



Tansley review

The origin and evolution of lignin biosynthesis

Author for correspondence:
Clint Chapple
Tel: +1 765 494 0494
Email: chapple@purdue.edu

Jing-Ke Weng and Clint Chapple
Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA

Received: 15 March 2010
Accepted: 7 May 2010

Contents

| | | | |
|---|-----|------------------------|-----|
| Summary | 273 | IV. Concluding remarks | 282 |
| I. Introduction | 273 | Acknowledgements | 282 |
| II. Emergence of the monolignol biosynthetic scaffold | 274 | References | 282 |
| III. Occurrence and elaboration of lignification in tracheophytes | 280 | | |

Summary

New Phytologist (2010) **187**: 273–285
doi: 10.1111/j.1469-8137.2010.03327.x

Key words: biofuel, cell wall, convergent evolution, diversification, land colonization, lycophyte, P450, phenylpropanoid.

Lignin, a phenolic polymer derived mainly from hydroxycinnamyl alcohols, is ubiquitously present in tracheophytes. The development of lignin biosynthesis has been considered to be one of the key factors that allowed land plants to flourish in terrestrial ecosystems. Lignin provides structural rigidity for tracheophytes to stand upright, and strengthens the cell wall of their water-conducting tracheary elements to withstand the negative pressure generated during transpiration. In this review, we discuss a number of aspects regarding the origin and evolution of lignin biosynthesis during land plant evolution, including the establishment of its monomer biosynthetic scaffold, potential precursors to the lignin polymer, as well as the emergence of the polymerization machinery and regulatory system. The accumulated knowledge on the topic, as summarized here, provides us with an evolutionary view on how this complex metabolic system emerged and developed.

I. Introduction

The earliest unequivocal fossil evidence suggests that the earliest land plants arose *c.* 450 million yr ago during the Late Ordovician (Stewart & Rothwell, 1993). The actual colonization of land by plants could have begun tens of millions of years earlier during the Early Cambrian, considering that the appearance of dispersed plant spores predated the first land plant megafossils by *c.* 50 million years

(Kenrick & Crane, 1997; Gensel, 2008). It is reasonable to envision that the algal ancestors of early land plants had been constantly washed onto freshwater and marine shores, thus providing the early populations on which Darwinian selection could operate. After arriving on or adjacent to these terrestrial environments, pioneering land plants were immediately confronted with several major challenges, including exposure to damaging UV-B radiation from which water had protected their ancestors, lack of structural

support once provided by buoyancy, desiccation stress and, eventually, co-evolving herbivores and pathogens (Raven, 1984). To cope with many of these stresses, a series of specialized metabolic pathways, often referred to as secondary metabolism, evolved in early land plants, among which phenylpropanoid metabolism was probably one of the most critical. The acquisition of the ability to deaminate the amino acid phenylalanine and hydroxylate the aromatic ring of subsequent metabolites led to the ability to accumulate simple phenylpropanoids with absorbance maxima in the UV-B range (280–320 nm). Such newly acquired features imparted early land plants, especially their vulnerable haploid spores, with the ability to resist UV irradiation, which made survival on land possible (Lowry *et al.*, 1980).

Although the occurrence of phenylpropanoid metabolism in early land plants facilitated their initial move onto land, for tens of millions of years their body plans remained small because of a lack of mechanical reinforcement (Bateman *et al.*, 1998). It was not until the rise of tracheophytes, which had developed the ability to deposit the phenylpropanoid polymer lignin in their cell wall, that land plants truly flourished and began their dominance of the terrestrial ecosystem. Lignin bestowed the early tracheophytes with the physical rigidity to stand upright, strengthened the water-conducting cells for long-distance water transport, and allowed plants to expand significantly in body size compared with their sister group, the bryophytes. The nature and randomness of the linkages in lignin also made lignin one of the hardest biopolymers to degrade, an ideal characteristic for a defensive barrier against the pathogens and herbivores that would soon co-evolve with vascular plants. Lignification consequently transformed phenylpropanoid metabolism into a major sink for carbon in plants, eclipsed only by cellulose, such that it has now been estimated to represent as much as 30% of the total biomass produced in the biosphere (Boerjan *et al.*, 2003).

The expansion of tracheophyte abundance and diversity, enabled by their ability to produce lignin and other factors, has had far-reaching consequences on terrestrial ecosystems (Kenrick & Crane, 1997). Through photosynthesis, early tracheophytes provided the basis of net primary production for the development of almost all the early life forms on land, including arthropods, fungi and microorganisms (Beerling *et al.*, 2001). The enhanced chemical weathering of rocks by the organic acids released from the roots of early tracheophytes, as well as the accumulation of organic matter from plant decay, greatly contributed to the early soil formation on earth (Gensel & Edwards, 2001). Because lignin degrades only slowly, large amounts of atmospheric carbon were fixed by early tracheophytes, resulting in the significant drop in atmospheric CO₂ levels and a corresponding increase in O₂ levels during the late Paleozoic era (Berner, 1993; Berner *et al.*, 2000). These changes in atmospheric composition, in turn, precipitated many

physiological innovations, for example the evolution of flight in insects (Dudley, 1998) and the origin of megaphylls (true leaves) in plants (Beerling *et al.*, 2001). The lignin-rich biomass buried in swamps and peat bogs during the Carboniferous period fossilized over hundreds of millions of years to become coal, the most widely distributed fossil fuel reserve, and one that has greatly propelled human industrialization (Hayatsu *et al.*, 1979; Freese, 2003).

In recent years, there has been an explosion of genomic resources for plant species, including those from the lower branches of the plant phylogeny (Bowman *et al.*, 2007). These advances have greatly facilitated comparative genomics studies that have interrogated the evolutionary history of a number of plant processes. Here, we discuss issues relating to the origin of lignin biosynthesis and its subsequent evolution. We hope this review will not only help the reader attain a more comprehensive understanding of how evolution has shaped cell wall biochemistry, but will also illuminate opportunities for genetic engineering of lignin biosynthesis that could improve desirable traits in economically important crops.

II. Emergence of the monolignol biosynthetic scaffold

Lignin is polymerized mainly from hydroxycinnamyl alcohols, typically *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, through radical coupling (Fig. 1) (Boerjan *et al.*, 2003). Lignins derived from these hydroxycinnamyl alcohols are commonly referred to as hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin, respectively. Except for S lignin, which is specific to certain lineages (e.g. flowering plants and the lycophyte *Selaginella*) (Weng *et al.*, 2008b), H and G lignins are fundamental to all the tracheophytes (Fig. 2). Bryophytes do not synthesize lignin, but are widely found to accumulate soluble phenylpropanoids, such as flavonoids and lignans (Basile *et al.*, 1999; Umezawa, 2003). Further, the genome of the reference moss *Physcomitrella patens* contains orthologs of all the eight core lignin biosynthetic enzymes required for the biosynthesis of *p*-coumaryl alcohol and coniferyl alcohol (Fig. 3), whereas the green algae *Chlamydomonas reinhardtii* contains none (Silber *et al.*, 2008; Weng *et al.*, 2008b; Xu *et al.*, 2009). This evidence suggests that the metabolic scaffold for monolignol biosynthesis evolved earlier than the rise of tracheophytes, and was probably established in the earliest land plants where some or all of the pathway served in the generation of UV-protectant molecules.

1. Recruitment of enzymes of primary metabolism to a new secondary metabolic pathway

The novel activities of the enzymes required for lignin deposition did not simply appear, but were gradually

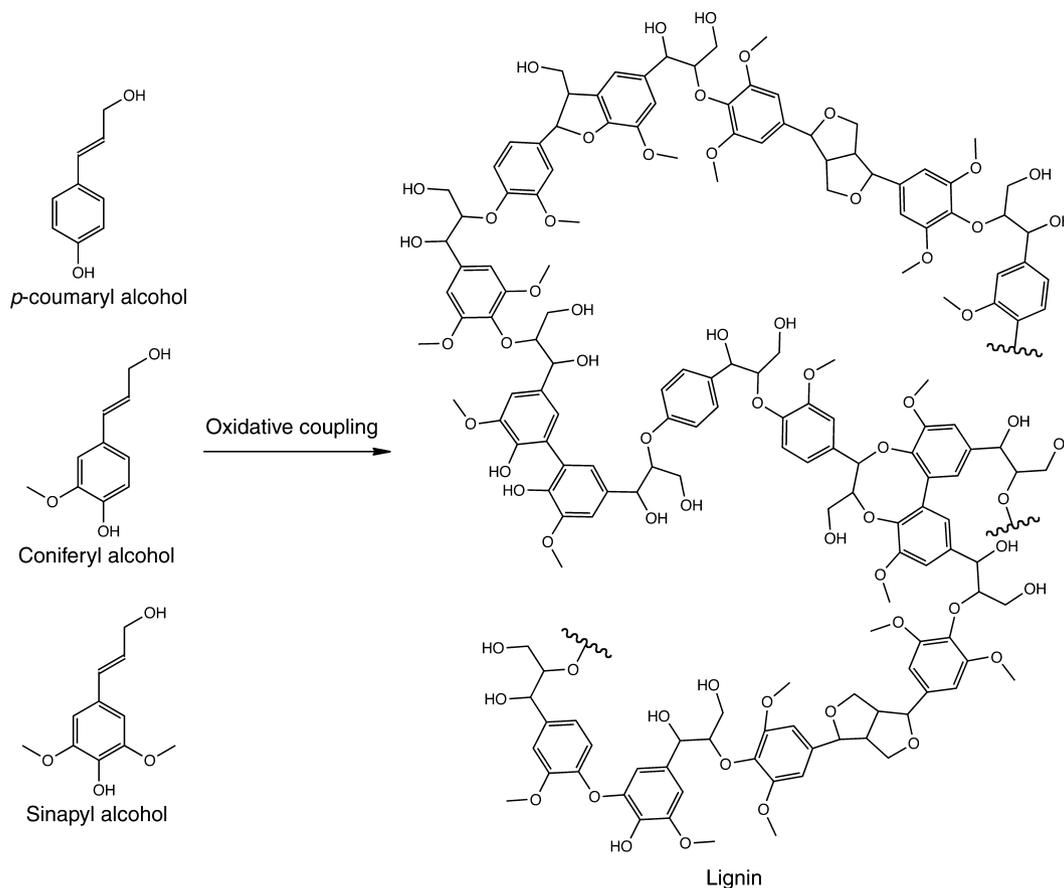


Fig. 1 The phenylpropanoid polymer lignin is primarily polymerized from the three hydroxycinnamyl alcohols.

