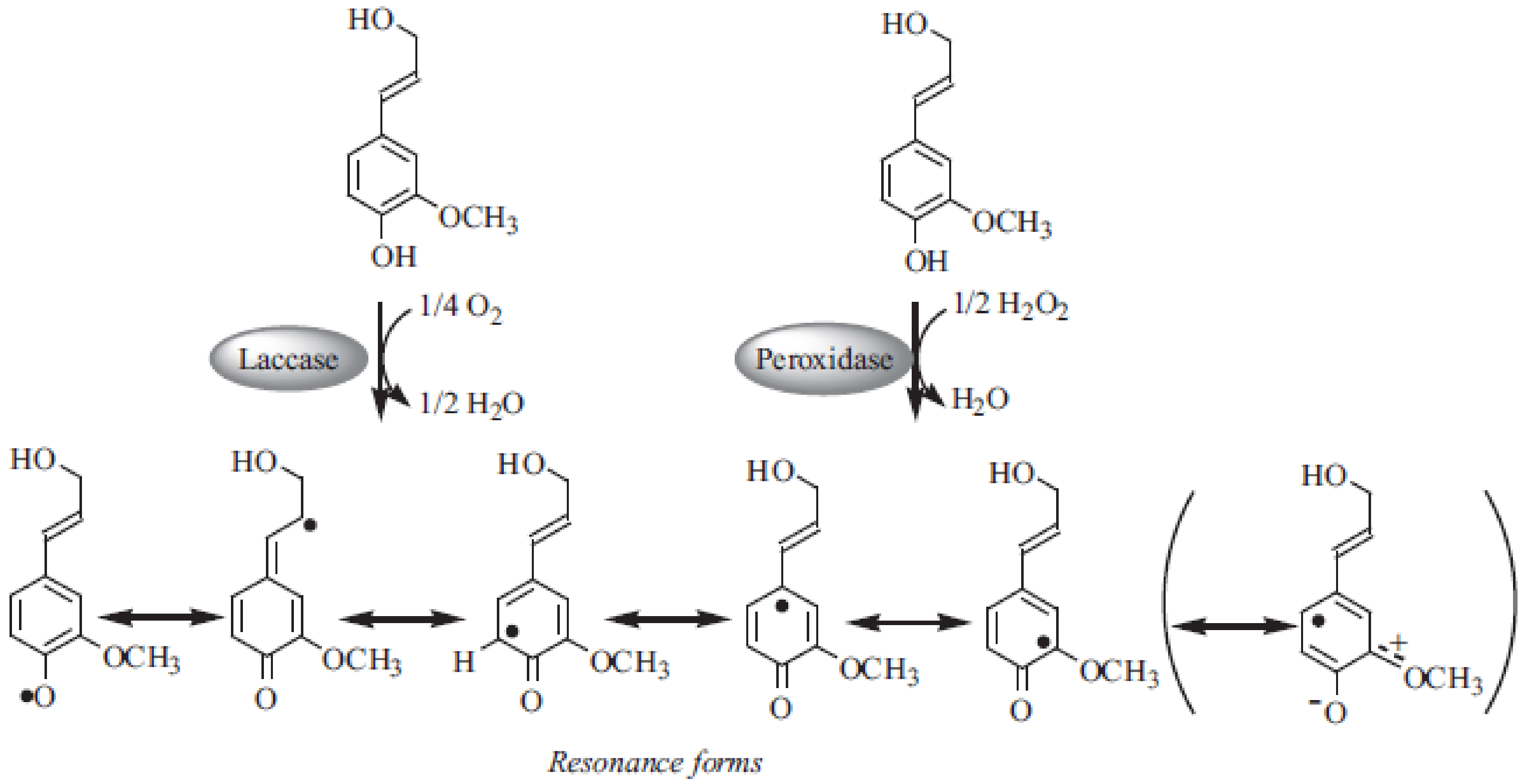


Figure 6.5. Enzymatic generation of resonance stabilized monolignol radicals.

Acoplamentos possíveis entre radicais

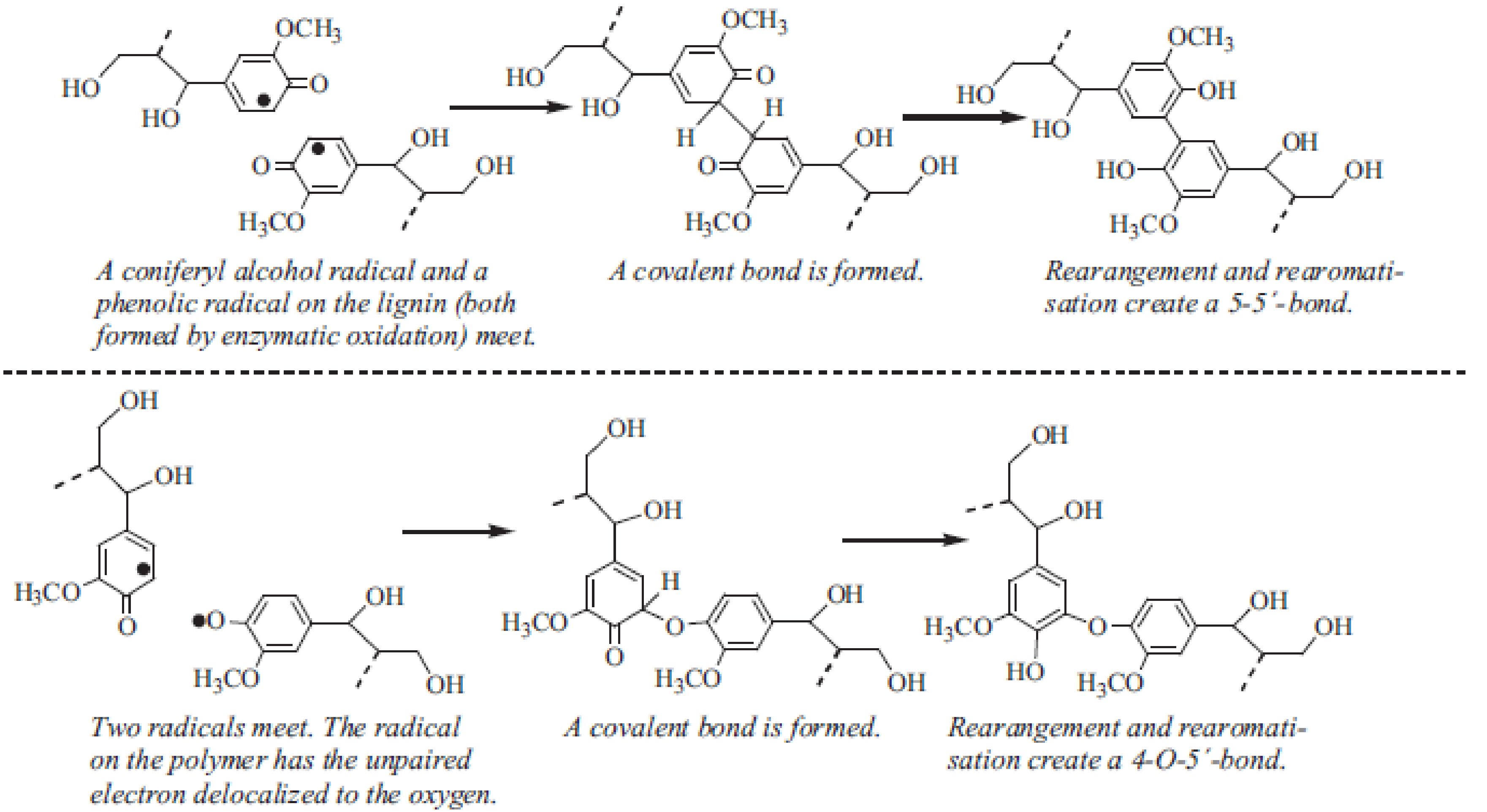


Figure 6.7. Formation of 5-5´- and 4-O-5´-bonds.

