Lignin and the General Phenylpropanoid Pathway

Introduction and Importance:

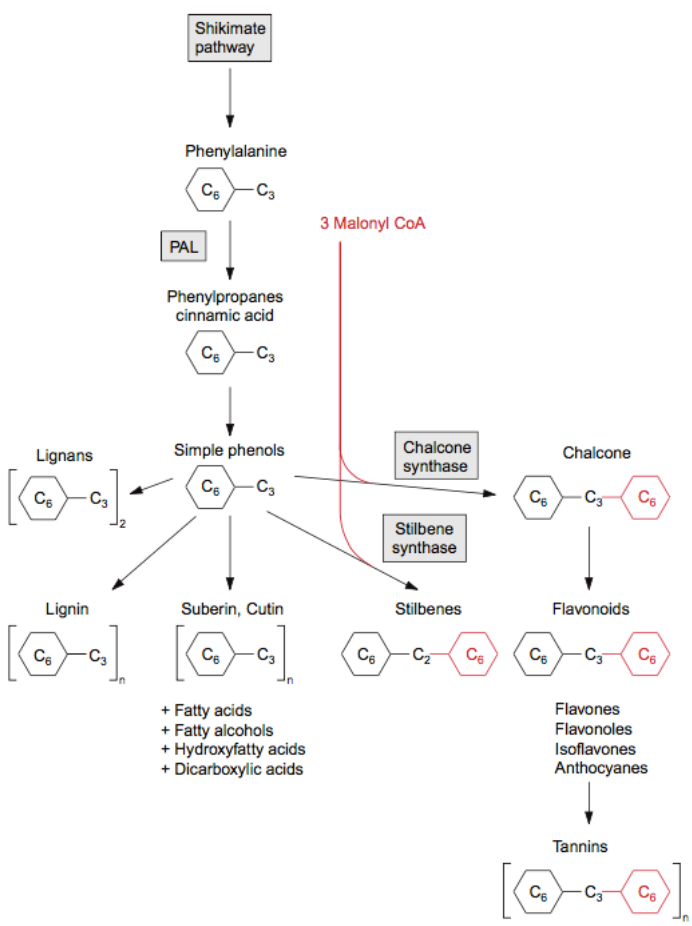
Phenolic: a compound consisting of an aromatic ring plus at least one hydroxyl [= phenyl group], or derived from such a compound.

Phenylpropanoid: a compound consisting of a phenyl plus a propane (3C), or derived from such a compound.

Phenylpropanoids and phenolics are a major biochemical pathway in plants
- 20,000 compounds, huge chemical diversity
- types: flavonoids, tannins, lignans, stilbenes, phenolic acids & **lignin**

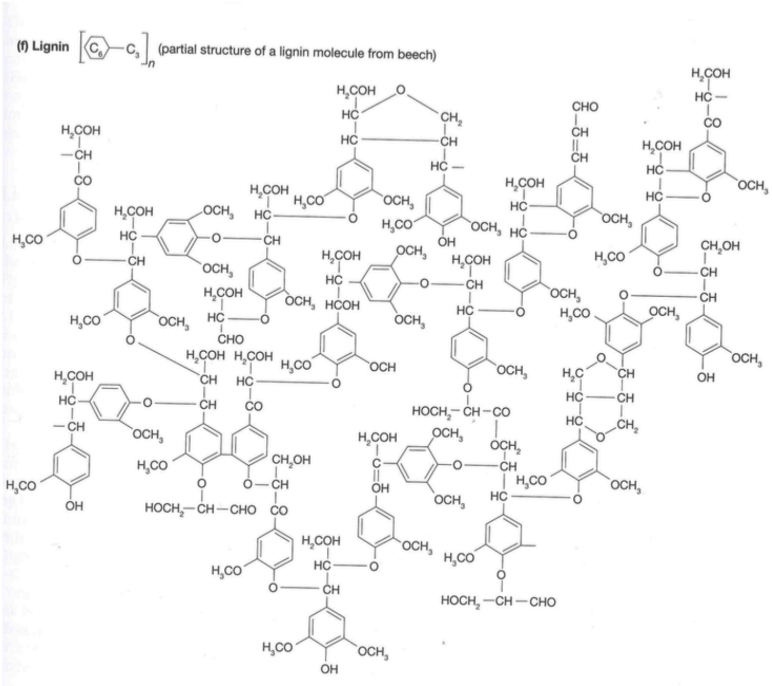
Ecological and evolutionary importance:
- functions: pigments and light screens, antimicrobials and toxins, signals
- closely linked to plants' invasion of the land
- UV light screens, lignin for xylem