acquired through the Darwinian process of mutation and selection (Tawfik, 2006). It has been widely accepted that novel enzyme functions usually arise after gene duplication events, where one copy maintains the ancestral function, and the other copy becomes less constrained and can evolve (Lynch & Conery, 2000). In rare cases, the newly derived gene provides certain selective advantages to the host, and becomes fixed in the population (Lynch & Conery, 2000). Presumably, the primitive forms of phenylpropanoid metabolism emerged from a much larger number of metabolic pathways accidentally assembled using the evolving duplicated genes encoding primary metabolic enzymes and/or enzymes acquired from symbiotic organisms via horizontal gene transfer (Richards *et al.*, 2006). It seems likely that specific combinations of new activities that yielded compounds with UV-absorbent properties would have been selected for in early terrestrial plants. Under subsequent purifying selection, those initial combinations could have converged into one particular metabolic scaffold, which has been preserved and elaborated on in land plants today.

Examination of the eight enzymes that constitute the core monolignol biosynthetic scaffold suggests that their ancestry is deeply rooted in the primary metabolism (Table 1,

Fig. 3). Phenylalanine ammonia-lyase (PAL), the enzyme that converts phenylalanine to *trans*-cinnamic acid at the entry point of the phenylpropanoid pathway, is homologous to histidine ammonia-lyase (HAL), an enzyme involved in histidine degradation in primary metabolism. PAL exhibits considerable structural conservation with HAL, and mirrors HAL in catalytic mechanism by adopting the electrophilic 4-methylidene-imidazole-5-one group derived from the conserved alanine-glycine-serine (Ala-Gly-Ser) tripeptide (Baedeker & Schulz, 2002; Ritter & Schulz, 2004). Cinnamate 4-hydroxylase (C4H) and *p*-coumaroyl shikimate 3'-hydroxylase (C3'H) are cytochrome P450 monooxygenases (P450) that catalyze the first two aromatic hydroxylation reactions in the monolignol biosynthetic pathway. Although C4H and C3'H have greatly deviated from their primary metabolic cousins, for example sterol 14-demethylase, in their substrate recognition sites, they maintain highly conserved motifs responsible for heme binding and a common catalytic mechanism (Werck-Reichhart & Feyereisen, 2000). 4-Hydroxycinnamoyl-CoA ligase (4CL) activates *p*-coumaric acid to the activated thioester form *p*-coumaroyl CoA, which can then be channeled either further downstream into monolignol biosynthesis or into flavonoid biosynthesis if acted on by chalcone synthase

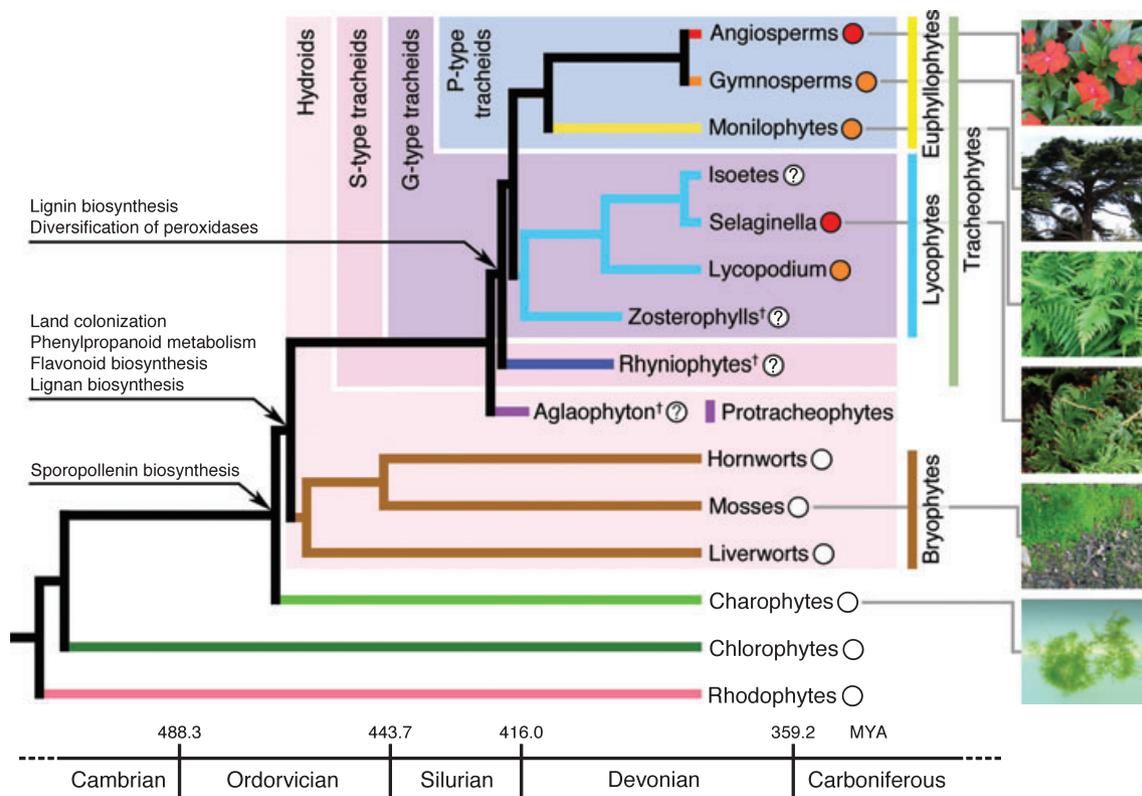


Fig. 2 A plant phylogenetic tree marked with the major milestones of evolution of lignin biosynthesis. The distribution of lignin and its monomeric composition across major plant lineages are denoted by a circle at each branch. Open circle, no lignin; orange circle, presence of H and G lignin; red circle, presence of S lignin in addition to H and G lignin; circle with question mark, unknown. Note that, within several groups with G lignin, S lignin-containing exceptions are known. †Extinct lineage.

(CHS). 4CL is homologous to long-chain fatty acyl-CoA synthetase, the first committed enzyme in the β -oxidation pathway for breaking down long-chain fatty acids. Like that of fatty acyl-CoA synthetase, the 4CL catalytic mechanism involves the formation of an acyl-adenylate intermediate (Schneider *et al.*, 2003; Hisanaga *et al.*, 2004). Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT) catalyzes the formation of *p*-coumarate esters using shikimate or quinate as an acyl acceptor. These specific *p*-coumarate esters then serve as the substrates for the phenylpropanoid *meta*-hydroxylation catalyzed by C3'H. HCT belongs to the BAHD acyltransferase superfamily, which has extensively diversified in plants, and has been suggested to be involved in the biosynthesis of a large number of secondary metabolites (D'Auria, 2006). Despite low primary sequence identity, the structure of the plant BAHD enzyme vinorine synthase shows a surprising overall similarity to the structures of primary metabolic CoA-dependent acyltransferases, such as carnitine acetyltransferase, which is required for the transport of long-chain acyl groups from fatty acids into mitochondria for β -oxidation (Wu *et al.*, 2003; Ma *et al.*, 2005). Caffeoyl-CoA *O*-methyltransferase (CCoAOMT) catalyzes the first transmethylation reaction

on the aromatic 3-hydroxyl using *S*-adenosyl methionine (SAM) as the methyl donor. CCoAOMT shares a common architecture with mammalian catechol-*O*-methyl transferase, and contains a highly conserved α/β Rossmann fold, which forms the catalytic divalent cation and SAM-binding sites (Vidgren *et al.*, 1994; Ferrer *et al.*, 2005). Two oxidoreductases, (hydroxy)cinnamoyl-CoA reductase (CCR) and (hydroxy)cinnamyl alcohol dehydrogenase (CAD), are employed in the core monolignol biosynthetic pathway that reduces hydroxycinnamoyl-CoA esters to their corresponding alcohols. Whereas CCR shares significant similarities with mammalian 3- β -hydroxysteroid dehydrogenase responsible for steroid biosynthesis (Lacombe *et al.*, 1997), CAD is homologous to the ethanol-degrading alcohol dehydrogenase widely spread in bacteria and animals (Youn *et al.*, 2006). Like many of the oxidoreductases, both CCR and CAD contain highly conserved Rossmann fold NAD(P)H/NAD(P)(+) binding domains.

