Lignin Structure in a Mutant Pine Deficient in Cinnamyl Alcohol Dehydrogenase

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LIGNIN STRUCTURE IN A MUTANT PINE DEFICIENT IN CINNAMYL ALCOHOL DEHYDROGENASE

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LIGNIN STRUCTURE IN A MUTANT PINE DEFICIENT IN CINNAMYL ALCOHOL DEHYDROGENASE

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ABSTRACT

Cinnamyl alcohol dehydrogenase (CAD) activity is deficient in loblolly pine (*Pinus taeda* L.), harboring a mutated allele of the *cad* gene (*cad-n1*). We compared lignin structure of CAD-deficient and wild type pines, both types segregating within full-sib families obtained by controlled crosses. The type and frequency of lignin building units and distribution of interunit bonds were determined from the GC-MS analysis of thioacidolysis monomers and dimers. While the lignin content was only slightly reduced, the lignin structure was dramatically modified by the mutation in both mature and juvenile trees. Lignins from CAD-deficient pine displayed unusually high levels of coniferaldehyde and dihydroconiferyl alcohol (DHCA). In addition, biphenyl and biphenyl ether bonds were in large excess in these abnormal lignins. These results suggest that the CAD-deficient pines efficiently compensate for the shortage in normal lignin precursors by utilizing non traditional wall phenolics to construct unusual lignins particularly enriched in resistant interunit bonds.

Keywords. Lignification; altered lignin structure; pine (*Pinus taeda* L.); Thioacidolysis, dihydroconiferyl alcohol.
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INTRODUCTION

Lignins are cell wall polymers essential for mechanical support, water transport, and disease resistance in vascular terrestrial plants. In wood, lignins are particularly abundant, they typically account for 20 to 30% of the dry weight. Removal of lignin from wood is the basis of kraft pulping, the major chemical process used for the production of high quality pulp and paper. In recent years, studies of mutant and transgenic plants with altered monolignol biosynthesis have suggested that plants have a high level of metabolic plasticity in the formation of lignin (Whetten et al., 1998; Boudet, 1998). This means that unusual phenolics may be committed to the formation of lignins when there is a metabolic shortage in one of the \( p \)-hydroxycinnamyl alcohols, namely, \( p \)-coumaryl, coniferyl and sinapyl alcohol, the conventional monolignols involved in the lignin polymerization step. For example, a depressed caffeic acid \( O \)-methyltransferase (COMT) activity significantly increases the incorporation of 5-hydroxyconiferyl alcohol in angiosperm lignins (Lapierre et al., 1988; van Doorsselaere et al., 1995) while a reduction in cinnamyl alcohol dehydrogenase (CAD) and in cinnamyl-CoA reductase (CCR), respectively, increases the incorporation of cinnamaldehyde (Halpin et al., 1994; Hibino et al., 1995) and tyramine ferulate (Ralph et al., 1998). In addition, naturally occurring variation in lignin subunit composition that exists among diverse plant taxa happens to extend beyond the three traditional \( p \)-hydroxycinnamyl alcohols, again highlighting the ability of plants to adapt to diverse changes in lignin precursor supply. For example, grass lignins incorporate \( p \)-coumarate and ferulate units (Ralph et al., 1994; Jacquet et al., 1995). Thus, there is growing evidence that the structure of the polymers we call lignins may be dictated by precursor supply and variations therein (Sederoff et al., 1999).
Recently, a mutant loblolly pine (Pinus taeda L.) was discovered in which the expression of the gene encoding cinnamyl alcohol dehydrogenase (CAD; EC 1.1.1.195) is severely reduced due to the presence of a recessive (cad-n1) allele (MacKay et al., 1997). In the xylem of homozygous mutant seedlings, CAD activity was almost completely deficient (between 0 and 1% of wild type), while lignin content was only slightly decreased (MacKay et al., 1997). NMR analysis of an isolated milled wood lignin sample revealed the incorporation of high levels of coniferaldehyde, dihydroconiferyl alcohol (DHCA) and vanillin in the CAD-deficient pine (Ralph et al., 1997). The finding that DHCA was utilized as a major lignin precursor is very significant. However, it was based upon NMR analysis of lignin isolated from a single CAD-deficient tree and was not quantitative. This finding thus needs to be verified and confirmed in several specimens, preferably with a method that does not rely on lignin isolation.