Acoplamentos possíveis entre radicais

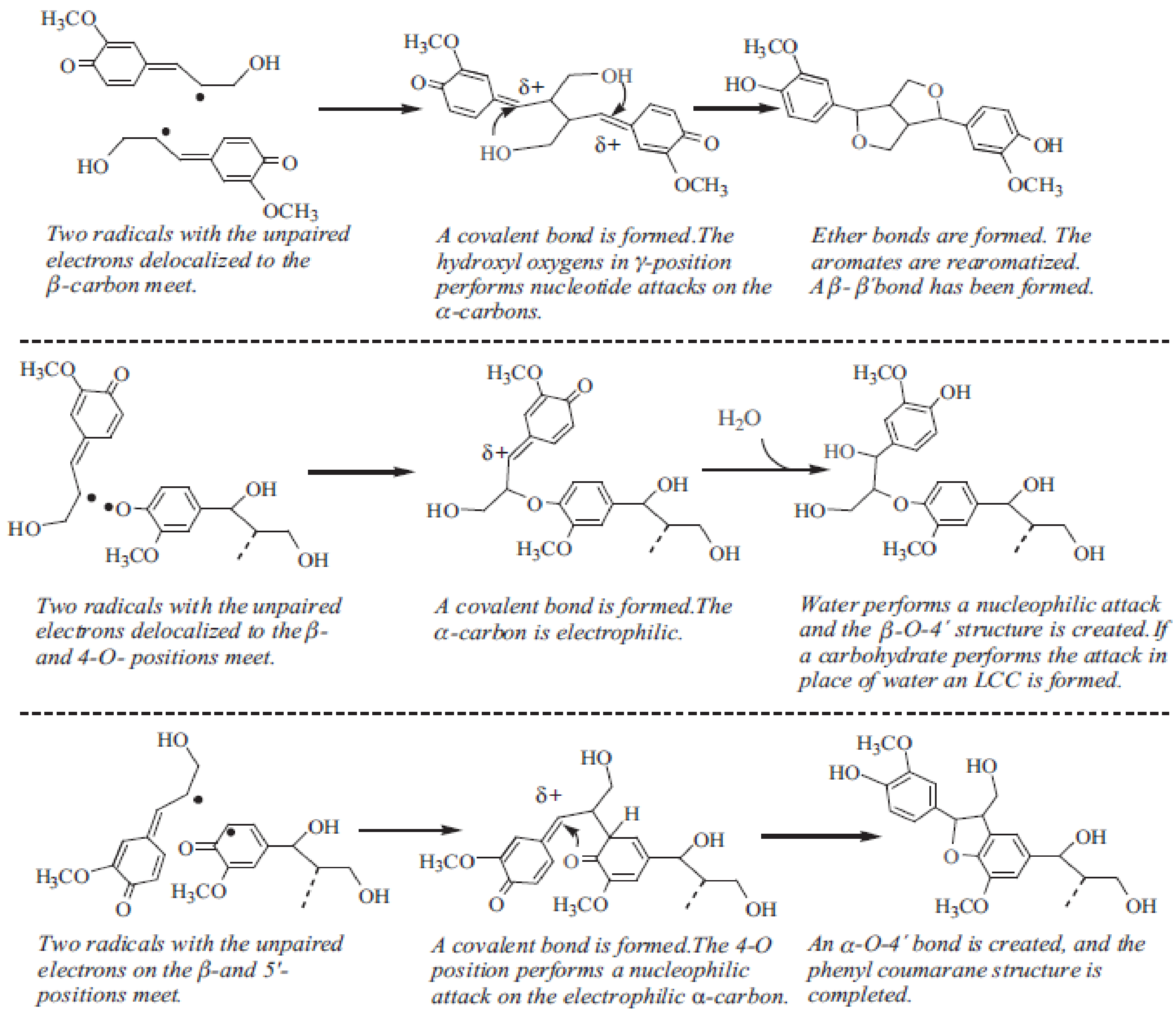


Figure 6.8. Mechanism of formation of β- β´, β-O-4´ and β-5´ bonds in lignin.

Acoplamentos possíveis entre radicais

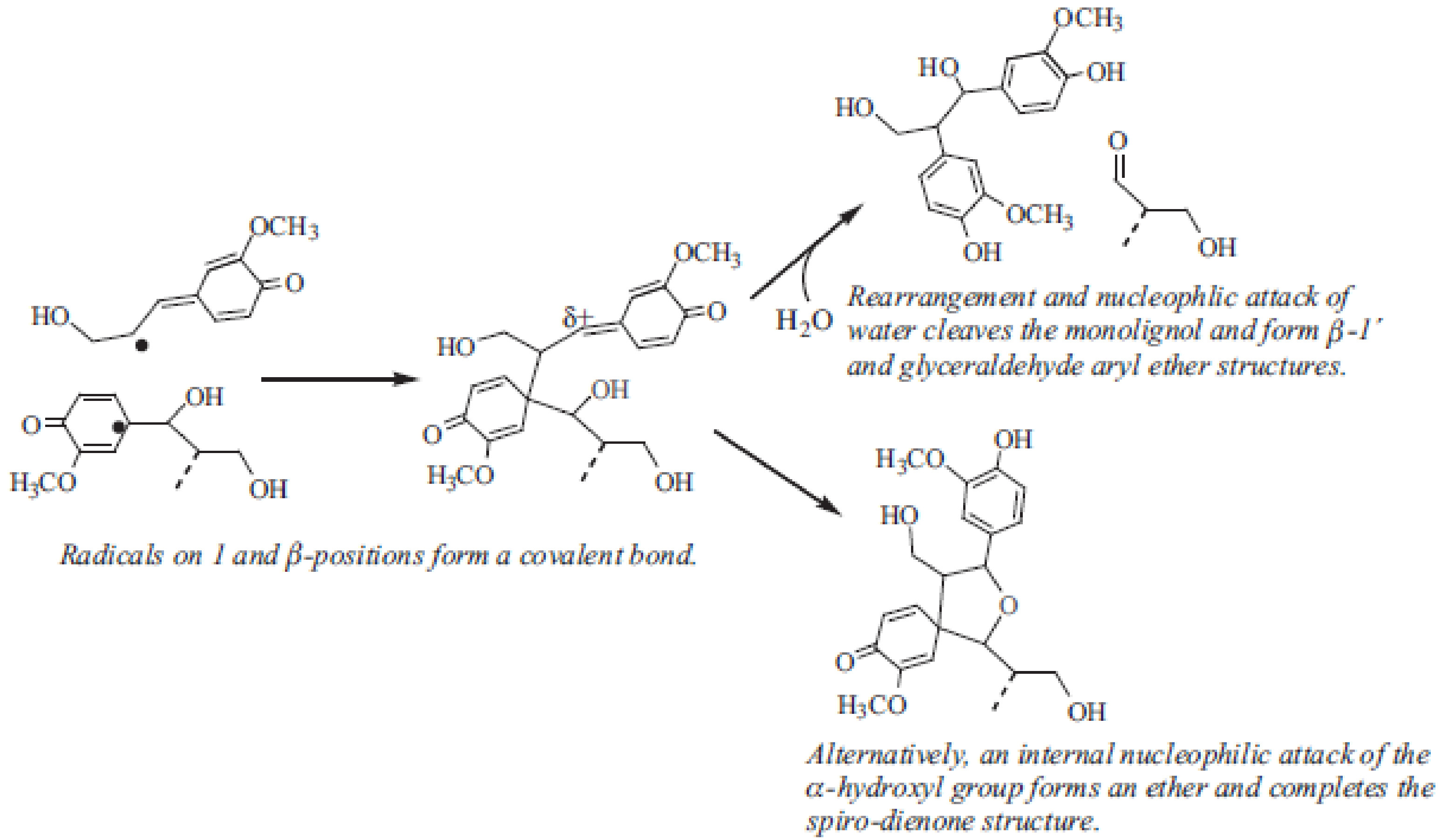


Figure 6.11. Formation of β-1′ and 4-=-1′structures.

Acoplamentos possíveis entre radicais

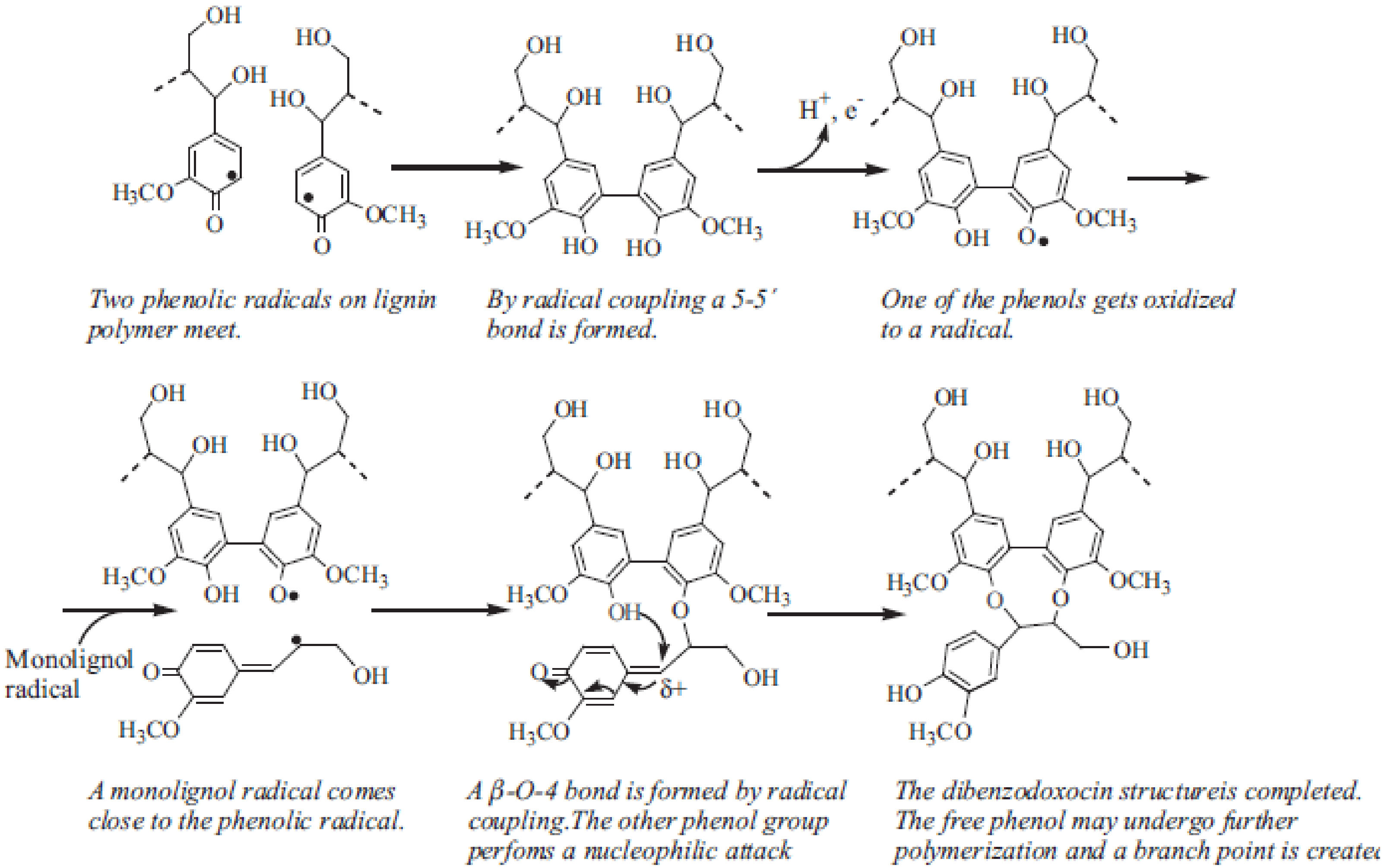


Figure 6.12. Formation of dibenzodoxocin, one of the most important branch points in lignin.

Frequência das ligações entre os precursores

Table 6.2. Bonds between monolignols and lignin functional groups.