Although difficult to address, it is intriguing to consider the progressive evolutionary paths by which individual enzymes were recruited and integrated into such a complex metabolic system. Based on a large-scale phylogenetic analysis, it was recently suggested that land plants obtained PAL

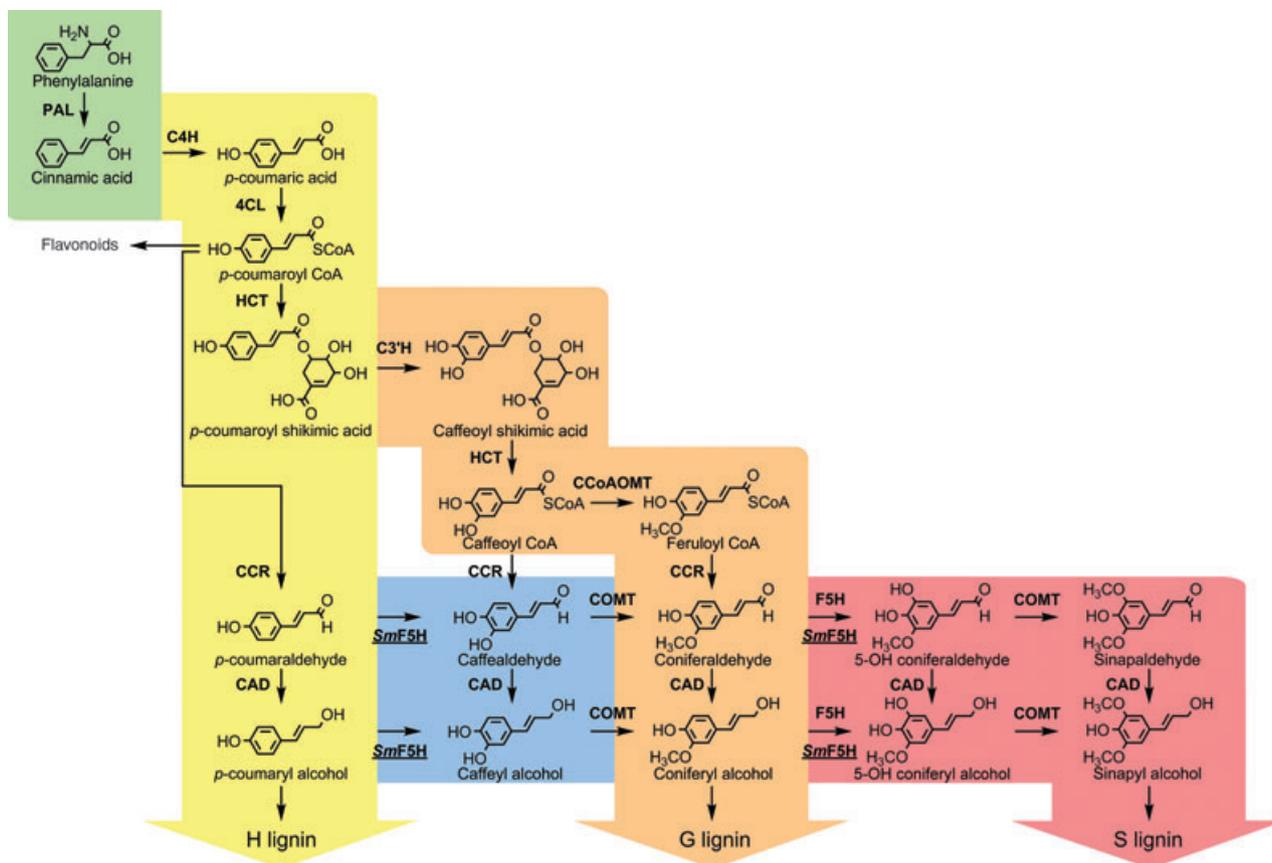


Fig. 3 The monolignol biosynthetic pathway. Note that the pathway branch leading to syringyl lignin biosynthesis (highlighted in red) is restricted to angiosperms and the lycophyte *Selaginella*. *Selaginella* contains a unique bifunctional *SmF5H* (underlined), defining a syringyl lignin pathway distinct from that of angiosperms. Enzymes and their abbreviations: CAD, (hydroxy)cinnamyl alcohol dehydrogenase; CCoAOMT, caffeoyl-CoA O-methyl transferase; CCR, (hydroxy)cinnamoyl-CoA reductase; C3'H, *p*-coumaroyl shikimate 3'-hydroxylase; C4H, cinnamate 4-hydroxylase; 4CL, 4-hydroxycinnamoyl-CoA ligase; COMT, caffeic acid O-methyltransferase; F5H, ferulic acid/coniferaldehyde/coniferyl alcohol 5-hydroxylase; HCT, hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase; PAL, phenylalanine ammonia-lyase.

Table 1 The eight core monolignol biosynthetic enzymes and their representative cousins in primary metabolism

| Enzyme | Enzyme family | Representative cousins in primary metabolism |
|---------|---------------------------------|--|
| PAL | Lyase, class I-like | Histidine ammonia-lyase |
| C4H | Cytochrome P450 monooxygenase | Sterol 14-demethylase |
| 4CL | Acyl-CoA synthetase | Long-chain fatty acyl-CoA synthetase |
| HCT | BAHD acyl transferase | Carnitine acetyltransferase |
| C3'H | Cytochrome P450 monooxygenase | Sterol 14-demethylase |
| CCoAOMT | SAM-dependent methyltransferase | Catechol-O-methyl transferase |
| CCR | Dehydrogenase | 3- β -Hydroxysteroid dehydrogenase |
| CAD | Dehydrogenase | Alcohol dehydrogenase |

from symbiotic bacteria or fungi via horizontal gene transfer, which could have initiated the early development of phenylpropanoid metabolism (Emiliani *et al.*, 2009). The

newly acquired ability to synthesize cinnamic acid from phenylalanine might have occurred concomitantly with the development of the activity of a promiscuous P450 that was the progenitor of C4H. On the one hand, cinnamic acid has been shown to act as a potent anti-auxin that inhibits plant growth (Vanoverbeek *et al.*, 1951; Wong *et al.*, 2005). Thus, an enzyme that can further metabolize cinnamic acid might have become immediately necessary to avoid growth aberrations (Schillmiller *et al.*, 2009). On the other hand, the addition of the *para*-hydroxy group to the phenylpropanoid backbone shifts its UV absorbance spectra to the UV-B range, which might have provided an obvious selective advantage to the emerging land plants. It has been shown that, at least in flowering plants, PAL and C4H have further evolved for the ability to physically interact with one another, resulting in the recruitment of soluble PAL tetramers to the cytosolic surface of endoplasmic reticulum (ER) membranes where C4H is localized (Rasmussen & Dixon, 1999; Achnine *et al.*, 2004). Although it remains to be tested whether this is a general phenomenon in land plants,

the interaction between PAL and C4H could sequester the potentially harmful intermediate cinnamic acid, which may have the potential to act as an ionophore (McLaughlin & Dilger, 1980). Alternatively, or in addition, by tethering PAL to the ER membrane through PAL–C4H interactions, phenylpropanoid pathway input could be restricted to subpools of phenylalanine in the vicinity of the ER, which could prevent direct competition for bulk cytosolic phenylalanine pools used in protein synthesis.

C4H was probably the first P450 to acquire a dedicated role in the phenylpropanoid pathway, and C3'H was likely to have been subsequently derived from it (Nelson, 2006). However, unlike C4H, which uses a free acid as substrate, C3'H acts on *p*-coumaroyl shikimate esters, the synthesis of which requires the activities of 4CL and HCT. Schoch *et al.* (2006) proposed that the involvement of shikimate, a key intermediate in the synthesis of aromatic amino acids, provides a layer of biochemical regulation on phenylpropanoid metabolism. As the availability of cytosolic pools of shikimate fluctuates in response to environmental cues, C3'H activity could then be fine-tuned accordingly (Schoch *et al.*, 2006). CCoAOMT activity may have evolved soon after the occurrence of C3'H, as the caffeoyl *ortho*-diphenol intermediates produced by C3'H are highly reactive, and are subjected to oxidation by various enzymes, such as polyphenol oxidases and catechol dioxygenases (Masai *et al.*, 1999; Cheynier & Moutounet, 2002). CCoAOMT converts caffeoyl-substituted intermediates to much more stable guaiacyl-substituted compounds, resulting in a ring modification pattern suitable for lignin biosynthesis later during plant evolution. It remains to be determined when the two oxidoreductases, CCR and CAD, evolved relative to the other enzymes in the phenylpropanoid pathway, but the profound impact of the occurrence of such a reductive pathway from phenolic acids to their alcohols is unmistakable. The addition of these hydroxycinnamyl alcohols to the biochemical repertoire of early land plants portended the origin of lignification later in the natural history of land plant evolution.