In this paper, we report on the quantitative evaluation of the lignin in several wild type and mutant seedlings or mature trees to further delineate the specific structural traits associated with CAD deficiency in gymnosperm lignin. This evaluation was based on the GC-MS analysis of lignin-derived monomers and dimers released upon thioacidolysis.

MATERIALS AND METHODS

Plant Material. Loblolly pine seedlings of a selfed family from a cad-n1 heterozygous mother-tree (7-56) were grown for 10 months under standard greenhouse conditions and then the cad genotype of each one was determined using RAPD markers as a fingerprinting method (MacKay et al., 1997). Five wild type and four homozygous cad-n1 seedlings were randomly selected for this study (Figure 1). Identification and characterization of two 12-year-old trees, one mutant (28% lignin) and one wild type (29.5% lignin) were also described previously.
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(Ralph et al., 1997; MacKay et al., 1999). In addition, one mutant tree (27.4% lignin) was selected from a 2-year-old field planting of a selfed family from the mother-tree 7-56 (L. Pearson, Westvaco). Wild type and mutant wood samples from 10-month-old seedlings and from 12- or 2-year-old trees were ground before successive solvent extractions (toluene-ethanol, 2/1, v/v; ethanol and water). The lignin content of these seedlings and other trees was determined on the dry extract-free wood and by the Klason method according to standard procedure (MacKay et al., 1997).

Analysis by Thioacidolysis. The determination of the lignin-derived monomers and dimers released by thioacidolysis of extract-free wood was done according to the standard procedure (Lapierre et al., 1999). These compounds were systematically identified by GC-MS of their trimethylsilylated (TMS) derivatives. The GC-FID and GC-MS quantitative evaluation of dihydroconiferyl alcohol was run by reference to a calibration carried out with the authentic compound. This authentic compound was obtained by Raney nickel reduction of commercial coniferyl alcohol. Thioacidolyses were run in duplicate. Standard errors between duplicate analyses were in the 3-5% range for the lignin-derived monomers and in the 5-10% range for the lignin-derived dimers.

RESULTS

Total Yield and Relative Distribution of Thioacidolysis Monomers. Analysis of lignin structure in extract-free wood of CAD-deficient and normal pine was performed by thioacidolysis. The key reaction of thioacidolysis (lignin depolymerization using BF₃ etherate in ethanethiol-dioxane mixture) is the specific cleavage of lignin β-O-4 ether bonds (Lapierre,
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1993; Lapierre et al., 1995). On this basis, the total yield in lignin-derived monomers is a close reflection of the lignin content in units only involved in β-O-4 bonds. In pine and other softwoods, thioacidolysis specifically releases phenolic monomers comprised of guaiacyl (G) G-CHR-CHR-CH₂R (R = SEt) as the major compounds (erythro and threo isomers), together with a series of other lignin-derived phenolics (discussed in the following section). From the total yield in lignin-derived monomers (Table 1), we calculated the percentage of lignin units in β-O-4 bonds based upon two assumptions: 1) that the recovery yield of these monomers is about 80% (including both the reaction and the extraction yields from model compound experiments), and 2) that the average molecular weight of the basic C₃C₆ unit is 180.

The total yield in lignin-derived monomers emphasizes a dramatic alteration of lignin profiles in mutant pine seedlings and mature tree relative to wild type samples (Table 1). Lignin units only involved in β-O-4 bonds are three times less abundant in mutant samples (7-8% versus 21-25% in wild type). Conversely, this result strongly supports the hypothesis that resistant carbon-carbon bonds are unusually abundant in mutant lignins - more than 90% of phenylpropane units are involved in such condensed bonds, versus the conventional 75% level observed for the normal gymnosperm lignin. This remarkably high condensation degree of the mutant lignin was consistent in all the samples but was slightly more pronounced in pine seedlings than in the older trees.