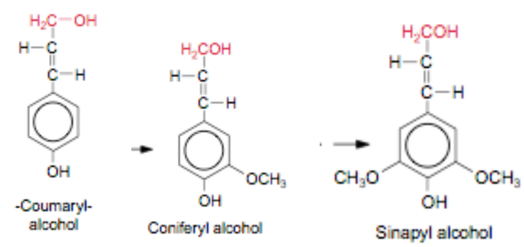
Functions and importance of lignin:
- reinforces CW in dead cells (xylem, wood)
  - -> efficient water conductance (all vascular plants)
  - -> allows for vertical growth (trees)
  - -> recalcitrant to degradation (C storage in ecosystems)
  - -> lignin a key part of wood - major building material



Lignin and its Synthesis

"A highly branched phenolic polymer with complex structures, made from phenylpropanoid alcohols, deposited in secondary cell walls"





I. Roadmap for biosynthesis (handout)

- synthesis from phenylalanine, via phenylpropanoid pathway
- need **monolignols** (*p*-coumaryl, coniferyl, sinapyl alcohols)
- these are then polymerized ***in situ*** to assemble lignin

Two trends within biosynthesis

i) hydroxylation and methoxylation

ii) reduction from acid to alcohol (via **CoA thioester**)

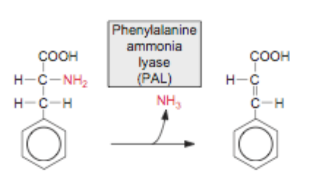
II. Key Steps in Monolignol Formation (to Coniferyl Alcohol)

i) General phenylpropanoid pathway [Phe → *p*-coumaroyl-CoA]

- these steps are also in for many other phenylpropanoid pathways (flavonoids, coumarins etc)

1. **Deamination** of Phe to **cinnamic acid**

*phenylalanine ammonia-lyase (**PAL**) [- famous enzyme, discovered in 1961, Eric Conn]*



2. **Hydroxylation** to ***p*-coumaric acid** by *cinnamate* 4'-*hydroxylase* (**C4H**)
3. **Ligation** with Coenzyme A to make coumaroyl-CoA (= activated precursor)
   4-coumarate:CoA ligase **(4CL)**

(NB: 4-coumaroyl-CoA is also a precursor for many other pathways)

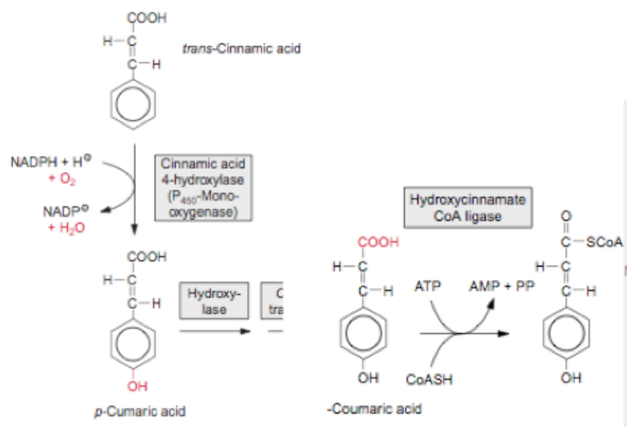
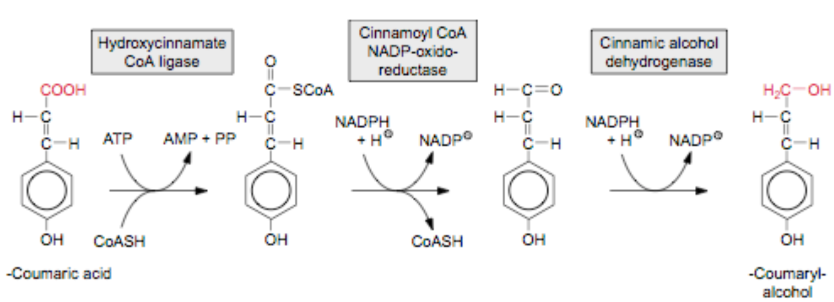


Figure 18.4 Synthesis of various hydroxycinnamic acids from *trans*-cin

ii) Additional hydroxylations and methylations (see handout)

1. the mysterious *3'-hydroxylase puts on the second -OH*
   (makes caffeic acid, but as the shikimic acid ester)
2. O-methylation
   *caffeoyl-CoA O-methyltransferase (**CCoAOMT**)*
3. Reduction to coniferaldehyde
   *cinnamoyl-CoA reductase (**CCR**)*
4. Reduction to alcohol
   cinnamyl alcohol dehydrogenase **(CAD)**
   coniferaldehyde-----> **coniferyl alcohol**
5. Additional steps to **sinapyl alcohol**
   - *coniferaldehyde 5' hydroxylase*
   - *aldehyde O-methyltransferase (**COMT**)*
   - ***CCR** & **CAD**/Sinapyl Alcohol Dehydrogenase)*