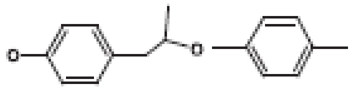
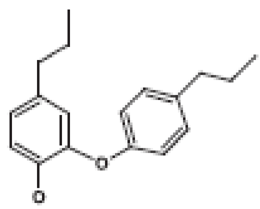
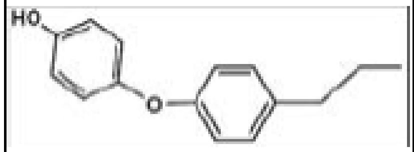
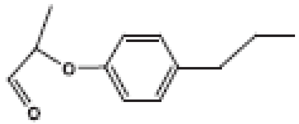
Name	Bonds	Structure*	Frequency softwood (%)	Frequency hardwood (%)
Ether bonds				
β-aryl ether	β-O-4′		35–60	50–70
Diaryl ether	4-O-5′		<4	7?
	1-O-4′		low	low
Glyceraldehyde aryl ether	β-O-4′		<1	<1

Table 6.2. Bonds between monolignols and lignin functional groups.

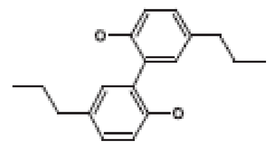
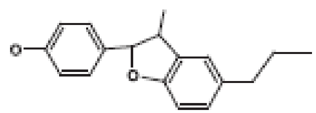
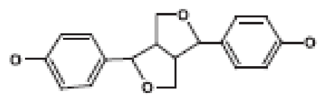
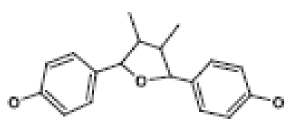
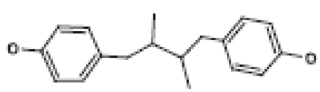
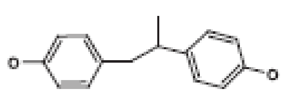
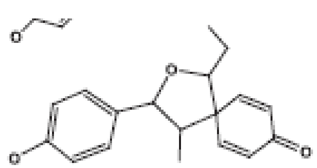
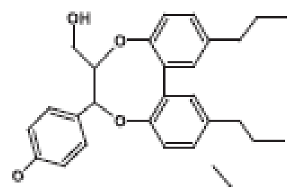
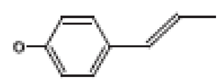
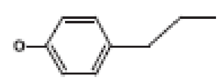
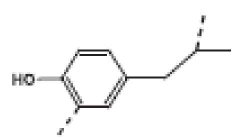
Name	Bonds	Structure*	Frequency softwood (%)	Frequency hardwood (%)
Carbon-carbon bonds (condensed bonds)				
Dihydroxy biphenyl	5-5´		10	~5
Phenyl coumarane	β-5´		11–12	4–9
Pinoresinol	ββ´		2–3	3–4
	ββ´		<1	none
Secoisolariciresinol			1–2	none
	β-1´		1–2	1
Spirodienon	β-1´		1–3	2–3

Table 6.2. Bonds between monolignols and lignin functional groups.

Name	Bonds	Structure*	Frequency softwood (%)	Frequency hardwood (%)

Name	Bonds	Structure*	Frequency softwood (%)	Frequency hardwood (%)

Name	Bonds	Structure*	Frequency softwood (%)	Frequency hardwood (%)
Dibenzo-dioxocin			4–5	trace
End groups				
Coniferyl alcohols			1–6	Trace–6
Dihydroconiferyl alcohol			2	none
Free phenol			11	9?

Modelo de polimerização baseado na difusão do radical no álcool coniferílico - polimerização "end-wise"

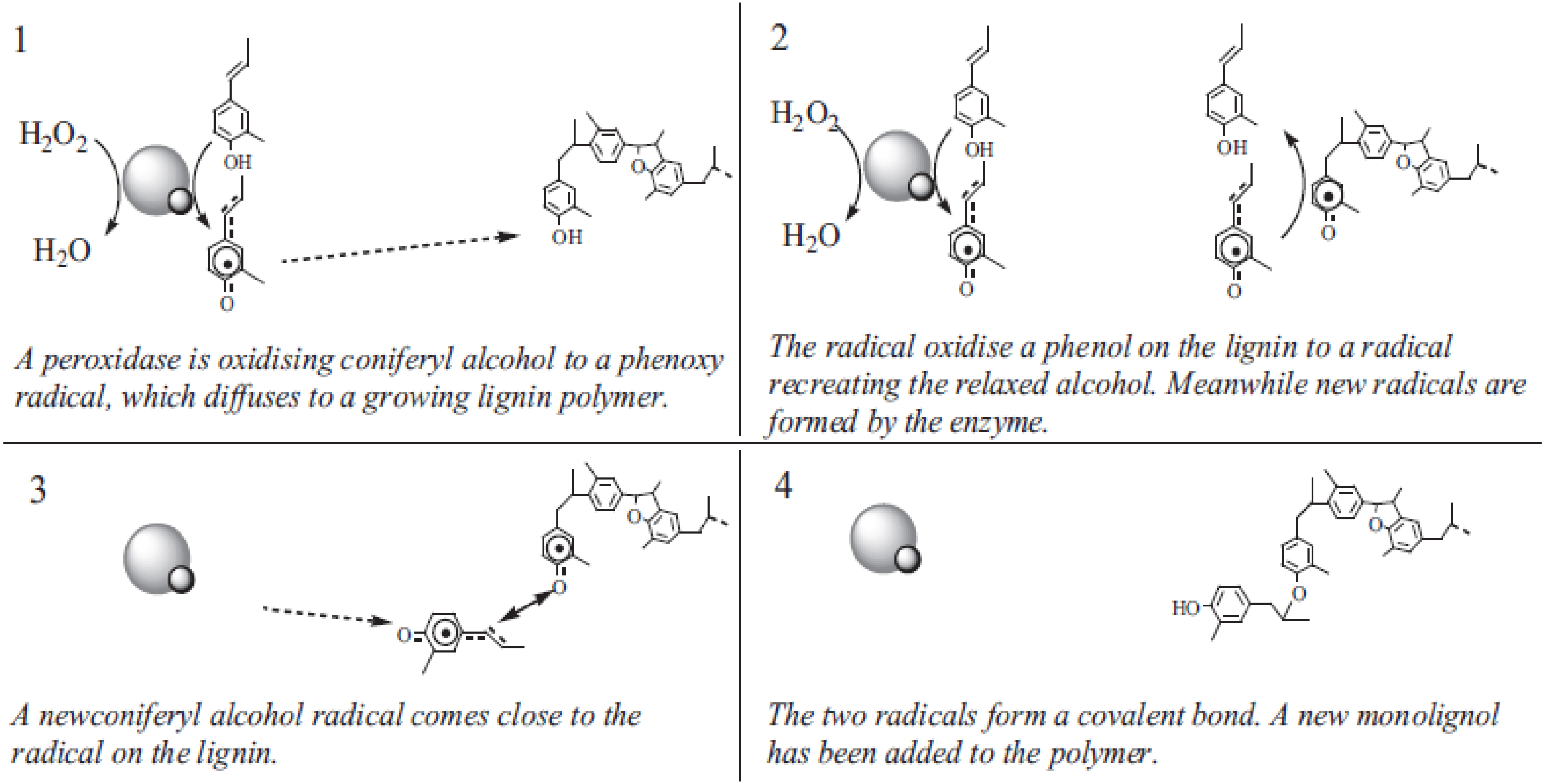


Figure 6.6. Principe for indirect enzymatic driven polymerization.

Modelo que ilustra a lignina preenchendo espaços entre as cadeias de hemicelulose com "cross-link" ocasional