2. Lignin-like compounds in nonvascular plants

Lignin has generally been considered to be a hallmark of tracheophytes, but there are sporadic reports in the literature describing the detection of lignin or 'lignin-like' compounds from nonvascular plants, including brown algae, charophytic algae and mosses (Siegel, 1969; Reznikov *et al.*, 1978; Delwiche *et al.*, 1989). Many of the early findings were based on relatively crude detection methods according to today's standard, and were either highly controversial or failed to stand further scrutiny (reviewed by Lewis & Yamamoto, 1990). For example, the lignin-like compounds found in the brown algae *Fucus* were later confirmed to be a series of phloroglucinol-derived polyphenols, which are

more likely to be synthesized from fatty acids via the activity of polyketide synthases rather than from phenylpropanoid metabolism (Ragan, 1984). Recently, Ligrone *et al.* (2008) revisited this issue by immunogold labeling approaches on several bryophyte species and the charophytic alga *Nitella flexilis* using polyclonal antibodies raised against synthetic lignin-like polymers. In this study, weakly reacting lignin-related epitopes could be detected in the cell wall of all the nonvascular plant species examined, among which the moss *Sphagnum cuspidatum* even exhibited a labeling intensity similar to that of the tracheophyte controls (Ligrone *et al.*, 2008). Although the specificity of the antibodies used in this study needs to be further tested, these data suggest that nonvascular plants could have evolved the ability to transport and accumulate polymerized phenolic compounds in the wall. These wall-bound phenolics could possibly exist as polyphenols that resemble genuine lignin to some extent. The detection of lignin-related epitopes in *Nitella*, a genus representing the closest lineage sister to land plants, further raised an interesting possibility that a primitive form of phenylpropanoid metabolism could have emerged before land colonization by plants. Based on these earlier findings, it was even more surprising when Martone *et al.* (2009) reported the identification of lignin in the red alga *Calliarthron cheilosporioides*, the ancestor of which diverged from all the other green plants over 1.3 billion yr ago. Using the derivatization followed by reductive cleavage (DFRC) method, a lignin analysis technique specific to the β -O-4 linkages found in lignin (Lu & Ralph, 1997), these authors showed that *Calliarthron* lignin contains H, G and S units, a composition typically found in angiosperms (Martone *et al.*, 2009). The authors explained the independent occurrence of the angiosperm-type lignin in red algae by convergent evolution (Martone *et al.*, 2009). This hypothesis requires *Calliarthron* to have independently evolved not only the core monolignol biosynthetic pathway necessary for the synthesis of H and G lignin, but also the additional branch for S lignin synthesis that is only present in limited land plant lineages. It will be of great importance to interrogate the genome of this species to determine definitively whether it encodes the enzymes necessary for monolignol synthesis or whether the presence of low levels of lignin in this species (only *c.* 1% of the amount found in terrestrial plants) has another explanation.

It has been suggested that the UV autofluorescent lignin-like material surrounding the zygotes of several charophytic algae species, such as *Coleochaete*, is sporopollenin, a biopolymer that constitutes the outer wall of noneuphyllphyte spores and euphyllphyte pollen (Delwiche *et al.*, 1989; Kroken *et al.*, 1996). Sporopollenin is a constituent of all known land plants, and is polymerized from hydroxylated fatty acids and phenolics (Ahlers *et al.*, 2003). Its occurrence predated lignin (Morant *et al.*, 2007; Dobritsa *et al.*,

2009). The presence of sporopollenin in freshwater algae suggests that the initial selective advantage for phenylpropanoid metabolism could well have been that it provided algal zygotes with a UV-protecting outer layer that ultimately facilitated their movement onto the land (Morant *et al.*, 2007). The biochemical apparatus that initially functioned in the transport of sporopollenin phenolic monomers to the cell wall and the enzymes involved in subsequent polymerization processes may have been the ancestral forms of those that were later recruited and optimized for lignification.

Another group of phenylpropanoids, lignans, are often compared with, and even confused with, lignin. Lignans are dimers derived from hydroxycinnamyl alcohols, allylphenols or hydroxycinnamic acids, which are oxidatively coupled from radicals produced by the actions of laccases or peroxidases (Davin & Lewis, 2003). Examination of the phylogenetic distributions of lignans across land plants reveals an interesting pattern. Whereas hydroxycinnamyl alcohols are widely involved in the biosynthesis of various classes of lignans in tracheophytes, the lignans reported from bryophytes to date are only derived from hydroxycinnamic acids (Tazaki *et al.*, 1995; Cullmann *et al.*, 1999; Scher *et al.*, 2003; Umezawa, 2003). This observation is correlated with the occurrence of lignin biosynthesis in tracheophytes, which requires a major redirection of metabolic flux towards the reduction of hydroxycinnamic acids and aldehydes into their corresponding alcohols. The presence of CCR and CAD orthologs in the *Physcomitrella* genome suggests that bryophytes may contain the biochemical capacity to synthesize hydroxycinnamyl alcohols; however, the major offshoots from phenylpropanoid metabolism remain at the level of the acids and are directed to the biosynthesis of flavonoids and lignans. Although the evolution of lignan

biosynthesis via radical coupling of phenylpropanoids in bryophytes may represent early steps on the evolutionary path towards lignification, several fundamental differences exist between lignan and lignin biosynthesis. First, in comparison with lignan linkages that are solely derived from monomer–monomer coupling reactions, lignin linkages are primarily formed through monomer–oligomer or oligomer–oligomer coupling reactions (Ralph *et al.*, 2004). Second, the majority of the linkages found in lignans are based on β - β' with no involvement of the 4-hydroxyl of monomers in linkage formation (Fig. 4a). By contrast, lignin contains a much richer variety of interunit linkages, including β -O-4', β -5', 5-5', 4-O-5', β - β' and β -1' (Fig. 4b). The prevalent β - β' linkages found in lignans only account for a minor proportion of the total linkages in lignin (Ralph *et al.*, 2004). Third, it has been shown that the stereospecificity of certain lignans, for example (+)-pinosresinol, is controlled by dirigent proteins during coupling reactions (Davin *et al.*, 1997). By contrast, the various linkages in the lignin polymer are formed via a combinatorial process, controlled only by the chemical properties and concentrations of the reactants and the conditions in the surrounding matrix (Ralph *et al.*, 2004). To date, no evidence has been found that suggests that any agent, such as dirigent proteins, is involved in lignin polymerization. Fourth, lignans and their glucosides are primarily stored in the vacuole, indicating that lignans could be synthesized either in that compartment or in the cytosol, and then transported into the vacuole by specific transporters localized at the vacuole membrane. Alternatively, the dimerization and subsequent glycosylation could take place on the vacuole membrane or inside the vacuole. During lignification, monolignols need to be transported to the apoplast, where they encounter the enzymes that lead to their polymerization.

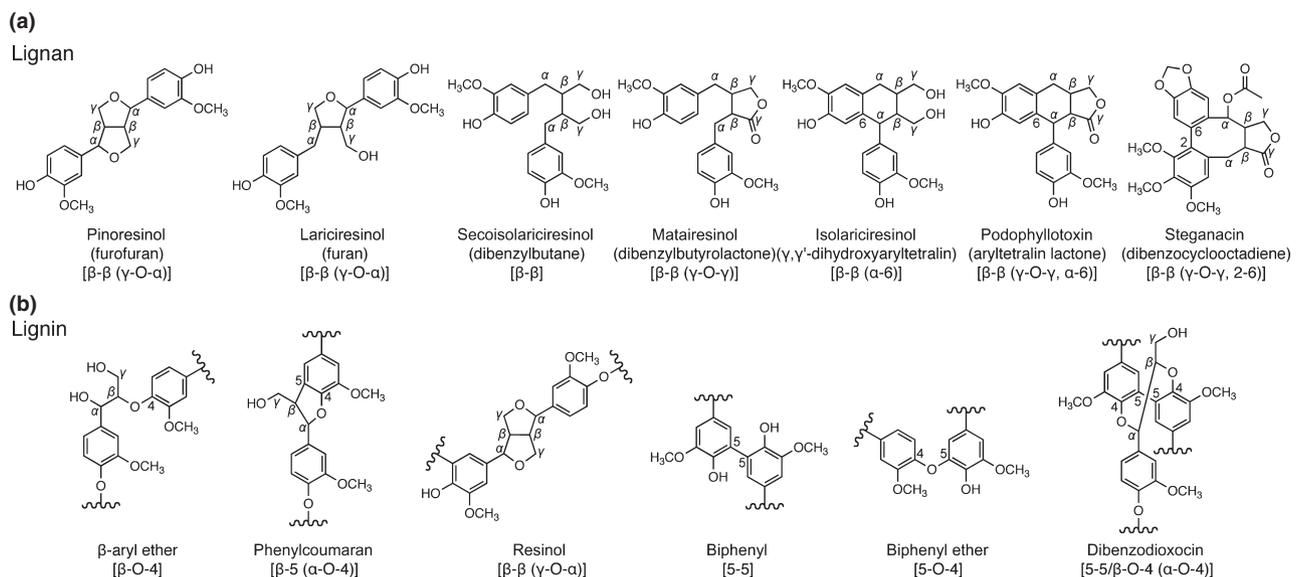


Fig. 4 Major linkages present in lignans (a) and lignins (b).