The relative distribution of the main lignin-derived monomers was severely affected by the cad-n1 allele. Monomers diagnostic for the β-O-4 linked structures coniferaldehyde, vanillin and DHCA were released in low proportion from the wild type samples, but recovered in large relative amounts from extract-free mutant samples (Figure 2). In a previous study, lignin was
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isolated from one normal and one cad-n1 mutant 12-year-old tree, the same trees as used here (Ralph et al., 1997). Upon NMR analysis of soluble lignin samples isolated with a 12-17% yield relative to the total lignin amount, it was shown that the lignin subunits coniferaldehyde, vanillin and dihydroconiferyl alcohol were increased in the CAD-deficient pine, with DHCA signals increasing the most. Increased incorporation of aromatic aldehyde (e.g. coniferaldehyde and vanillin) and decreased coniferyl content were also clearly supported by FTIR analysis extractive-free wood samples from the same trees (MacKay et al., 1997; MacKay et al., 1999). FTIR spectra did not appear to be diagnostic for DIICA. Thioacidolysis quantitatively confirmed the altered subunit composition in the native lignins of mature trees, analyzed in situ without any isolation step. Data obtained for the 10-month-old seedlings revealed that the increased incorporation of coniferaldehyde and DHCA occurred at early developmental stages and was more pronounced in juvenile seedlings relative to 12-year-old trees. Thus, it is clearly established that the increased incorporation of DHCA and coniferaldehyde is indeed specifically linked to the homozygous cad-n1 genotype.

In addition, we observed that the ratio of p-hydroxyphenyl to guaiacyl thioacidolysis monomers (H/G ratio, Table 2) was systematically higher in the mutant samples relative to wild type ones, with some values (S5 and 2-year-old tree) similar to the levels characteristic of compression wood lignins (Table 2). With the reservation that this II/G ratio does not reveal the trend for H units in the whole polymer but only in β-O-4 linked lignin structures, this result suggests an increased incorporation of H units with conventional lignin side chain in the mutant lignin. On this basis and in agreement with NMR results (Ralph et al., 1997), it seems that the mutation does not affect the formation of p-coumaryl alcohol, the precursor of H units. Taken
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together, these results show that lignin units usually present in relatively minor amounts in gymnosperms (namely coniferaldehyde, vanillin, DHCA and H units) have been extensively incorporated into the lignin of the mutant pine. The incorporation of these monomers provides a mechanism to compensate for the shortage in coniferyl alcohol, the traditional precursor of conifer lignins.

Thioacidolysis of pre-methylated samples is an effective method of determining free phenolic groups in lignin samples (Lapierre et al., 1995). This method was applied with wood samples from the normal and CAD-deficient pine. The proportion of free phenolic groups within the β-O-4 linked lignin moiety was twofold higher in the lignin of 12-year-old CAD-deficient tree (38% of β-O-4 G units were free phenolic) relative to the analogous wild type sample (17%). Such an increase in free phenolic groups was also reported in the lignins of CAD down-regulated poplar trees, which made them more amenable to kraft delignification (Lapierre et al., 1999). This increase may originate from a higher crosslinking degree or from a lower average molecular weight of the native lignin polymers. The latter hypothesis is supported by gel permeation chromatography of isolated lignins (Dimmel et al., 1999). It, therefore, seems that CAD deficiency induces important alterations of native lignins not only at the chemical, but also at the macromolecular level.

Distribution of Lignin Resistant Interunit Bonds Revealed by the Analysis of Lignin-derived Dimers. The GC-MS analysis of lignin-derived dimers released after thioacidolysis and desulfurization further pointed at the unusual structural traits of the lignin in mutant pine samples. Many of the various dimers recovered from mutant samples (Figure 3) were original structures characterized hercin for the first time.
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Dimers 3, 11, 16, and 17, which are representatives of conventional 5-5, 4-O-5, β-1 and β-5 guaiacylpropane substructures, were recovered as major components from normal loblolly pine samples and as minor compounds from mutant samples (Table 3). In contrast, these mutant samples gave rise to unusual dimers 4-6 and 12-15, provided with a Cγ hydroxymethyl group. As the survival of this CH$_2$OH terminal group to the entire thioacidolysis-desulfurization procedure is only possible with –CH$_2$-CH$_2$-CH$_2$OH reduced side chains, these dimers are representative of lignin substructures comprising a DHCA unit. The mass spectra of their TMS derivatives displayed specific fragmentation patterns with losses of 90 amu (loss of TMSOH) and of 116 amu (loss of C$_2$H$_4$OTMS plus H) fragments. Not surprisingly, DHCA was incorporated into lignin only at its phenolic group or at its C-5 aromatic carbon.