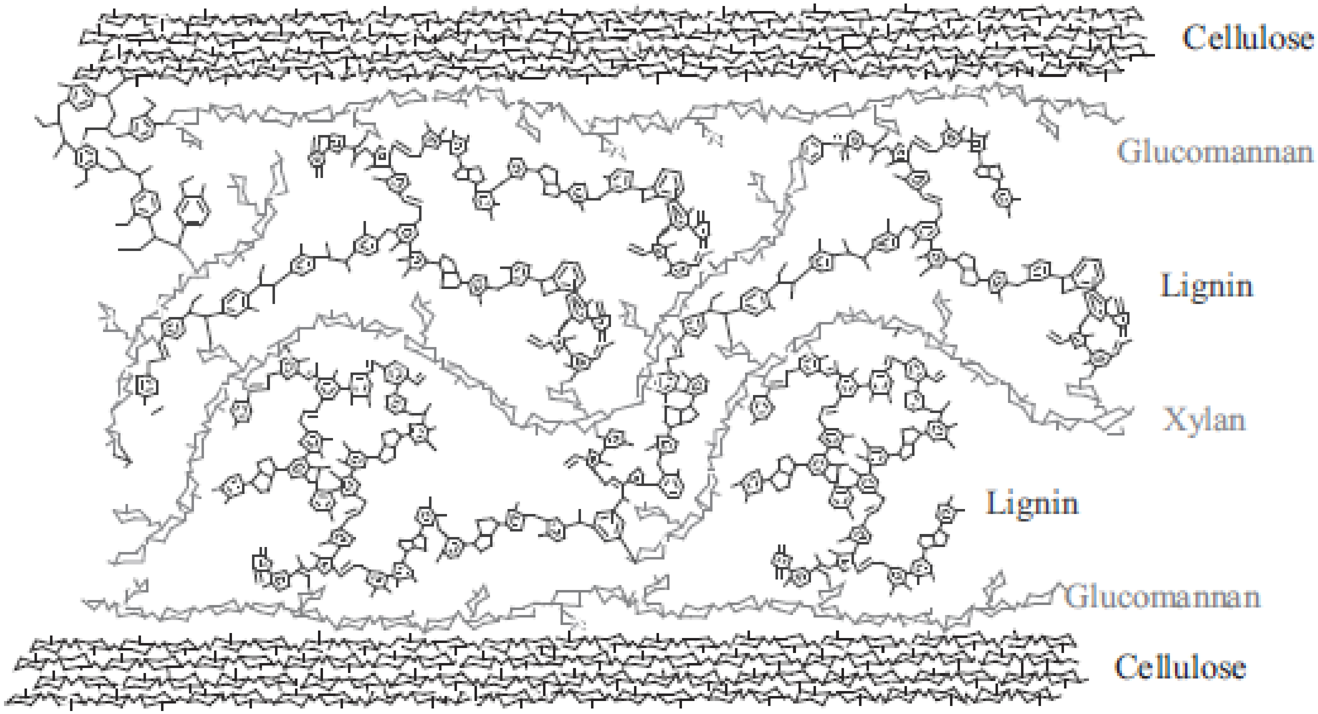
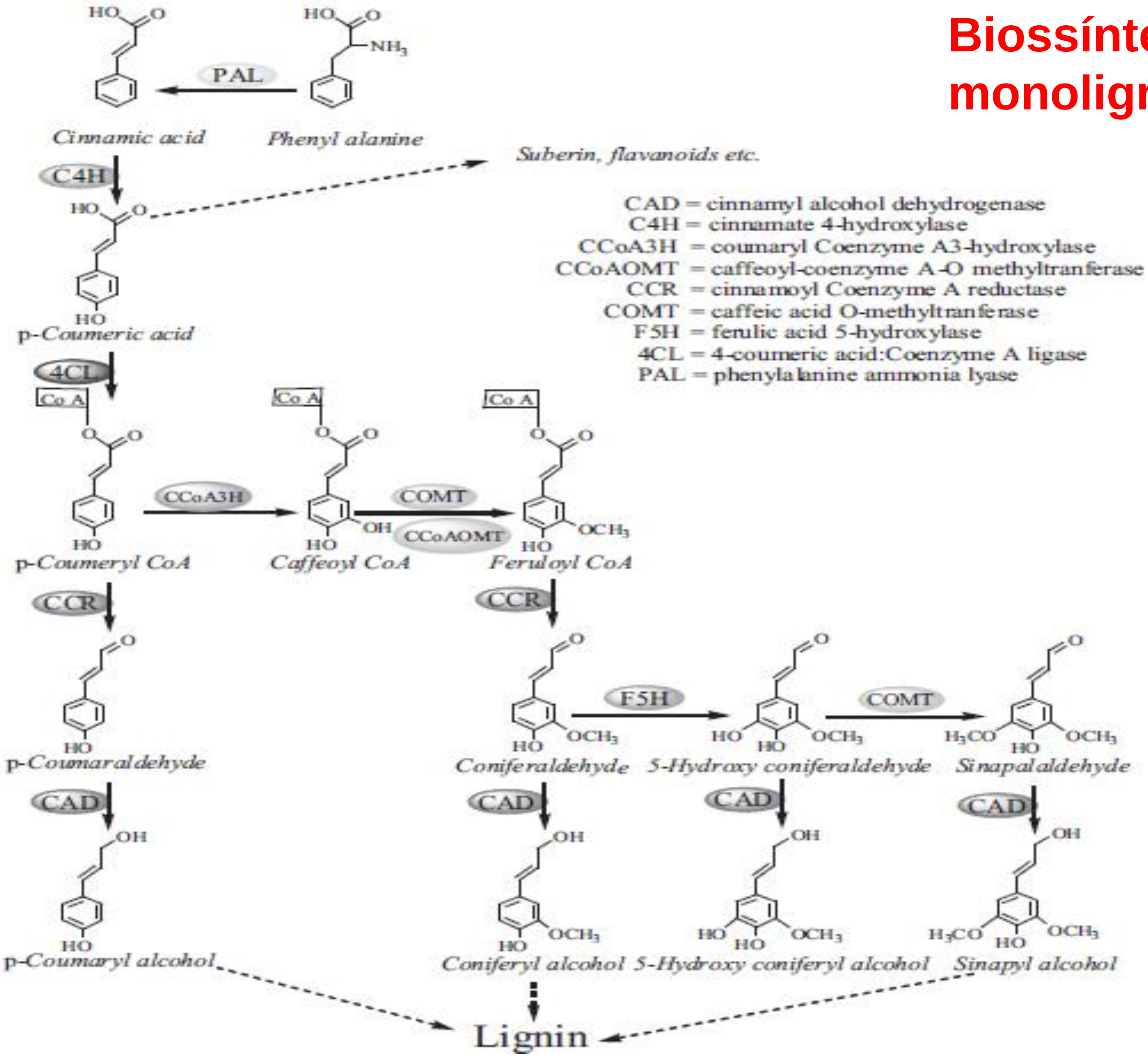
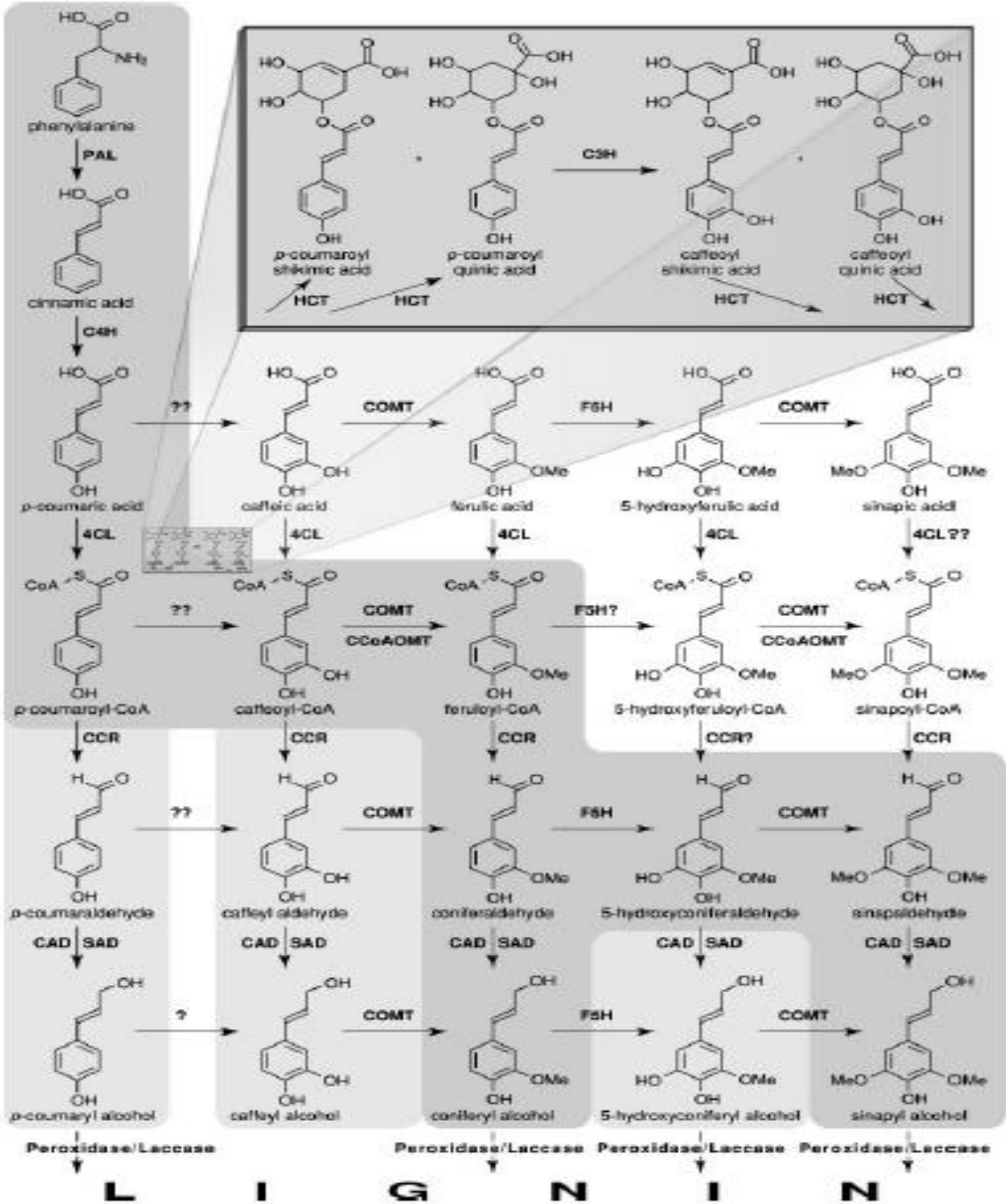


Figure 6.15. Distribution of lignin and polysaccharides on molecular level. This model for the S2 layers is based on studies by Sahlmén and coworkers and Lawoko and coworkers. Most of the lignin is believed to be located between glucomannan and xylan, but there are also some direct contacts between lignin and cellulose. In softwood covalent bonds between lignin and both hemicelluloses (glucomannan and xylan) are frequent. In hardwood the glucomannan content is very low and thus the LCC between lignin and xylans dominates. For both types of wood, the lignin covalently cross-links the different polysaccharides forming networks.

Biossíntese de monolignóis




phenylalanine
PAL
cinnamic acid
C4H
p-coumaric acid
??
caffeic acid
COMT
ferulic acid
F5H
5-hydroxyferulic acid
COMT
sinapic acid
p-coumaroyl shikimic acid
p-coumaroyl quinic acid
C3H
caffeoyl shikimic acid
caffeoyl quinic acid
HCT
HCT
HCT
HCT
4CL
4CL
4CL
4CL
4CL??
p-coumaroyl-CoA
??
caffeoyl-CoA
COMT
CCoAOMT
feruloyl-CoA
F5H?
5-hydroxyferuloyl-CoA
COMT
CCoAOMT
sinapoyl-CoA
CCR
CCR
CCR
CCR?
CCR
p-coumaraldehyde
??
caffeyl aldehyde
COMT
coniferaldehyde
F5H
5-hydroxyconiferaldehyde
COMT
sinapaldehyde
CAD SAD
CAD SAD
CAD SAD
CAD SAD
CAD SAD
p-coumaryl alcohol
?
caffeyl alcohol
COMT
coniferyl alcohol
F5H
5-hydroxyconiferyl alcohol
COMT
sinapyl alcohol
Peroxidase/Laccase
Peroxidase/Laccase
Peroxidase/Laccase
Peroxidase/Laccase
L I G N I N