III. Occurrence and elaboration of lignification in tracheophytes

1. Developmental innovations of water-conducting tracheids: a foundation for lignin deposition

Tracheophytes are typified by the presence of the specialized water-conducting tracheary elements in their vasculature, including tracheids, and larger, open-ended cells known as vessel elements which have also evolved in some lineages. Both cell types have thickened secondary cell walls, impregnated by lignin. On maturation, tracheids and vessel elements undergo programmed cytoplasmic degeneration, resulting in xylem tissues composed of cell walls, optimal for long-distance water transport. Comparative studies on the wall structure of tracheary elements from various extant land plant lineages and well-preserved early land plant fossils have suggested a gradual evolutionary path towards the origin of lignified xylem (Fig. 2). Indeed, highly specialized water-conducting cells probably first emerged in bryophytes. Tansley & Chick (1901) reported the identification of a water-conducting tissue system in mosses. They noted that these conducting cells, referred to as hydroids, lose their cell contents during development, and could be involved in water transport (Tansley & Chick, 1901). Further studies using electron microscopy by several groups confirmed Tansley and Chick's observations in an extended number of mosses and liverworts (Smith, 1964; Hebant, 1974; Ligrone *et al.*, 2000). These studies revealed that bryophyte hydroids can be found in the gametophyte leafy stem and/or sporophyte seta, and, in some species, hydroids even possess thickened secondary walls with plasmodesmata-derived wall perforations (Smith, 1964; Hebant, 1974; Ligrone *et al.*, 2000). The fossilized sporophyte of *Aglaophyton major*, an extinct species from Rhynian chert thought to be an evolutionary intermediate between bryophytes and tracheophytes, contains bryophyte-type hydroids without secondary thickened walls (Edwards, 1986; Boyce *et al.*, 2003). Several members of Rhyniopsida, an extinct class considered to be the earliest tracheophytes, contain S-type tracheids (named after the genus *Sennicaulis*) with characters similar to the hydroids of certain extant bryophytes, but with secondary walls (Kenrick & Crane, 1991; Edwards & Axe, 2000). Both extinct and extant members of the lycophyte clade possess G-type tracheids (named after the genus *Gosslingia*), which exhibit annular or helical thickenings in the secondary wall with pores distributed between thickenings (Kenrick & Crane, 1991; Friedman & Cook, 2000; Schneider & Carlquist, 2000a,b). The P-type tracheids (named after the genus *Psilophyton*) are restricted in euphyllophytes, which have characteristic bordered pits (Kenrick & Crane, 1997). All three types of tracheids contain secondary wall thickenings composed of a degradation-prone template layer (next to the primary cell wall)

and a lignin-rich resistant layer (next to the lumen). Compared with the resistant layer in S-type tracheids of rhyniophytes, which accounts for only a small proportion of the total thickness of their secondary wall, the relative thickness of the resistant layer in G- and P-type tracheids increased significantly in eutracheophytes of the Late Silurian and Early Devonian (Cook & Friedman, 1998). Extant tracheophytes, such as *Huperzia* and *Equisetum*, contain an even thicker resistant layer (Friedman & Cook, 2000). Concomitant with the trend of an increasing degree of secondary wall lignification, the degradation-prone template layer has been greatly reduced in extant lycophytes and ferns, and is absent in seed plants (Friedman & Cook, 2000).

In addition to tracheary elements, some cell types in the stem, such as the interfascicular fibers of many herbaceous dicots, also develop secondary wall thickening and are lignified, primarily for structural support (Ye, 2002). Compared with the evolution of lignified tracheids, which have been the focus of most research, little is known about the evolutionary origin of lignification in fibers. By measuring and comparing the $\delta^{13}\text{C}$ isotope abundance in the dissected fossilized tissue from the central cylinder, inner and outer cortex of an *Aglaophyton* stem fossil, Boyce *et al.* (2003) concluded that the outer cortex of *Aglaophyton* stem might have been lignified, although its tracheids had not yet evolved thickened secondary walls. This result suggests that the initial function of lignin could have been in structural support for the plant body to enhance light interception, or as Raven (1984) suggested, as a chemical barrier that deters pathogens. It may not have been until later that the lignification pathway was recruited to tracheids to strengthen their walls for long-distance water transport.

2. Recruitment of specific monolignol polymerization enzymes for lignin biosynthesis in tracheophytes

It has been proposed that the occurrence of lignification through oxidative coupling of monolignols was initially permitted by the increase in the oxygen concentration in the atmosphere during the Ordovician and Silurian periods (Lowry *et al.*, 1980). The synthesis of simple primitive forms of lignin via this process, catalyzed by laccases at first, could have provided great selective advantages to the group of plants that eventually gave rise to tracheophytes (Lowry *et al.*, 1980). Laccases belong to a class of multicopper oxidases widely distributed in plants, fungi, bacteria and animals, and perform one-electron oxidation of substrates of a broad range at the expense of O_2 (Solomon *et al.*, 1996; Baldrian, 2006). In plants, laccases are not only involved in monolignol polymerization (Sterjiades *et al.*, 1992), but have also been reported to participate in the polymerization of other polyphenols, such as proanthocyanidins, flavonoid-derived pigments accumulated in the

seed coats of many plants (Ranocha *et al.*, 2002; Pourcel *et al.*, 2005). In fungi, laccases were in turn exploited to degrade lignin (Vanden Wymelenberg *et al.*, 2006). There was a significant increase in the diversity of laccases associated with the rise of land plants, but not with the rise of tracheophytes (Table 2). Indeed, a recent expression analysis of the 17 laccase family members from *Arabidopsis* revealed that only a few among them are specifically expressed in lignifying inflorescence, probably representing those lignification-related isoforms (McCaig *et al.*, 2005).

Although laccases may have been the earliest oxidases involved in lignin polymerization, the primary responsibility for this process may have soon shifted by the emergence of class III peroxidases, a group of heme-containing oxidases that catalyze one-electron oxidation of substrates at the expense of H₂O₂. The class III peroxidases are homologous to the more widely distributed class I peroxidases, such as ascorbate peroxidase, a key enzyme in the glutathione–ascorbate cycle that detoxifies excessive H₂O₂ produced in plant cells (Sharp *et al.*, 2003). Therefore, it has been postulated that the initial roles of the class III peroxidases in early land plants could have been to relieve the increasing oxidative stress resulting from the rising atmospheric O₂ levels during the Late Ordovician by exploiting phenylpropanoids as reducing agents (Kawaoka *et al.*, 2003). This development inevitably generated phenylpropanoid radicals with the potential to polymerize into higher order polyphenols, including lignin. Genes encoding class III peroxidases are absent from the available genome sequences of marine algae, and the gene family appears to have greatly expanded since the rise of tracheophytes (Table 2) (Passardi *et al.*, 2004; Bakalovic *et al.*, 2006). An increasing body of evidence supports the idea that class III peroxidases are the dominant forms of lignin polymerization enzymes. For example, using a 3,3'-diaminobenzidine cytochemistry method, Barcelo (1995) reported that the H₂O₂-dependent peroxidases, but not laccases, accounted for the primary oxidative activities in the lignifying hypocotyls of lupin. Genetic studies in tobacco and aspen further showed that knockdown of certain class III peroxidases had an impact on both lignin content and composition (Blee *et al.*, 2003; Li *et al.*, 2003).

Although the precise functions of individual class III peroxidase isoforms during lignification are largely unknown

because of the high level of redundancy within this family, recent studies have revealed the presence of sinapyl alcohol-specific peroxidases (Sasaki *et al.*, 2004; Gabaldon *et al.*, 2005, 2006), which distinguishes them from most of the previously characterized class III peroxidases that are more efficient towards coniferyl alcohol (Ralph *et al.*, 2004). The motifs in the primary sequence that potentially dictate this substrate specificity have also been mapped through sequence comparison (Barcelo *et al.*, 2007). Interestingly, these sinapyl alcohol-related motifs were also found in peroxidases from plant lineages that do not synthesize S lignin, and even in mosses, suggesting that the evolution of S lignin biosynthesis is not restricted by the lack of polymerization enzymes that can generate radicals from sinapyl alcohol (Gomez Ros *et al.*, 2007).

3. Multiple biosynthetic strategies for S lignin synthesis

In addition to H and G units, which are ubiquitous across all tracheophyte lineages, lignins from angiosperms also contain S units. Extensive genetic and biochemical studies over the past decade have revealed that angiosperms have evolved an offshoot from the core monolignol biosynthetic pathway that can divert G-substituted phenylpropanoid intermediates to produce sinapyl alcohol (Humphreys & Chapple, 2002). This unique S lignin pathway branch is composed of two enzymes: a cytochrome P450-dependent monooxygenase (P450), known as ferulic acid/coniferaldehyde/coniferyl alcohol 5-hydroxylase (F5H), and a SAM-dependent *O*-methyltransferase, known as caffeic acid *O*-methyltransferase (COMT) (Fig. 2). The combined activities of these two enzymes lead to the addition of a 5-methoxy group to coniferaldehyde/coniferyl alcohol to form sinapaldehyde/sinapyl alcohol (Humphreys *et al.*, 1999; Parvathi *et al.*, 2001). Although S lignin has been traditionally considered to be a characteristic unique to angiosperms, the detection of S lignin has been reported from certain species of lycophytes, ferns and gymnosperms, including *Selaginella*, cuplet fern (*Dennstaedtia bipinnata*), yew plum pine (*Podocarpus macrophyllus*), sandarac-cypress (*Tetraclinis articulata*) and all three extant genera within the division Gnetophyta (Creighton *et al.*, 1944; Towers & Gibbs, 1953; White & Towers, 1967; Erickson & Miksche, 1974; Miksche & Yasuda, 1977; Kuroda, 1983; Logan &

Table 2 The distribution of laccases and class III peroxidases in representative sequenced genomes of green plants

| Organism | Laccases | % of total gene complement | Class III peroxidases | % of total gene complement |
|-----------------------------------|----------|----------------------------|-----------------------|----------------------------|
| <i>Chlamydomonas reinhardtii</i> | 3 | 0.18 | 0 | 0.0 |
| <i>Physcomitrella patens</i> | 12 | 0.33 | 43 | 1.2 |
| <i>Selaginella moellendorffii</i> | 10 | 0.45 | 79 | 3.5 |
| <i>Oryza sativa</i> | 20 | 0.35 | 138 | 2.4 |
| <i>Arabidopsis thaliana</i> | 17 | 0.65 | 73 | 2.8 |
| <i>Populus trichocarpa</i> | 39 | 0.87 | 105 | 2.3 |

Thomas, 1985; Jin *et al.*, 2005; Weng *et al.*, 2008a). These findings suggest that S lignin could have been lost in most of the species in lycophytes, ferns and gymnosperms, or was invented multiple times in various lineages of tracheophytes.