Experiments with authentic 5-5' bisconiferaldehyde subjected to the whole thioacidolysis-desulfurization procedure revealed that the conjugated double bonds survived the Raney nickel reduction step to a very large extent (70%), probably due to the extended conjugated system (Jacquet, 1997). On this basis, unsaturated dimers 7-10 and 15 most likely originate from lignin substructures involving a coniferaldehyde unit. Dimers 10 and 15 are unique representatives of novel lignin substructures associating DHCA and coniferaldehyde. The recovery of such heterodimers diagnostic for the radical coupling of unusual lignin precursors further emphasizes the extent of the shortage in coniferyl alcohol. In addition to these unusual DHCA or unsaturated dimers, dimers with shortened C$_6$C$_1$ side chains (dimers 1, 4, 7 and 12) were recovered in unusually high proportion from mutant pine samples (data not shown). These dimers may stem from lignin substructures provided with a vanillin end group or from the degradation of coniferaldehyde structures through reverse aldol condensation.
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The quantitative evaluation of the lignin-derived dimers clearly showed the overwhelming importance of biphenyl bonds in the distribution of resistant linkages of the mutant lignin. Biphenyl dimers and, to a lesser extent, biphenyl ether dimers were recovered in relatively high amounts from mutant seedlings or mature trees (Table 3 and Figure 4). Conversely, β-5 and β-1 dimers were recovered in very low amounts from the mutant samples. The lignins of normal and mutant loblolly pines, therefore, have distinct patterns of interunit linkages. This difference is directly related to the incorporation of DHCA and coniferaldehyde units into the mutant lignins and to the radical coupling modes of these molecular species.

DISCUSSION

Previous characterization of lignin from CAD-deficient pine by NMR identified DHCA as a component of lignin. This finding came from the solution state NMR spectra of a milled wood lignin sample isolated from a single tree and with a 17% isolation yield. Furthermore, the genotype of the mature tree subjected to lignin isolation was simply inferred from CAD allozyme profiles of both its parents in addition to the brown-reddish coloration of its wood. Here, we studied several seedlings randomly selected from a larger population in which the genotype had been unambiguously determined by genetic finger-printing with RAPD markers (MacKay et al., 1997). The evaluation of lignin structure was directly performed by thioacidolysis of the extract-free wood from pine seedlings and mature trees without any isolation step. Similar to other degradative methods, thioacidolysis does not afford the whole lignin polymer characterization, but only that of its labile fraction, which further emphasizes the necessity for combined physical and chemical approaches. However, the somehow limited
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recovery yield of thioacidolysis monomers may be viewed not only as a limitation, but also as a structural information. In the present work, the lower thioacidolysis yield observed for the mutant, relative to the wild type samples, straightforwardly reflects the lignin structural alteration, namely the enrichment in resistant interunit bonds. In this study, we have demonstrated that the substantial incorporation of DIICA into lignins is specifically related to CAD deficiency in loblolly pine and linked, as is the brown wood color, to the cad-nl homozygous genotype. It is unlikely that DHCA would be formed from coniferyl alcohol as a post-polymerization modification because DHCA was involved in a high proportion of biphenyl 5-5 and biphenyl ether 4-O-5 linkages, untypical for coniferyl alcohol.

In agreement with recent literature evaluating lignin in plants displaying reduced CAD activity, this study further confirmed that a severe reduction in CAD activity induces dramatic changes in lignin structure while the lignin content is not altered to any great extent. High levels of coniferaldehyde and biphenyl interunit bonds were evidenced in lignin of the sorghum bm6 mutant showing depressed CAD activity (Jacquet, 1997), similar to the CAD-deficient pine. In CAD down-regulated transgenic poplars, the extent to which the lignin structure was altered was related to the residual CAD activity. When CAD was moderately decreased, i.e., the residual activity was 30% of the wild type, the main structural change was an enrichment in free phenolic groups which proved beneficial to kraft pulping efficiency (Lapierre et al., 1999). When CAD activity was more drastically reduced, i.e., to 3-8% of the wild type level, more dramatic alterations occurred, namely, the incorporation of sinapaldehyde units into the lignin together with a substantial enrichment in free phenolic groups and in carbon-carbon interunit resistant bonds (Lapierre et al., unpublished data). Similarly, downregulation of CAD in transgenic tobacco to approximately 7% of wild type significantly increased the incorporation
of cinnamaldehyde groups (Halpin et al., 1994). The incorporation of cinnamaldehyde into lignins seems therefore to be a common strategy employed by CAD-deficient pine, tobacco, sorghum, and poplar plants in order to maintain a lignin level compatible with their growth and development. In contrast, the massive incorporation of DHCA in addition to coniferaldehyde seems more specific of CAD-deficient pine. It is noteworthy that this building unit is systematically found in relatively small amounts in the lignins of normal gymnosperms (Gellerstedt et al., 1984) while found as a trace component in angiosperm lignins (Lapierre, unpublished results). Thus, it seems possible to achieve different lignin profiles depending on the level of CAD activity and, perhaps, through different responses to low CAD activity in different taxa (e.g. poplar vs pine).