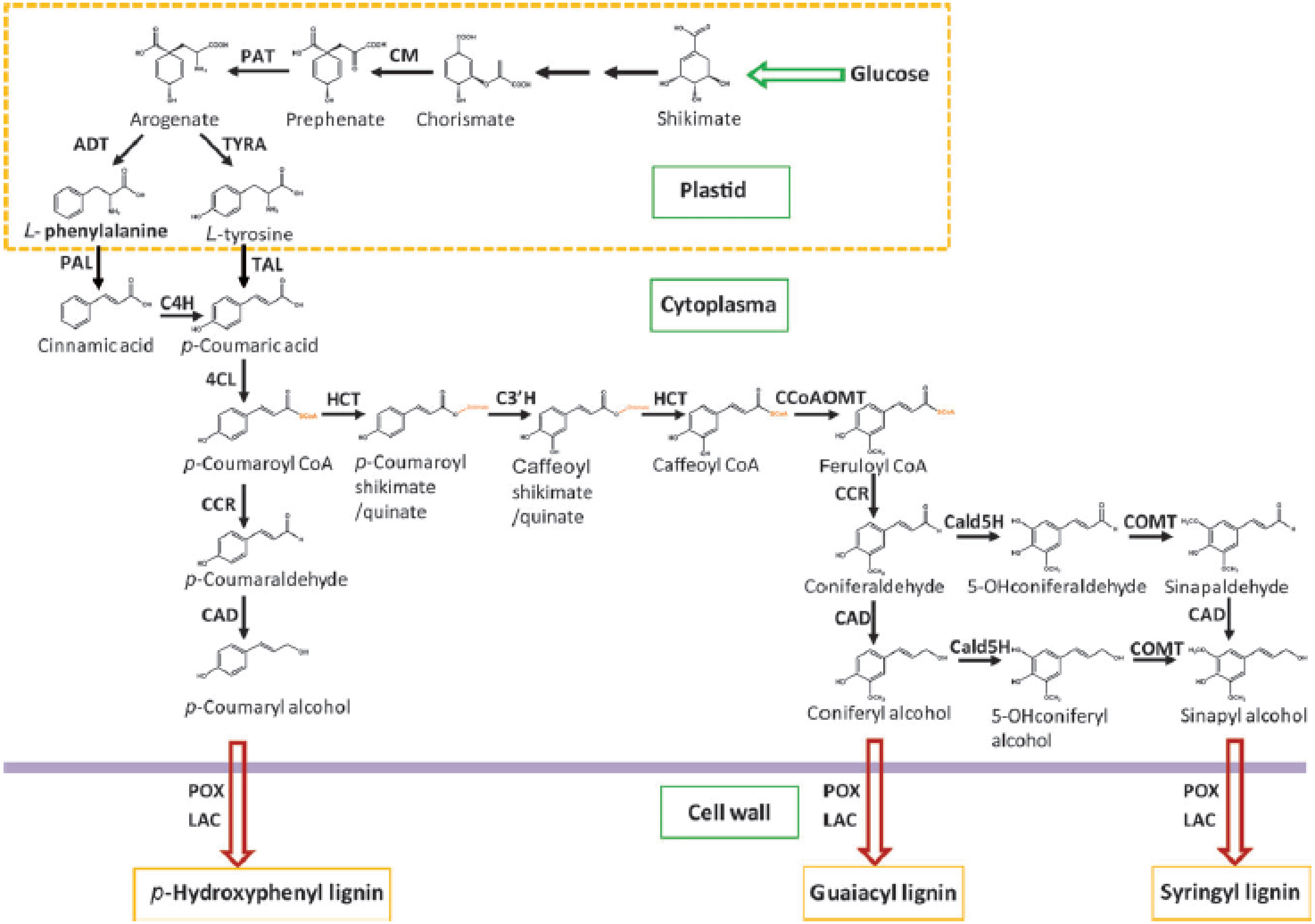
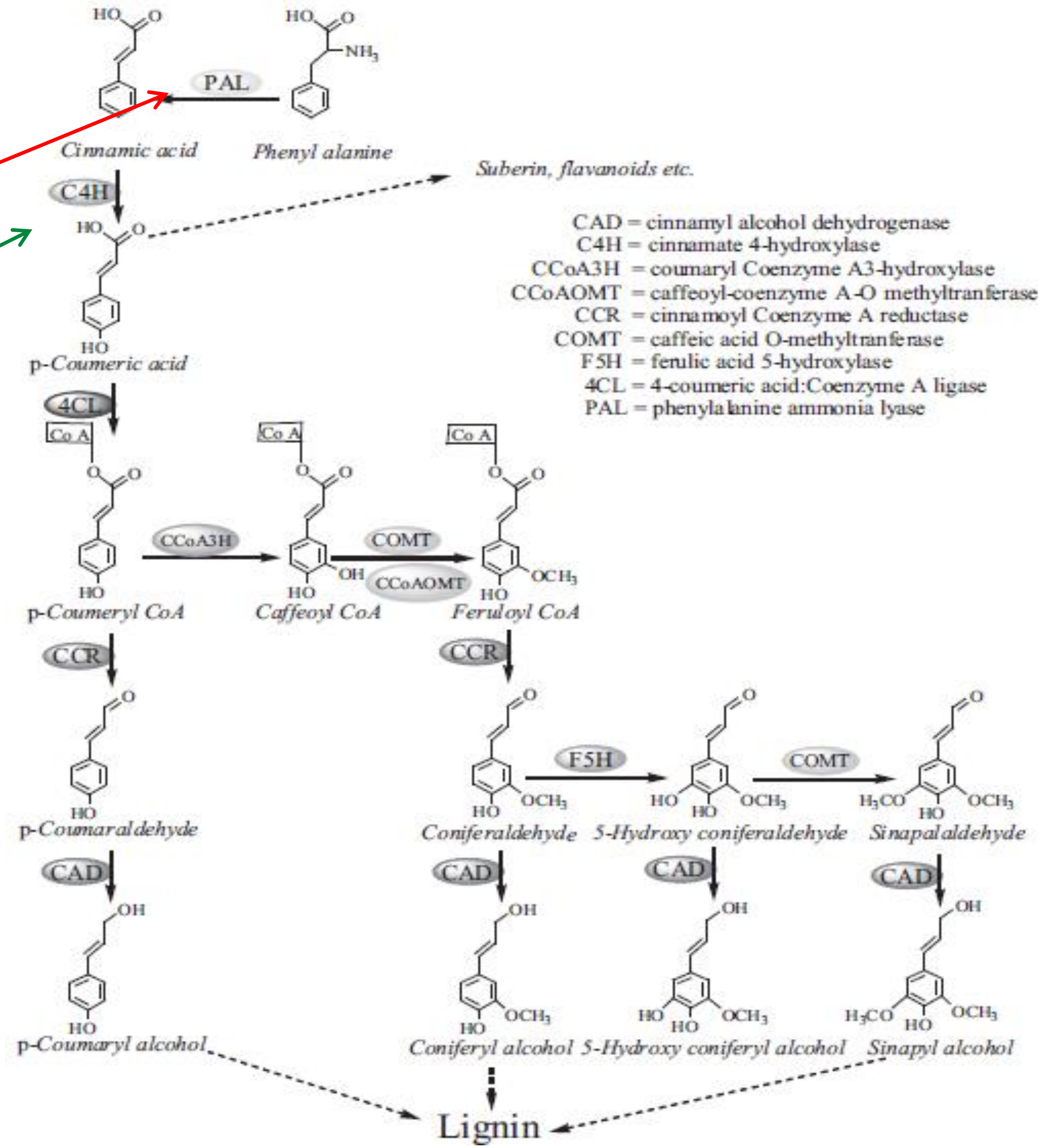


Figure 1. The Scheme of the Simplified Shikimate–Phenylpropanoid–Lignin Biosynthetic Pathway, Illustrating Different Compartmentalization of the Biosynthesis.

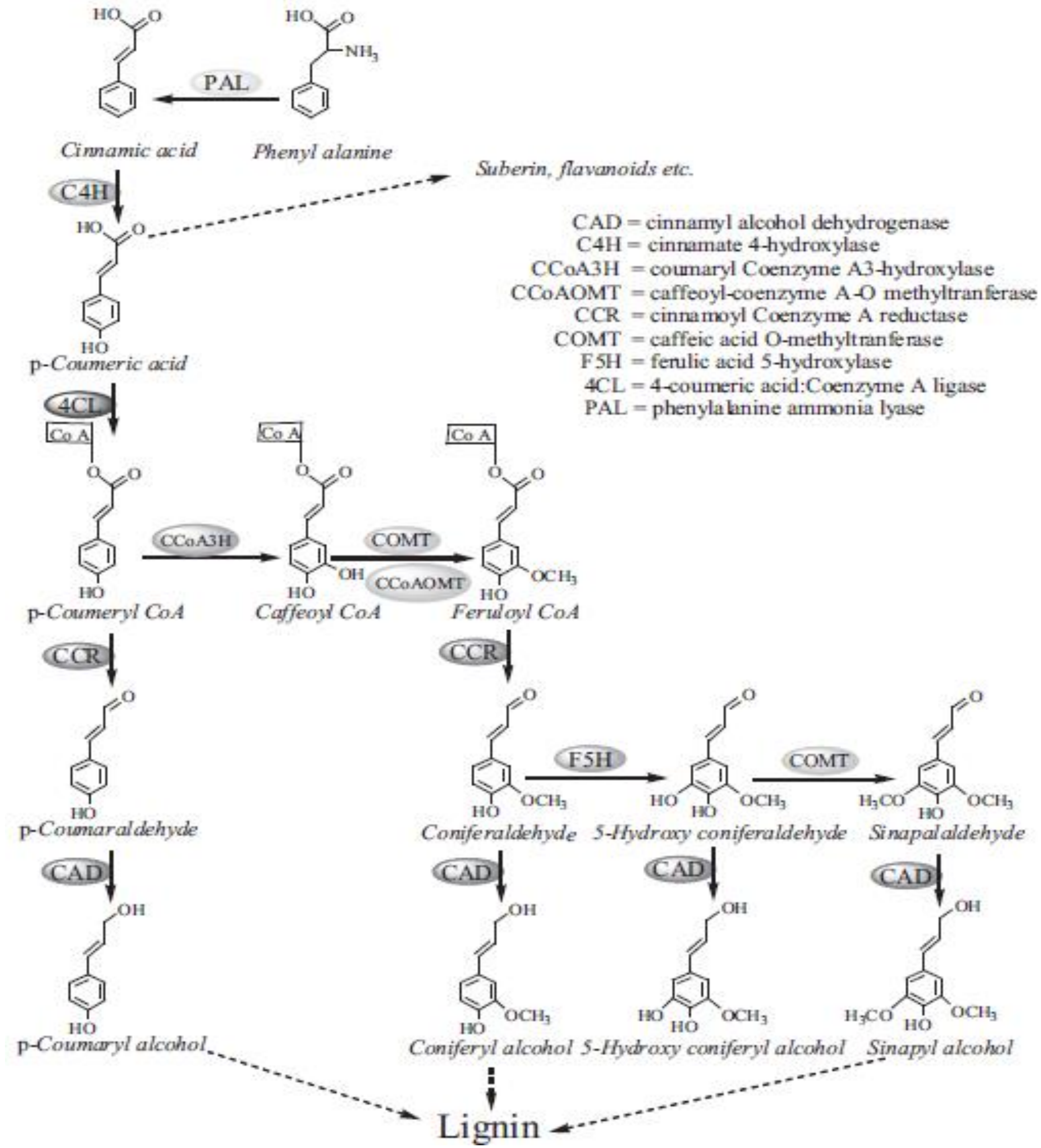
CM, chorismate mutase; TYRA, arogenage dehydrogenase; ADT, arogenate dehydratase; PAT, prephenate aminotransferase; PAL, phenylalanine ammonia lyase; TAL, tyrosine ammonia lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-hydroxycinnamoyl CoA ligase; HCT, hydroxycinnamoyl; CoA, shikimate/quinate hydroxycinnamoyltransferase; C3′H, *p*-coumaroylshikimate 3′-hydroxylase; CCoAOMT, caffeoyl CoA *O*-methyltransferase; CCR, cinnamoyl CoA reductase; Cald5H/F5H, coniferaldehyde/ferulate 5-hydroxylase; COMT, caffeic acid/5-hydroxyferulic acid *O*-methyltransferase; CAD, (hydroxy)cinnamyl alcohol dehydrogenase; POX, peroxidase; LAC, laccase.