*NB: There is the analogous path to **coumaryl alcohol**. CCR and CAD work in several places i.e., take several substrates.*



III. Lignin Assembly and Composition

[Three monolignols: (***p*-coumaryl alcohol**, **coniferyl** alcohol & **sinapyl** alcohol)

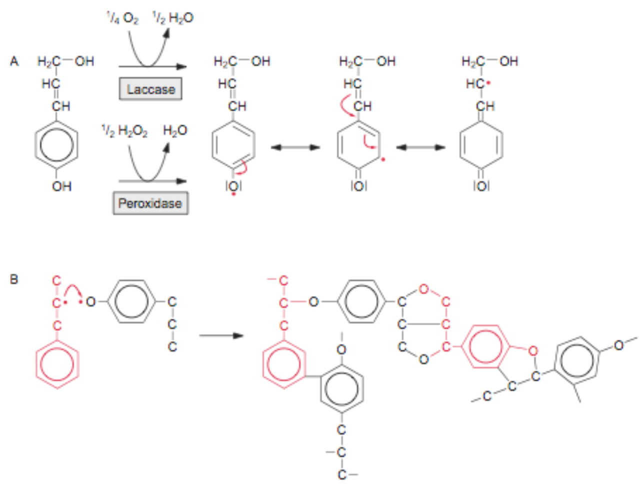
1. Reversible glucosylation (transport and storage)
   coniferyl alcohol glucosyl transferase (UDP-glucose), and *ß-glucosidase*

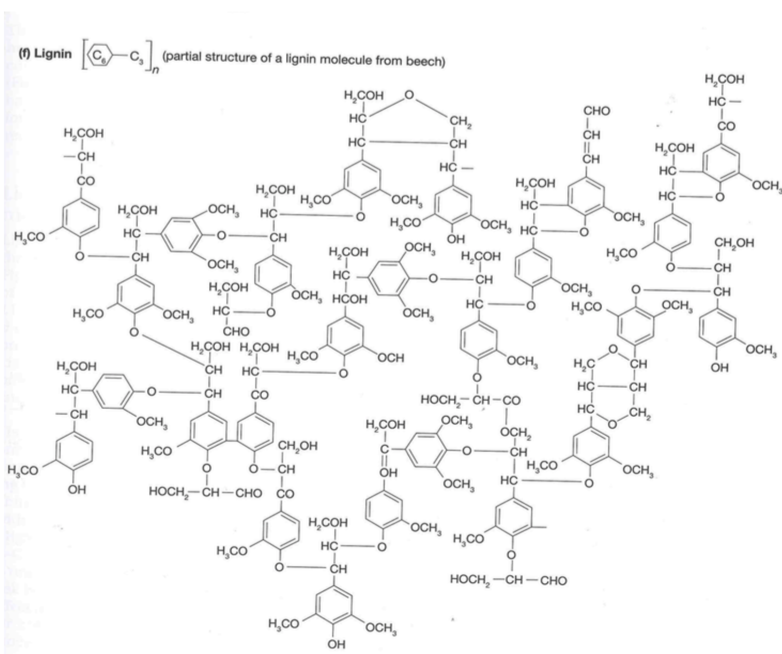
2. <u>Oxidation</u> gives rise to **resonance-stabilized** <u>free radicals</u> of monolignols

   Enzymes: ***laccase*** and ***peroxidase***

3. <u>Polymerisation</u> of "activated" monolignols (possibly in somewhat random patterns)

4. Variation in lignin composition is common:
   - **guaiacyl** vs **syringyl** lignin types
   - variable in species, cell types, tissues etc.
   - impact on pulping efficiency of types:
     G lignin (softwood) but harder to digest.
     S lignin (hardwoods) is easier to pulp
   - makes lignin interesting <u>biotechnological</u> <u>targets</u>

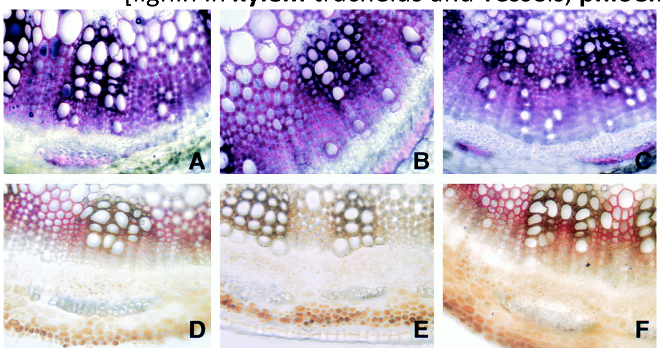




IV. Control of Lignin Synthesis

1. Developmentally-regulated (via gene expression). There are master transcription factors (ie NAC) that respond to plant hormones (auxins, ethylene), to trigger tissue-specific gene expression.