Important changes in the type of bonds within the lignin polymer accompanied the increased incorporation of coniferaldehyde and DHCA and the decreased utilization of coniferyl alcohol as lignin precursors. The altered bonding structure is evidenced by the frequency of biphenyl and biphenyl other dimers being approximately tripled among thioacidolysis dimers in CAD-deficient pine. This shift is consistent with the fact that within the pool of lignin precursors there are far fewer reactive C₅ available to form β-O-4 bonds as well as β-5 and β-1 linkages. Clearly, the C₅ of DHCA is unreactive because the side chain lacks a double bond. The doubling of free phenolic hydroxyl groups in the lignins is most likely a consequence of the shift toward more C-C linkages and lower average molecular weight of the polymers. It is well established that β-O-4 bonds are the most labile of the lignin polymer and thus it is assumed that decreasing their abundance would have adverse effects on delignification during chemical pulping. However, the decrease in molecular weight and increase in free phenolic hydroxyl
groups may have the opposite effect on lignin removal. Indeed, data from recent pulping studies are now indicating that such a change in lignin structure may have some clear benefits. Increased lignin removal from CAD-deficient pine and poplar was observed in kraft and soda pulping, as well as in alkali treatment at room temperature (Baucher et al., 1995; Lapierre et al., 1999; Dimmel et al., 1999; MacKay et al., 1999). The common structural alteration of the pine and poplar lignins which can directly account for this phenomenon is their increased content in free phenolic groups associated with the CAD deficiency. Taken together, the data presented here point to significant opportunities to manipulate lignin subunit composition and structure with the potential for simplified pulping.

ACKNOWLEDGEMENTS

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REFERENCES


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**Figure 1.** Inheritance of the cad-n1 allele and genetic control of altered lignin. Inheritance of the cad-n1 allele was followed in a selfed family from a parent tree, which was heterozygous cad-n1. The cad genotype of several progeny was determined by genetic finger-printing in the region of the cad gene locus, by using RAPD (randomly amplified polymorphic DNAs) markers (MacKay et al., 1997). The three genotypes illustrated above segregated in a 1:2:1 ratio, consistent with Mendelian expectations. Only homozygous cad-n1 seedlings had brown-reddish wood, linking the altered color (and lignin) with that specific genotype. Several homozygous wild type (Cad/Cad) and homozygous cad-n1 (cad-n1/cad-n1) seedlings were selected, for lignin determination and further analyzed by thioacidolysis.

**Figure 2.** Importance (% molar) of various thioacidolysis monomers relative to the guaiacyl G-CHR-CHR-CH₃R major isomers (R=SEt) released from mutant and wild type extract-free pine woods.

**Figure 3.** Main dimers recovered from mutant and wild type pine samples. R = H in 11, 14, 17 and 22; R = CH₃ in 12, 15, 18 and 23; R = C₂H₅ in 13, 16, 19 and 24. R= -CH₂-CH₂OH in 20. Dimers with CH₃OH at C or with unsaturated side chains originate only from mutant samples.

**Figure 4.** Relative frequency (% molar) of the main thioacidolysis dimers (Figure 3) recovered from normal and mutant pine seedlings and mature trees. These dimers are representative of the lignin condensed bonding patterns.
Table 1. Yield in Lignin-derived Thioacidolysis Monomers Recovered from Wild Type and Mutant Loblolly Pine Extract-free Wood Samples. Results are expressed in μmoles per gram of Klason Lignin or in percentage of units (per 100 C₆C₃) only involved in β-O-4 Bonds (values between brackets).