Uso de plantas transgênicas com deficiência em genes específicos permite avaliar o fenótipo gerado e buscar entender o papel de cada enzima na biossíntese de monolignóis

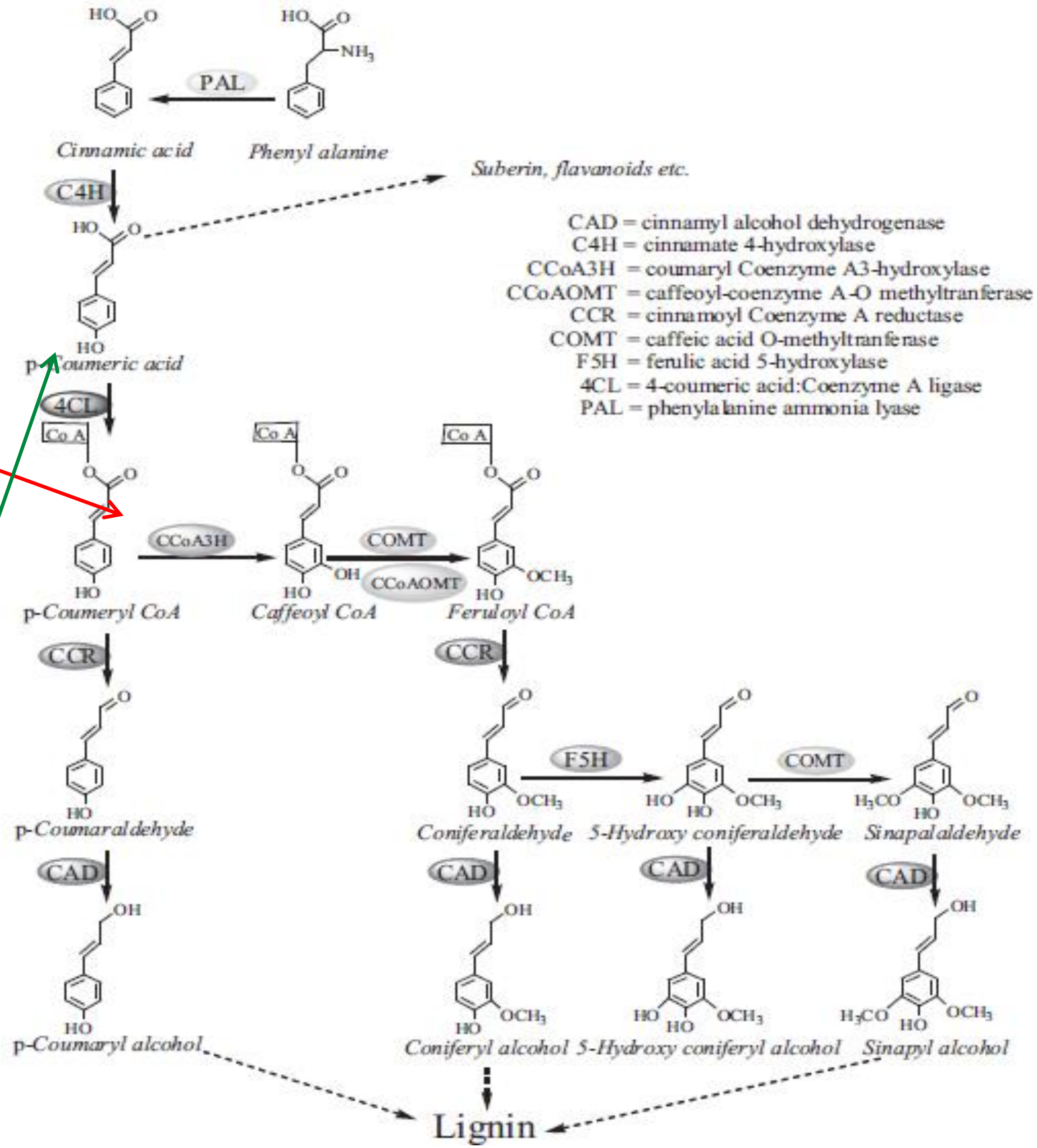
Transgênicos (ou mutantes) deficientes para **PAL** e **C4H** apresentam teor significativamente reduzido de lignina: condizente com o papel de enzimas de "entrada" na via de biossíntese de monolignóis



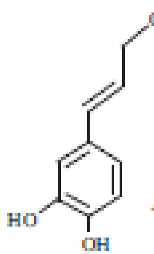
Transgênicos (ou mutantes) deficientes para **4CL** apresentam teor reduzido de lignina e incorporação dos ácidos precursores correspondentes na lignina formada

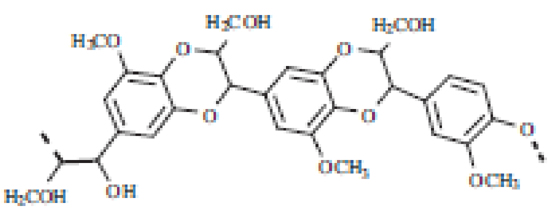


Transgênicos (ou mutantes) deficientes para **C3H** apresentam teor significativamente reduzido de lignina, lignina majoritariamente H e acúmulo de ácido p-cumárico solúvel;

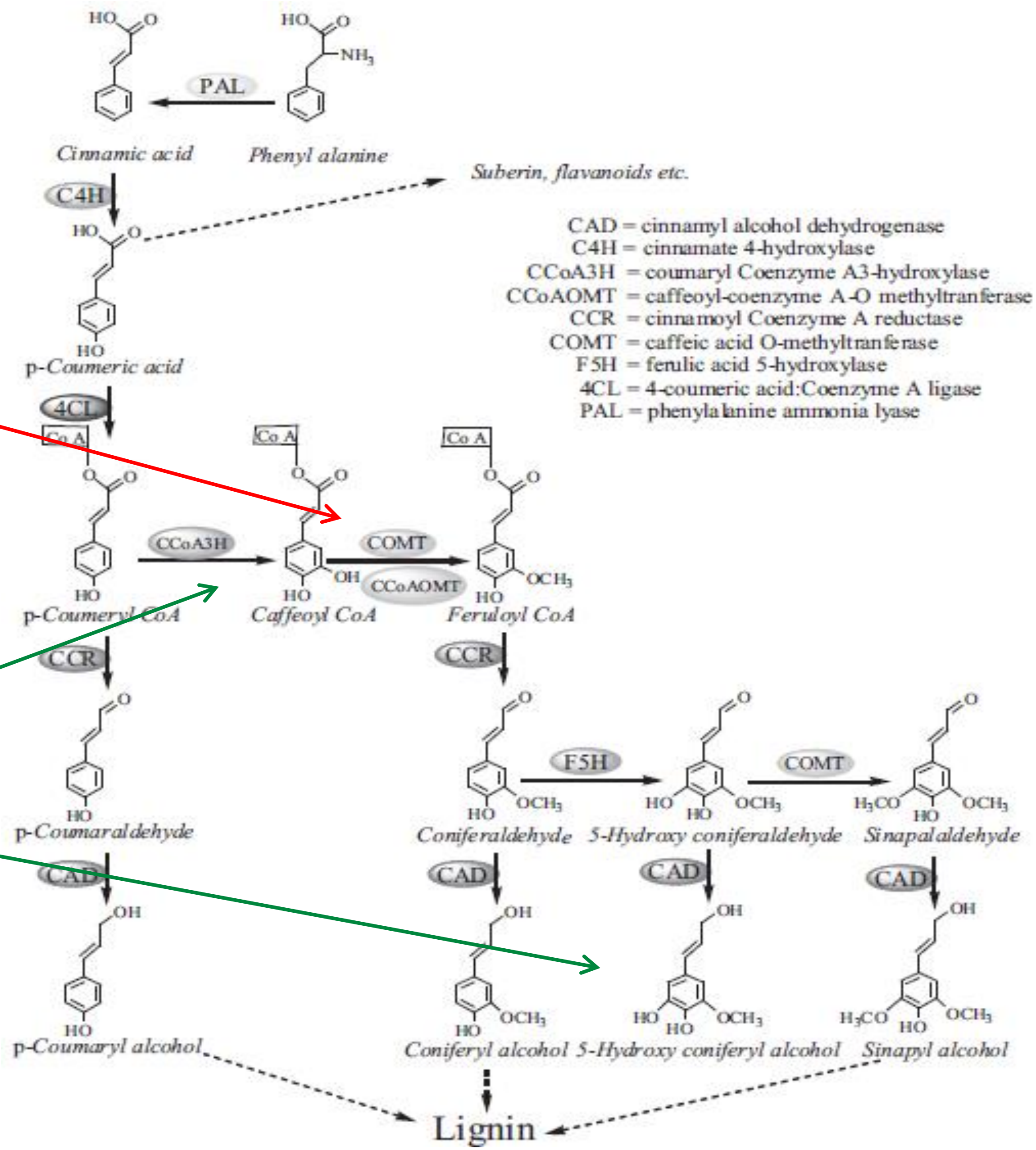


Transgênicos (ou mutantes) deficientes para **COMT** apresentam teor reduzido de lignina, e diminuição da proporção de lignina S e incorporação de 5-hidroxi-coniferil álcool na lignina;