A recent study in the lycophyte *Selaginella moellendorffii* identified a novel P450 (*SmF5H*) that is involved in S lignin biosynthesis. Kinetic characterization of *SmF5H* revealed that it is capable of the 5-hydroxylation of coniferaldehyde and coniferyl alcohol *in vitro*, like its angiosperm counterpart, and that it can also restore S lignin biosynthesis when expressed in a *Arabidopsis* F5H-deficient mutant (Weng *et al.*, 2008b). Phylogenetic analysis suggested that *SmF5H* evolved independently from its angiosperm counterpart, and was probably derived from an independent radiation of P450 enzymes unique to lycophytes (Weng *et al.*, 2008b). A follow-up study further revealed that *SmF5H* also has phenylpropanoid 3-hydroxylase activity, a catalytic property absent in its angiosperm counterpart. Expression of *SmF5H* in an *Arabidopsis* double-mutant deficient in both C3'H and F5H restores its S lignin biosynthesis via a route not normally employed in angiosperms (Fig. 3) (Weng *et al.*, 2010). These results revealed a remarkable divergence in choices of biosynthetic strategies to synthesize S lignin in lycophytes and angiosperms. In angiosperms, the S lignin pathway diverts G-substituted intermediates, namely coniferaldehyde and coniferyl alcohol, towards S subunits by employing a 5-specific F5H. By contrast, by employing the bifunctional phenylpropanoid *meta*-hydroxylase *SmF5H*, *Selaginella* can synthesize S subunits from *p*-coumaraldehyde and *p*-coumaryl alcohol via G-substituted intermediates, making this pathway independent of C3'H, HCT and CCoAOMT (Fig. 2). As mentioned above, S lignin could have evolved multiple times in various vascular plant lineages, which leaves open the possibility that other plants have elaborated still more distinct S lignin biosynthetic pathways. Elucidation of the molecular mechanisms behind the occurrence of S lignin in these fern and gymnosperm species will provide more insights into how versatile metabolic systems can be adapted to elaborate on an established core pathway.

In angiosperms, S lignin is predominantly localized in fiber cells, versus the tracheary elements which are often enriched with G lignin (Chapple *et al.*, 1992; Nakashima *et al.*, 2008). Similar tissue specificity of the lignin subunit distribution pattern is also evident in *Selaginella*, where S lignin is abundant in the stem cortical cells but not in the xylem (Weng *et al.*, 2008b). These observations suggest that S lignin could have provided similar selective advantages to *Selaginella* and angiosperms. A lignin polymer containing sinapyl alcohol units may have superior mechanical support properties, a function previously inferred for these fibers in angiosperms (Li *et al.*, 2001). A G/S copolymer, with its increased linkage diversity, could also serve as a better

chemical barrier to protect the water- and nutrient-conducting systems from pathogen attacks (Moerschbacher *et al.*, 1990; Menden *et al.*, 2007).

IV. Concluding remarks

Many advances in modern biology have been made possible by comparative studies using model species from phylogenetically divergent lineages. For example, our knowledge of biological processes in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Danio rerio* and *Mus musculus* has helped us to understand the complex physiology of humans and the molecular mechanisms behind many human diseases. Plant biology of the last century has witnessed a revolutionary advancement in functional genomics in flowering plants, including the well-established model species *Arabidopsis thaliana* and those species directly related to agriculture. In recent years, significant progress has been made in plant genome sequencing projects, including those that occupy particularly interesting positions within the evolutionary history of plants. The development of these genomic resources, together with increasing functional studies in species other than flowering plants, have now allowed us to evaluate many plant biological processes in an evolutionary context. In the past, lignin biosynthesis has attracted a great deal of attention because of its agricultural and economic importance. We are now in a position to develop a better understanding of how lignin biosynthesis originated and evolved, which will, in turn, provide greater insights into how land plants adapted to the terrestrial environment and dealt with the challenges it presented.

Acknowledgements

This work was funded by the National Science Foundation, Grant No. IOB-0450289.

References

- Achnine L, Blancaflor EB, Rasmussen S, Dixon RA. 2004. Colocalization of L-phenylalanine ammonia-lyase and cinnamate 4-hydroxylase for metabolic channeling in phenylpropanoid biosynthesis. *Plant Cell* 16: 3098–3109.
- Ahlers F, Lambert J, Wiermann R. 2003. Acetylation and silylation of piperidine solubilized sporopollenin from pollen of *Typha angustifolia* L. *Zeitschrift für Naturforschung C, Journal of Biosciences* 58: 807–811.
- Baedeker M, Schulz GE. 2002. Autocatalytic peptide cyclization during chain folding of histidine ammonia-lyase. *Structure* 10: 61–67.
- Bakalovic N, Passardi F, Ioannidis V, Cosio C, Penel C, Falquet L, Dunand C. 2006. Peroxibase: a class III plant peroxidase database. *Phytochemistry* 67: 534–539.
- Baldrian P. 2006. Fungal laccases – occurrence and properties. *FEMS Microbiology Reviews* 30: 215–242.
- Barcelo AR. 1995. Peroxidase and not laccase is the enzyme responsible for cell wall lignification in the secondary thickening of xylem vessels in *Lupinus*. *Protoplasma* 186: 41–44.