<table>
<thead>
<tr>
<th>10-month-old seedlings</th>
<th>Wild type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₁₁ : 1080 (24.3%)</td>
<td>S₃ : 329 (7.4%)</td>
<td></td>
</tr>
<tr>
<td>S₂₁ : 1076 (24.2%)</td>
<td>S₅ : 298 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>S₁₁₇ : 1111 (25.0%)</td>
<td>S₁₃₂ : 289 (6.5%)</td>
<td></td>
</tr>
<tr>
<td>S₁₃₈ : 973 (21.9%)</td>
<td>S₁₆₀ : 280 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>S₁₃₉ : 1062 (23.9%)</td>
<td>Mean value : 299 ± 21</td>
<td></td>
</tr>
<tr>
<td><strong>Mean value : 1060 ± 60</strong></td>
<td>Mean value : 299 ± 21</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mature trees</th>
<th>Wild type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild type</strong></td>
<td></td>
<td>2-year-old : 360 (8.1%)</td>
</tr>
<tr>
<td>12-year-old: 991 (22.3)%</td>
<td>12-year-old: 373 (8.4%)</td>
<td></td>
</tr>
</tbody>
</table>
Lignin structure in mutant pine.

Table 2. Molar Ratio H/G of \( p \)-hydroxyphenyl H Monomers H-CHR-CHR-CH\(_2\)R Relative to Analogous Guaiacyl G-CHR-CHR-CH\(_2\)R Isomers Released from Wild Type and Mutant Samples (\( S = 10 \)-month-old Seedlings).

<table>
<thead>
<tr>
<th>Wild type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S_{11} ) : 0.029</td>
<td>( S_3 ) : 0.047</td>
</tr>
<tr>
<td>( S_{21} ) : 0.015</td>
<td>( S_5 ) : 0.184</td>
</tr>
<tr>
<td>( S_{117} ) : 0.008</td>
<td>( S_{152} ) : 0.058</td>
</tr>
<tr>
<td>( S_{138} ) : 0.009</td>
<td>( S_{160} ) : 0.067</td>
</tr>
<tr>
<td>( S_{139} ) : 0.019</td>
<td>2-year-old : 0.275</td>
</tr>
<tr>
<td>12-year-old : 0.033</td>
<td>12-year-old : 0.11</td>
</tr>
</tbody>
</table>
Table 3. Main Dimers (Figure 3) Recovered from Wild Type or Mutant Pine Seedlings (10-month-old S) or Mature Tree. Results are expressed in μmoles per gram of Klason lignin.

<table>
<thead>
<tr>
<th>Dimer and Sample</th>
<th>Pine</th>
<th>Wild type</th>
<th>Mutant</th>
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<tbody>
<tr>
<td></td>
<td>S138</td>
<td>S139</td>
<td>S152</td>
</tr>
<tr>
<td>1-3 biphenyl</td>
<td>51.5</td>
<td>48.8</td>
<td>47.9</td>
</tr>
<tr>
<td>DHCA biphenyl 4-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unsaturated biphenyl 9-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11 biphenyl ether</td>
<td>11.5</td>
<td>13.8</td>
<td>14</td>
</tr>
<tr>
<td>DHCA biphenyl ether 12-14</td>
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<td>-</td>
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</tr>
<tr>
<td>Unsaturated biphenyl ether 15</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-1 16</td>
<td>48.2</td>
<td>58.9</td>
<td>56.1</td>
</tr>
<tr>
<td>β-5 17</td>
<td>51</td>
<td>71.6</td>
<td>65.9</td>
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<tr>
<td>Total dimers</td>
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<td>193</td>
<td>184</td>
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Parent tree (Clone 7-56)

<table>
<thead>
<tr>
<th>Parent tree</th>
<th>Cad / cad-n1*</th>
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<tr>
<td></td>
<td>Cad / Cad</td>
</tr>
<tr>
<td></td>
<td>Cad / cad-n1</td>
</tr>
<tr>
<td>Progeny</td>
<td>cad-n1 / cad-n1</td>
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</table>

CAD Activity

<table>
<thead>
<tr>
<th>CAD Activity</th>
<th>Normal</th>
<th>Partially CAD-deficient</th>
<th>Completely CAD-deficient</th>
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<tbody>
<tr>
<td>Wood Color</td>
<td>Nearly white</td>
<td>Nearly white</td>
<td>Brown</td>
</tr>
</tbody>
</table>

| Seedlings | S11 : 31.7% | S3 : 24.9% |
| &         | S21 : 29.5% | S4 : 28.5% |
| Lignin    | S117 : 31.2% | S152 : 26.8% |
| Content (%) | S138 : 32.3% | S160 : 28.2% |
|           | S139 : 30.4% |               |

* Cad: normal cad gene; cad-n1: mutant cad gene

Figure 1
Figure 3
Figure 4