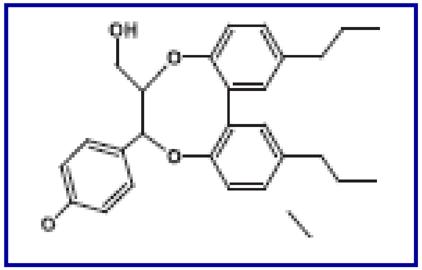


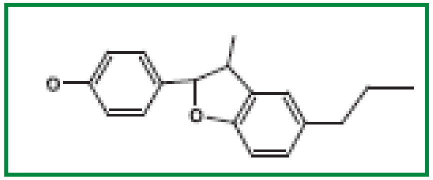


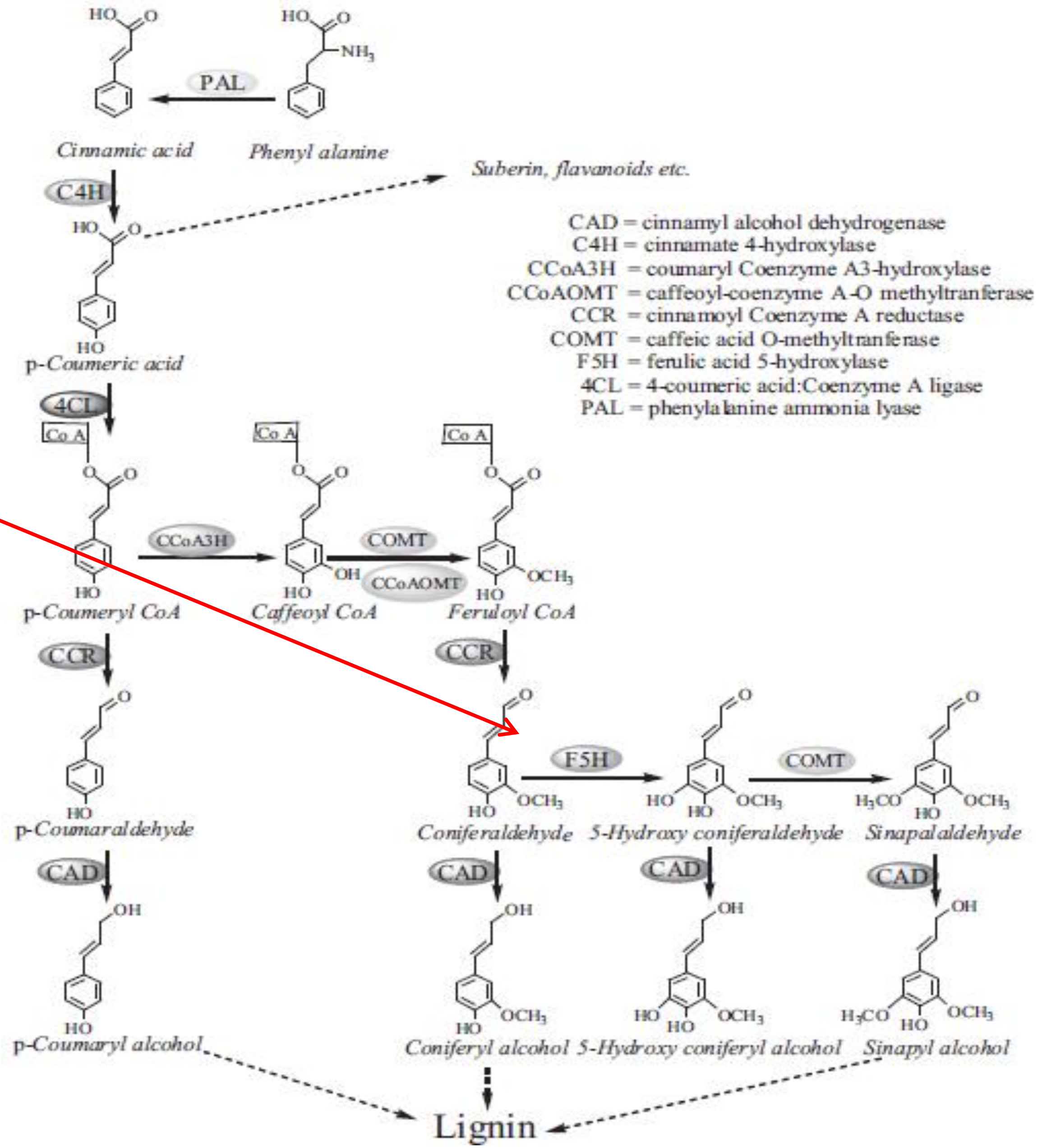
Lignin from COMT-downregulated plant



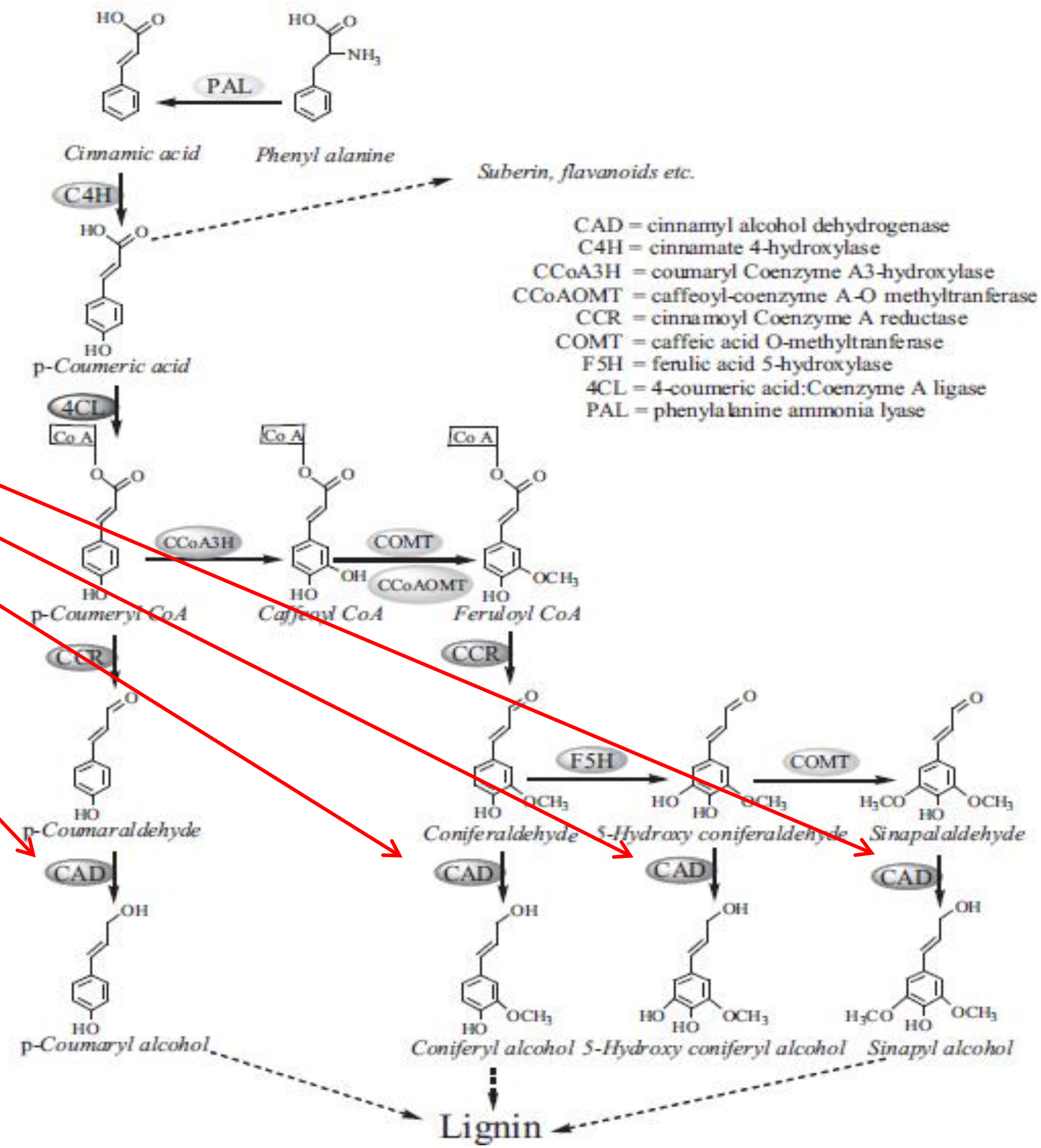
Transgênicos (ou mutantes) deficientes para **F5H** apresentam lignina sem estruturas S e de estruturas condensadas (dibenzodioxocinas e fenilcumaranos)







Transgênicos (ou mutantes) deficientes para **CAD** apresentam lignina com acúmulo de subestruturas contendo cinamaldeídos, o que confere **coloração avermelhada às plantas**;



Populus deficiente em CAD

Figure 4 Stem xylem phenotype of transgenic poplar downregulated for CAD. Debarked stems of 4-year-old, field-grown wild type (*left*) and transgenic poplar with reduced CAD activity (*right*), displaying the bright-red xylem coloration typical of plants with reduced CAD activity. Note: Cut transverse ends have been marked with paint to identify the line; see inset for unpainted transverse section showing restriction of the red color to developing xylem (© Nature McMillan, reprinted with permission).