- Barcelo AR, Ros LV, Carrasco AE. 2007. Looking for syringyl peroxidases. *Trends in Plant Science* 12: 486–491.
- Basile A, Giordano S, Lopez-Saez JA, Cobiánchi RC. 1999. Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry* 52: 1479–1482.
- Bateman RM, Crane PR, DiMichele WA, Kenrick PR, Rowe NP, Speck T, Stein WE. 1998. Early evolution of land plants: phylogeny, physiology, and ecology of the primary terrestrial radiation. *Annual Review of Ecology and Systematics* 29: 263–292.
- Beerling DJ, Osborne CP, Chaloner WG. 2001. Evolution of leaf-form in land plants linked to atmospheric CO₂ decline in the late Palaeozoic era. *Nature* 410: 352–354.
- Berner RA. 1993. Paleozoic atmospheric CO₂: importance of solar radiation and plant evolution. *Science* 261: 68–70.
- Berner RA, Petsch ST, Lake JA, Beerling DJ, Popp BN, Lane RS, Laws EA, Westley MB, Cassar N, Woodward FI *et al.* 2000. Isotope fractionation and atmospheric oxygen: implications for phanerozoic O₂ evolution. *Science* 287: 1630–1633.
- Blee KA, Choi JW, O'Connell AP, Schuch W, Lewis NG, Bolwell GP. 2003. A lignin-specific peroxidase in tobacco whose antisense suppression leads to vascular tissue modification. *Phytochemistry* 64: 163–176.
- Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 54: 519–546.
- Bowman JL, Floyd SK, Sakakibara K. 2007. Green genes – comparative genomics of the green branch of life. *Cell* 129: 229–234.
- Boyce CK, Cody GD, Fogel ML, Hazen RM, Alexander CMO, Knoll AH. 2003. Chemical evidence for cell wall lignification and the evolution of tracheids in early Devonian plants. *International Journal of Plant Sciences* 164: 691–702.
- Chapple CC, Vogt T, Ellis BE, Somerville CR. 1992. An Arabidopsis mutant defective in the general phenylpropanoid pathway. *Plant Cell* 4: 1413–1424.
- Cheynier V, Moutounet M. 2002. Oxidative reactions of caffeic acid in model systems containing polyphenol oxidase. *Journal of Agricultural and Food Chemistry* 40: 2038–2044.
- Cook ME, Friedman WE. 1998. Tracheid structure in a primitive extant plant provides an evolutionary link to earliest fossil tracheids. *International Journal of Plant Sciences* 159: 881–890.
- Creighton RHJ, Gibbs RD, Hibbert H. 1944. Studies on lignin and related compounds. LXXV. Alkaline nitrobenzene oxidation of plant materials and application to taxonomic classification. *Journal of the American Chemical Society* 66: 32–37.
- Cullmann F, Schmidt A, Schulz F, Trennheuser ML, Becker H. 1999. Lignans from the liverworts *Lepidozia incurvata*, *Chiloscyphus polyanthos* and *Jungermannia exsertifolia* ssp. *cordifolia*. *Phytochemistry* 52: 1647–1650.
- D'Auria JC. 2006. Acyltransferases in plants: a good time to be BAHD. *Current Opinion in Plant Biology* 9: 331–340.
- Davin L, Lewis N. 2003. An historical perspective on lignan biosynthesis: monolignol, allylphenol and hydroxycinnamic acid coupling and downstream metabolism. *Phytochemistry Reviews* 2: 257–288.
- Davin LB, Wang HB, Crowell AL, Bedgar DL, Martin DM, Sarkanen S, Lewis NG. 1997. Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. *Science* 275: 362–366.
- Delwiche CF, Graham LE, Thomson N. 1989. Lignin-like compounds and sporopollenin coelocchaete, an algal model for land plant ancestry. *Science* 245: 399–401.
- Dobritsa AA, Shrestha J, Morant M, Pinot F, Matsuno M, Swanson R, Moller BL, Preuss D. 2009. Cyp704b1 is a long-chain fatty acid omega-hydroxylase essential for sporopollenin synthesis in pollen of Arabidopsis. *Plant Physiology* 151: 574–589.
- Dudley R. 1998. Atmospheric oxygen, giant paleozoic insects and the evolution of aerial locomotor performance. *Journal of Experimental Biology* 201: 1043–1050.
- Edwards D, Axe L. 2000. Novel conducting tissues in lower Devonian plants. *Botanical Journal of the Linnean Society* 134: 383–399.
- Edwards DS. 1986. *Aglaophyton major*, a non-vascular land-plant from the Devonian rhynie chert. *Botanical Journal of the Linnean Society* 93: 173–204.
- Emiliani G, Fondi M, Fani R, Gribaldo S. 2009. A horizontal gene transfer at the origin of phenylpropanoid metabolism: a key adaptation of plants to land. *Biology Direct* 4: 7.
- Erickson M, Miksche GE. 1974. Characterization of gymnosperm lignins by oxidative-degradation. *Holzforschung* 28: 135–138.
- Ferrer JL, Zubieta C, Dixon RA, Noel JP. 2005. Crystal structures of alfalfa caffeoyl coenzyme a 3-O-methyltransferase. *Plant Physiology* 137: 1009–1017.
- Freese B. 2003. *Coal: a human history*. Cambridge, MA, USA: Perseus Publishing.
- Friedman WE, Cook ME. 2000. The origin and early evolution of tracheids in vascular plants: integration of palaeobotanical and neobotanical data. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 355: 857–868.
- Gabaldon C, Lopez-Serrano M, Pedreno MA, Barcelo AR. 2005. Cloning and molecular characterization of the basic peroxidase isoenzyme from *Zinnia elegans*, an enzyme involved in lignin biosynthesis. *Plant Physiology* 139: 1138–1154.
- Gabaldon C, Lopez-Serrano M, Pomar F, Merino F, Cuello J, Pedreno MA, Barcelo AR. 2006. Characterization of the last step of lignin biosynthesis in *Zinnia elegans* suspension cell cultures. *FEBS Letters* 580: 4311–4316.
- Gensel PG. 2008. The earliest land plants. *Annual Review of Ecology, Evolution, and Systematics* 39: 459–477.
- Gensel PG, Edwards D. 2001. *Plants invade the land: evolutionary and environmental perspectives*. New York, NY, USA: Columbia University Press.
- Gomez Ros LV, Gabaldon C, Pomar F, Merino F, Pedreno MA, Barcelo AR. 2007. Structural motifs of syringyl peroxidases predate not only the gymnosperm–angiosperm divergence but also the radiation of tracheophytes. *New Phytologist* 173: 63–78.
- Hayatsu R, Winans RE, McBeth RL, Scott RG, Moore LP, Studier MH. 1979. Lignin-like polymers in coals. *Nature* 278: 41–43.
- Hebant C. 1974. Studies on the development of the conducting tissue-system in the gametophytes of some polytrichales. II. Development and structure at maturity of the hydroids central strand. *Journal of the Hattori Botanical Laboratory* 38: 565–607.
- Hisanaga Y, Ago H, Nakagawa N, Hamada K, Ida K, Yamamoto M, Hori T, Arai Y, Sugahara M, Kuramitsu S *et al.* 2004. Structural basis of the substrate-specific two-step catalysis of long chain fatty acyl-CoA synthetase dimer. *Journal of Biological Chemistry* 279: 31717–31726.
- Humphreys JM, Chapple C. 2002. Rewriting the lignin roadmap. *Current Opinion in Plant Biology* 5: 224–229.
- Humphreys JM, Hemm MR, Chapple C. 1999. New routes for lignin biosynthesis defined by biochemical characterization of recombinant ferulate 5-hydroxylase, a multifunctional cytochrome P450-dependent monooxygenase. *Proceedings of the National Academy of Sciences, USA* 96: 10045–10050.
- Jin ZF, Matsumoto Y, Tange T, Akiyama T, Higuchi M, Ishii T, Iiyama K. 2005. Proof of the presence of guaiacyl-syringyl lignin in *Selaginella tamariscina*. *Journal of Wood Science* 51: 424–426.
- Kawaoka A, Matsunaga E, Endo S, Kondo S, Yoshida K, Shinmyo A, Ebinuma H. 2003. Ectopic expression of a horseradish peroxidase enhances growth rate and increases oxidative stress resistance in hybrid aspen. *Plant Physiology* 132: 1177–1185.

- Kenrick P, Crane PR. 1991. Water-conducting cells in early fossil land plants: implications for the early evolution of tracheophytes. *Botanical Gazette* 152: 335–356.
- Kenrick P, Crane PR. 1997. The origin and early evolution of plants on land. *Nature* 389: 33–39.
- Kroken SB, Graham LE, Cook ME. 1996. Occurrence and evolutionary significance of resistant cell walls in charophytes and bryophytes. *American Journal of Botany* 83: 1241–1254.
- Kuroda H. 1983. Comparative studies on O-methyltransferases involved in lignin biosynthesis. *Wood Research* 69: 91–135.
- Lacombe E, Hawkins S, Van Doorselaere J, Piquemal J, Goffner D, Poeydomenge O, Boudet AM, Grima-Pettenati J. 1997. Cinnamoyl CoA reductase, the first committed enzyme of the lignin branch biosynthetic pathway: cloning, expression and phylogenetic relationships. *Plant Journal* 11: 429–441.
- Lewis NG, Yamamoto E. 1990. Lignin – occurrence, biogenesis and biodegradation. *Annual Review of Plant Physiology and Plant Molecular Biology* 41: 455–496.
- Li L, Cheng XF, Leshkevich J, Umezawa T, Harding SA, Chiang VL. 2001. The last step of syringyl monolignol biosynthesis in angiosperms is regulated by a novel gene encoding sinapyl alcohol dehydrogenase. *Plant Cell* 13: 1567–1586.
- Li Y, Kajita S, Kawai S, Katayama Y, Morohoshi N. 2003. Down-regulation of an anionic peroxidase in transgenic aspen and its effect on lignin characteristics. *Journal of Plant Research* 116: 175–182.
- Ligrone R, Carafa A, Duckett J, Renzaglia K, Ruel K. 2008. Immunocytochemical detection of lignin-related epitopes in cell walls in bryophytes and the charalean alga *Nitella*. *Plant Systematics and Evolution* 270: 257–272.
- Ligrone R, Duckett JG, Renzaglia KS. 2000. Conducting tissues and phyletic relationships of bryophytes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 355: 795–813.
- Logan KJ, Thomas BA. 1985. Distribution of lignin derivatives in plants. *New Phytologist* 99: 571–585.
- Lowry B, Hebant C, Lee D. 1980. The origin of land plants – a new look at an old problem. *Taxon* 29: 183–197.
- Lu FC, Ralph J. 1997. Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: protocol for analysis of dfrc monomers. *Journal of Agricultural and Food Chemistry* 45: 2590–2592.
- Lynch M, Conery JS. 2000. The evolutionary fate and consequences of duplicate genes. *Science* 290: 1151–1155.
- Ma X, Koepke J, Panjikar S, Fritsch G, Stöckigt J. 2005. Crystal structure of vinorine synthase, the first representative of the BAHD superfamily. *Journal of Biological Chemistry* 280: 13576–13583.
- Martone PT, Estevez JM, Lu F, Ruel K, Denny MW, Somerville C, Ralph J. 2009. Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture. *Current Biology* 19: 169–175.
- Masai E, Shinohara S, Hara H, Nishikawa S, Katayama Y, Fukuda M. 1999. Genetic and biochemical characterization of a 2-pyrone-4,6-dicarboxylic acid hydrolase involved in the protocatechuate 4,5-cleavage pathway of *Sphingomonas paucimobilis* syk-6. *Journal of Bacteriology* 181: 55–62.
- McCaig BC, Meagher RB, Dean JF. 2005. Gene structure and molecular analysis of the laccase-like multicopper oxidase (LMCO) gene family in *Arabidopsis thaliana*. *Planta* 221: 619–636.
- McLaughlin SG, Dilger JP. 1980. Transport of protons across membranes by weak acids. *Physiological Reviews* 60: 825–863.
- Menden B, Kohlhoff M, Moerschbacher BM. 2007. Wheat cells accumulate a syringyl-rich lignin during the hypersensitive resistance response. *Phytochemistry* 68: 513–520.
- Miksche GE, Yasuda S. 1977. Gas-chromatography analysis of lignin oxidation-products. 16. Lignin in leaves of several softwoods and hardwoods. *Holzforschung* 31: 57–59.
- Moerschbacher BM, Noll U, Gorrichon L, Reisener HJ. 1990. Specific inhibition of lignification breaks hypersensitive resistance of wheat to stem rust. *Plant Physiology* 93: 465–470.
- Morant M, Jorgensen K, Schaller H, Pinot F, Moller BL, Werck-Reichhart D, Bak S. 2007. Cyp703 is an ancient cytochrome P450 in land plants catalyzing in-chain hydroxylation of lauric acid to provide building blocks for sporopollenin synthesis in pollen. *Plant Cell* 19: 1473–1487.
- Nakashima J, Chen F, Jackson L, Shadle G, Dixon RA. 2008. Multi-site genetic modification of monolignol biosynthesis in alfalfa (*Medicago sativa*): effects on lignin composition in specific cell types. *New Phytologist* 179: 738–750.
- Nelson DR. 2006. Plant cytochrome P450s from moss to poplar. *Phytochemistry Reviews* 5: 193–204.
- Parvathi K, Chen F, Guo D, Blount JW, Dixon RA. 2001. Substrate preferences of O-methyltransferases in alfalfa suggest new pathways for 3-O-methylation of monolignols. *Plant Journal* 25: 193–202.
- Passardi F, Longet D, Penel C, Dunand C. 2004. The class III peroxidase multigenic family in rice and its evolution in land plants. *Phytochemistry* 65: 1879–1893.
- Pourcel L, Routaboul JM, Kerhoas L, Caboche M, Lepiniec L, Debeaujon I. 2005. Transparent testa10 encodes a laccase-like enzyme involved in oxidative polymerization of flavonoids in Arabidopsis seed coat. *Plant Cell* 17: 2966–2980.
- Ragan MA. 1984. *Fucus* 'lignin': a reassessment. *Phytochemistry* 23: 2029–2032.
- Ralph J, Lundquist K, Brunow G, Lu F, Kim H, Schatz PF, Marita JM, Hatfield RD, Ralph SA, Christensen JH *et al.* 2004. Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids. *Phytochemistry Reviews* 3: 29–60.
- Ranocha P, Chabannes M, Chamayou S, Danoun S, Jauneau A, Boudet AM, Goffner D. 2002. Laccase down-regulation causes alterations in phenolic metabolism and cell wall structure in poplar. *Plant Physiology* 129: 145–155.
- Rasmussen S, Dixon RA. 1999. Transgene-mediated and elicitor-induced perturbation of metabolic channeling at the entry point into the phenylpropanoid pathway. *Plant Cell* 11: 1537–1552.
- Raven JA. 1984. Physiological correlates of the morphology of early vascular plants. *Botanical Journal of the Linnean Society* 88: 105–126.
- Reznikov V, Mikhaseva M, Zil'bergleit M. 1978. The lignin of the alga *Fucus vesiculosus*. *Chemistry of Natural Compounds* 14: 554–556.
- Richards TA, Dacks JB, Campbell SA, Blanchard JL, Foster PG, McLeod R, Roberts CW. 2006. Evolutionary origins of the eukaryotic shikimate pathway: gene fusions, horizontal gene transfer, and endosymbiotic replacements. *Eukaryotic Cell* 5: 1517–1531.
- Ritter H, Schulz GE. 2004. Structural basis for the entrance into the phenylpropanoid metabolism catalyzed by phenylalanine ammonia-lyase. *Plant Cell* 16: 3426–3436.
- Sasaki S, Nishida T, Tsutsumi Y, Kondo R. 2004. Lignin dehydrogenative polymerization mechanism: a poplar cell wall peroxidase directly oxidizes polymer lignin and produces *in vitro* dehydrogenative polymer rich in beta-O-4 linkage. *FEBS Letters* 562: 197–201.
- Scher JM, Zapp J, Becker H. 2003. Lignan derivatives from the liverwort *Bazzania trilobata*. *Phytochemistry* 62: 769–777.
- Schillmiller AL, Stout J, Weng JK, Humphreys J, Ruegger MO, Chapple C. 2009. Mutations in the cinnamate 4-hydroxylase gene impact metabolism, growth and development in Arabidopsis. *Plant Journal* 60: 771–782.