[lignin in **xylem** tracheids and vessels, **phloem** fibers]



2. Environmentally-regulated (via gene expression): stress-responses to pathogens ("stress-lignin") (see Campbell & Ellis, *Planta* 184:49)'

Induced lignification in cell cultures: - why use cell cultures?
- pathogen responses (recall cell wall responses) ; elicitor vs living pathogen

M.M. Campbell and B.E. Ellis: Elicited phenylpropanoid metabolism

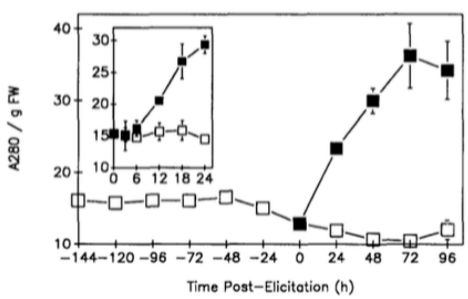


Fig. 2. The effect of elicitation on the accumulation of thioglycolic-acid-extractable complexes in *Pinus banksiana* cell cultures. Elicited (■) and control (□) cultures were monitored for the accumulation of TGA-extractable material at 24-h intervals. The accumulation of TGA-extractable material within the first 24 h post-elicitation was monitored at 0, 3, 6, 12, 18 and 24 h (*inset*, same axes). Each graph was prepared from an independently derived time-course. Error bars = 1 SD

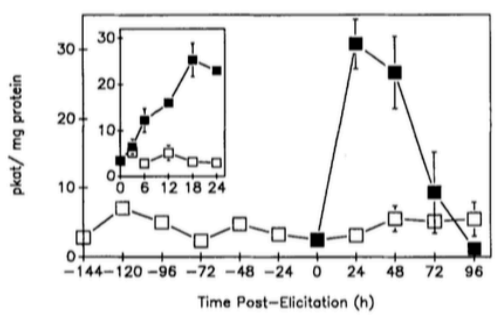


Fig. 4. The effect of elicitation on PAL activity in *Pinus banksiana* cell cultures. Soluble protein extracts from elicited (■) or control (□) cultures were assayed for PAL activity at 24-h intervals. The change in extractable PAL activity within the first 24 h post-elicitation was monitored at 0, 3, 6, 12, and 24 h (*inset*, same axes). Each graph was obtained from an independently derived time-course. Error bars = 1 SD

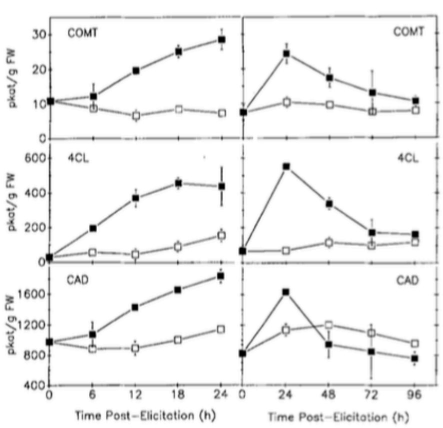


Fig. 5. The effect of elicitation on the activities of enzymes related to lignification in *Pinus banksiana* cells. S-adenosyl-L-methionine:caffeate O-methyltransferase (COMT), 4-coumarate:CoA ligase (4CL), and coniferyl alcohol dehydrogenase (CAD) activities were measured in soluble protein extracts from elicited (■) or control (□) cultures at various times post-elicitation. Cultures were

Genetic Engineering of Altered Lignin in Trees

- reduction of lignin content (pulp, forages, biofuel) could have huge benefit, as long as the plants are still viable.
  - it is possible to genetically transform poplar / aspen, which are fast growing and suitable for plantations.

Examples:

i) RNAi down regulation of *p-coumaroyl shikimate 3-hydroxylase* (hybrid poplar (Coleman et al, PNAS 2008)
- reduce lignin content, can get novel H-lignin

ii) *4CL and coniferaldehyde 5-hydroxylase*[Li L et al. PNAS 2003]
- > double transformation with both genes, and separate transgenics.
- >*4CL* suppression reduces lignin content
  , but *coniferaldehyde 5-hydroxylase* suppression led to greater S/G ratio