A alteração no acúmulo de diferentes subsestruturas nas ligninas de plantas alteradas geneticamente indica que a biossíntese de lignina apresenta certa "plasticidade", ou seja, a planta incorpora os monolignóis disponíveis quando não dispõe do monolignol tradicional

Lignification: Precursor Transport, Oxidation, and the Topochemistry of Lignin Assembly

>> A biossíntese de monolignóis é relativamente bem compreendida com base no estudo dos fenótipos de transgênicos

Como os monolignóis são **transportados** para a parede celular (para fora da membrana citoplasmática) e como a **polimerização dos monolignóis é controlada**, envolve **sistemas biológicos menos compreendidos** até o momento

Transporte dos monolignóis para o exterior >> **várias possibilidades**

1. *Exocytosis via ER-Golgi-derived vesicles* >> similar ao descrito para a "secreção" de hemicelulose

2. *Passive diffusion via hydrophobic reactions through the plasma membrane*

3. *Active transport via plasma membrane* >> located transporters or the other facilitators >> ABC transporters >> **mais provável devido ao acúmulo de evidências experimentais positivas**

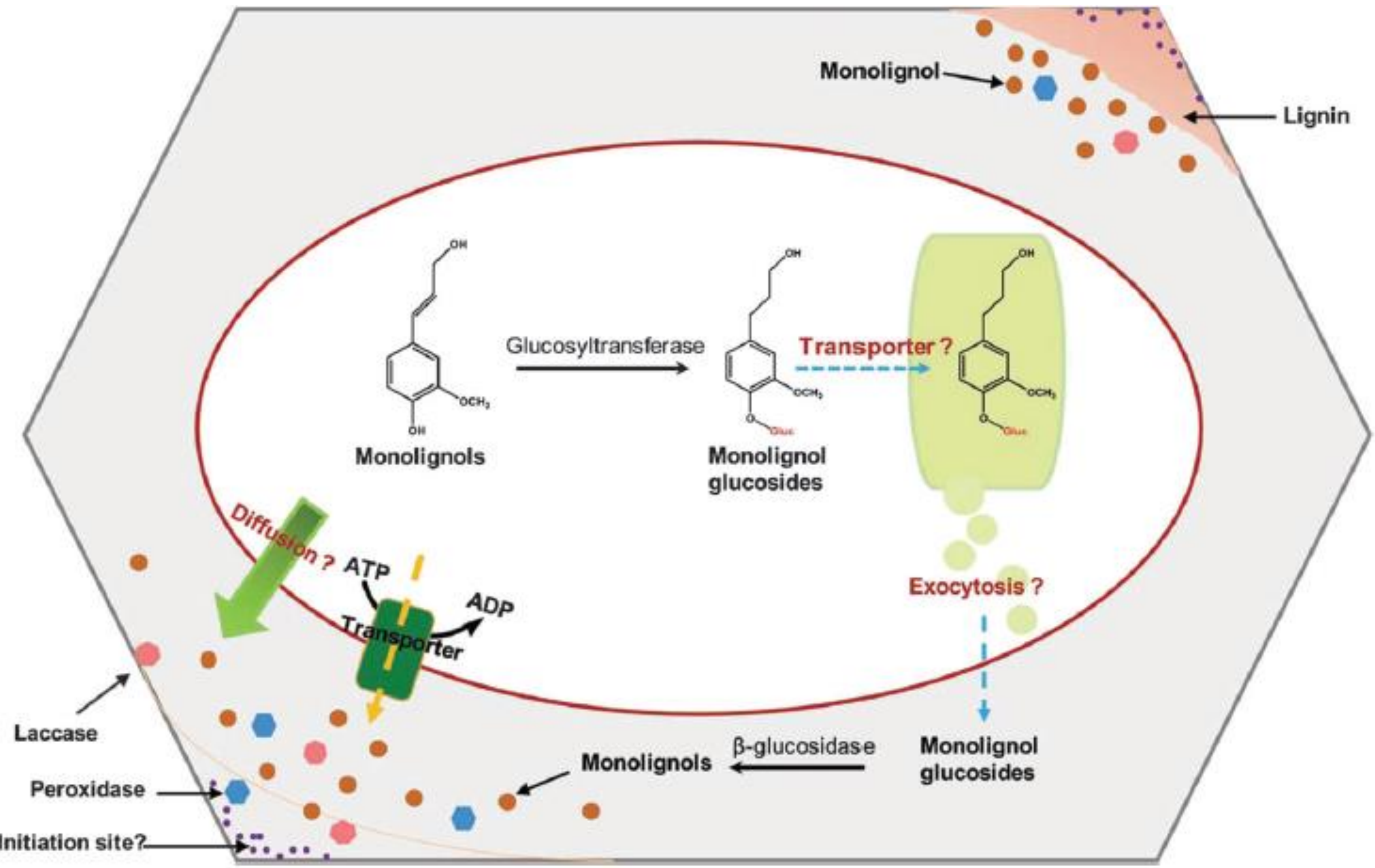


Figure 2. The Scheme for Monolignol Deposition and the Subsequent Initiation of Lignin Polymerization within the Cell Wall.

Symplastic transport of monolignols may export them to the cell wall through active transport or by passive diffusion. Alternatively, they may be sequestrated and stored as glucoconjugates into the vacuoles in gymnosperms, before their subsequent transport to the cell wall and hydrolysis to free monolignols for polymerization. The deposited monolignols in the cell wall diffuse to initiation sites where the polymerization process begins. The polymerization to form different bond-linkages of lignin is known to be a random chemical process. However, the nature of initiating sites and the way in which the amount and type of lignin formation is controlled across the cell wall are poorly understood.

Composição química da biomassa vegetal lignificada

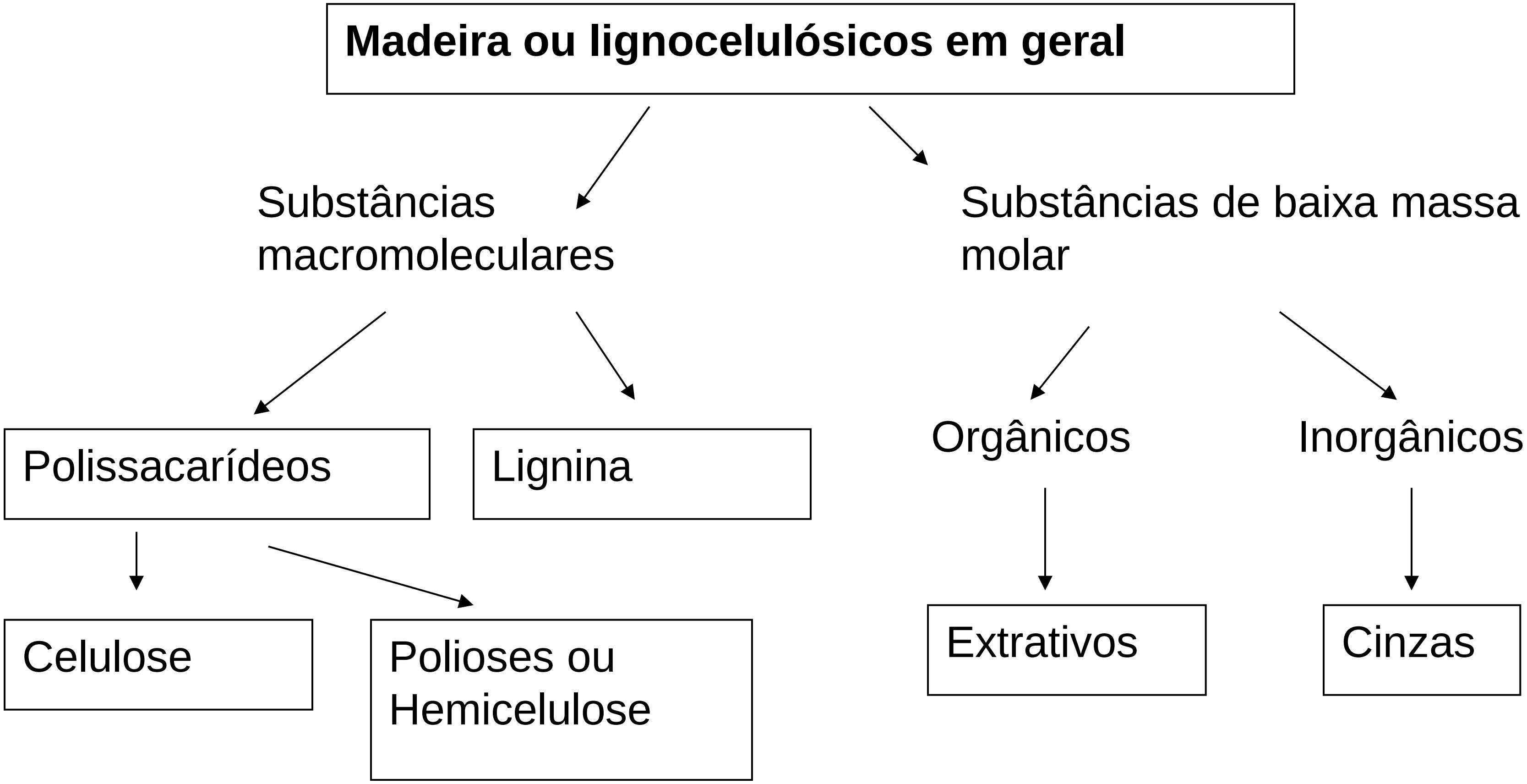


Table 2.2. Chemical composition of some wood species (mass %).

Species	Extractives	Lignin	Cellulose	Gluco-mannan	Xylan	Other poly-sacch.	others
Softwoods							
Norway Spruce (*Picea abies*)	1.7	27.4	41.7	16.3	8.6	3.4	0.9
Scots Pine (*Pinus sylvestris*)	3.5	27.7	40.0	16.0	8.9	3.6	0.3
Hardwoods							
Birch (*Betula verrucosa*)	3.2	22.0	41.0	2.3	27.5	2.6	1.4
Beech (*Fagus sylvatica*)	1.2	24.8	39.4	1.3	27.8	4.2	1.3
River red gum (*Eucalyptus calm-aldulensis*)	2.8	31.3	45.0	3.1	14.1	2.0	1.7
Red maple (*Acer rubrum*)	3.2	25.4	42.0	3.1	22.1	3.7	0.5