- Schneider EL, Carlquist S. 2000a. SEM studies on the vessels of heterophyllous species of *Selaginella*. *Journal of the Torrey Botanical Society* 127: 263–270.
- Schneider EL, Carlquist S. 2000b. SEM studies on vessels of the homophyllous species of *Selaginella*. *International Journal of Plant Sciences* 161: 967–974.
- Schneider K, Hovel K, Witzel K, Hamberger B, Schomburg D, Kombrink E, Stuible HP. 2003. The substrate specificity-determining amino acid code of 4-coumarate:CoA ligase. *Proceedings of the National Academy of Sciences, USA* 100: 8601–8606.
- Schoch G, Morant M, Abdulrazzak N, Asnaghi C, Goepfert S, Petersen M, Ullmann P, Werck-Reichhart D. 2006. The meta-hydroxylation step in the phenylpropanoid pathway: a new level of complexity in the pathway and its regulation. *Environmental Chemistry Letters* 4: 127–136.
- Sharp KH, Mewies M, Moody PC, Raven EL. 2003. Crystal structure of the ascorbate peroxidase–ascorbate complex. *Nature Structural Biology* 10: 303–307.
- Siegel SM. 1969. Evidence for the presence of lignin in moss gametophytes. *American Journal of Botany* 56: 175–179.
- Silber MV, Meimberg H, Ebel J. 2008. Identification of a 4-coumarate:CoA ligase gene family in the moss, *Physcomitrella patens*. *Phytochemistry* 69: 2449–2456.
- Smith JL. 1964. Water-conducting system of *Symphyogyna*. *Nature* 202: 617.
- Solomon EI, Sundaram UM, Machonkin TE. 1996. Multicopper oxidases and oxygenases. *Chemical Reviews* 96: 2563–2606.
- Sterjiades R, Dean JF, Eriksson KE. 1992. Laccase from sycamore maple (*Acer pseudoplatanus*) polymerizes monolignols. *Plant Physiology* 99: 1162–1168.
- Stewart WN, Rothwell GW. 1993. *Paleobotany and the evolution of plants*. New York, NY, USA: Cambridge University Press.
- Tansley AG, Chick E. 1901. Notes on the conducting tissue-system in Bryophyta. *Annals of Botany* 15: 1–38.
- Tawfik DS. 2006. Loop grafting and the origins of enzyme species. *Science* 311: 475–476.
- Tazaki H, Adam KP, Becker H. 1995. Five lignan derivatives from in vitro cultures of the liverwort *Jamesoniella autumnalis*. *Phytochemistry* 40: 1671–1675.
- Towers GHN, Gibbs RD. 1953. Lignin chemistry and the taxonomy of higher plants. *Nature* 172: 25–26.
- Umezawa T. 2003. Diversity in lignan biosynthesis. *Phytochemistry Reviews* 2: 371–390.
- Vanden Wymelenberg A, Sabat G, Mozuch M, Kersten PJ, Cullen D, Blanchette RA. 2006. Structure, organization, and transcriptional regulation of a family of copper radical oxidase genes in the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology* 72: 4871–4877.
- Vanoverbeek J, Blondeau R, Horne V. 1951. Trans-cinnamic acid as an anti-auxin. *American Journal of Botany* 38: 589–595.
- Vidgren J, Svensson LA, Liljas A. 1994. Crystal structure of catechol O-methyltransferase. *Nature* 368: 354–358.
- Weng JK, Akiyama T, Bonawitz ND, Li X, Ralph J, Chapple C. 2010. Convergent evolution of syringyl lignin biosynthesis via distinct pathways in the lycophyte *Selaginella* and flowering plants. *Plant Cell* 22: 1033–1045.
- Weng JK, Banks JA, Chapple C. 2008a. Parallels in lignin biosynthesis: a study in *Selaginella moellendorffii* reveals convergence across 400 million years of evolution. *Communicative and Integrative Biology* 1: 20–22.
- Weng JK, Li X, Stout J, Chapple C. 2008b. Independent origins of syringyl lignin in vascular plants. *Proceedings of the National Academy of Sciences, USA* 105: 7887–7892.
- Werck-Reichhart D, Feyereisen R. 2000. Cytochromes P450: a success story. *Genome Biology* 1: REVIEWS3003.
- White E, Towers GHN. 1967. Comparative biochemistry of lycophytes. *Phytochemistry* 6: 663–667.
- Wong WS, Guo D, Wang XL, Yin ZQ, Xia B, Li N. 2005. Study of *cis*-cinnamic acid in *Arabidopsis thaliana*. *Plant Physiology and Biochemistry* 43: 929–937.
- Wu D, Govindasamy L, Lian W, Gu Y, Kukar T, Agbandje-McKenna M, McKenna R. 2003. Structure of human carnitine acetyltransferase. Molecular basis for fatty acyl transfer. *Journal of Biological Chemistry* 278: 13159–13165.
- Xu Z, Zhang D, Hu J, Zhou X, Ye X, Reichel KL, Stewart NR, Syrenne RD, Yang X, Gao P *et al.* 2009. Comparative genome analysis of lignin biosynthesis gene families across the plant kingdom. *BMC Bioinformatics* 10(Suppl. 11): S3.
- Ye ZH. 2002. Vascular tissue differentiation and pattern formation in plants. *Annual Review of Plant Biology* 53: 183–202.
- Youn B, Camacho R, Moineddin SG, Lee C, Davin LB, Lewis NG, Kang C. 2006. Crystal structures and catalytic mechanism of the *Arabidopsis* cinnamyl alcohol dehydrogenases AtCAD5 and AtCAD4. *Organic and Biomolecular Chemistry* 4: 1687–1697.