Institute of Paper Science and Technology
Atlanta, Georgia

IPST Technical Paper Series Number 830

Pulping and Bleaching of CAD-Deficient Wood

D.R. Dimmel, J.J. MacKay, E.M. Althen, C. Parks, and R.R. Sederoff

December 1999

Submitted to
Journal of Wood Chemistry and Technology

INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY
PURPOSE AND MISSIONS

The Institute of Paper Science and Technology is an independent graduate school, research organization, and information center for science and technology mainly concerned with manufacture and uses of pulp, paper, paperboard, and other forest products and byproducts. Established in 1929, the Institute provides research and information services to the wood, fiber, and allied industries in a unique partnership between education and business. The Institute is supported by 52 North American companies. The purpose of the Institute is fulfilled through four missions, which are:

- to provide a multidisciplinary education to students who advance the science and technology of the industry and who rise into leadership positions within the industry;
- to conduct and foster research that creates knowledge to satisfy the technological needs of the industry;
- to serve as a key global resource for the acquisition, assessment, and dissemination of industry information, providing critically important information to decision-makers at all levels of the industry; and
- to aggressively seek out technological opportunities and facilitate the transfer and implementation of those technologies in collaboration with industry partners.

ACCREDITATION

The Institute of Paper Science and Technology is accredited by the Commission on Colleges of the Southern Association of Colleges and Schools to award the Master of Science and Doctor of Philosophy degrees.

NOTICE AND DISCLAIMER

The Institute of Paper Science and Technology (IPST) has provided a high standard of professional service and has put forth its best efforts within the time and funds available for this project. The information and conclusions are advisory and are intended only for internal use by any company who may receive this report. Each company must decide for itself the best approach to solving any problems it may have and how, or whether, this reported information should be considered in its approach.

IPST does not recommend particular products, procedures, materials, or service. These are included only in the interest of completeness within a laboratory context and budgetary constraint. Actual products, procedures, materials, and services used may differ and are peculiar to the operations of each company.

In no event shall IPST or its employees and agents have any obligation or liability for damages including, but not limited to, consequential damages arising out of or in connection with any company's use of or inability to use the reported information. IPST provides no warranty or guaranty of results.

PULPING AND BLEACHING OF CAD-DEFICIENT WOOD

D. R. Dimmel, J. J. MacKay, E. M. Althen, and C. Parks
Institute of Paper Science and Technology
500 10th Street NW, Atlanta, Georgia 30318 (U.S.A.)
E-mail: donald.dimmel@ipst.edu

R. R. Sederoff
North Carolina State University

ABSTRACT

This report concerns the pulpability and bleachability of wood from a mutant loblolly pine tree. The tree is deficient in the enzyme cinnamyl alcohol dehydrogenase (CAD) and, therefore, has a different pool of lignin precursors. Wood from a 12-year-old CAD-deficient loblolly pine has been pulped under soda and kraft conditions. In comparison to a normal 12-year-old loblolly pine, the CAD-deficient wood was much more easily delignified. In addition, the pulp from CAD-deficient wood was as easy to bleach as a control pulp. The high reactivity of CAD-deficient wood is surprising because its lignin is known to possess a large number of "stubborn" C-5 linkages. The explanation may be in the lignin size. The molecular weight of an isolated milled wood lignin from CAD-deficient pine was ~50% less than that from a normal pine tree.

INTRODUCTION

Trees that contain less lignin and/or lignins that are more easily degraded during pulping and bleaching could greatly improve production. Manipulation of lignin in trees has been achieved in poplar by genetic transformation (introduction of foreign genes); potential pulping benefits have been shown in small-scale cooks.[1,2] Genetic transformation of commercial softwoods is not yet possible on a routine basis; however, cross-breeding offers an alternative way to achieve a similar result. This report outlines the reactivity of an unusual wood that has been obtained through cross-breeding.

Softwood lignins are principally derived from coniferyl alcohol (**1**).[3,4] Oxidation of coniferyl alcohol to a radical, followed by coupling one radical form with another, leads to the lignin polymer network. The preferred coupling involves union of an O_4-radical with a C_β-radical; approximately 50% of the interunit linkages in softwood lignin are of this type (Figure 1).[5] Several other linkages are also present in varying amounts, including C_5-C_5 (Figure 1), C_1-C_β, C_β-C_β, C_5-O_4, etc.[5]

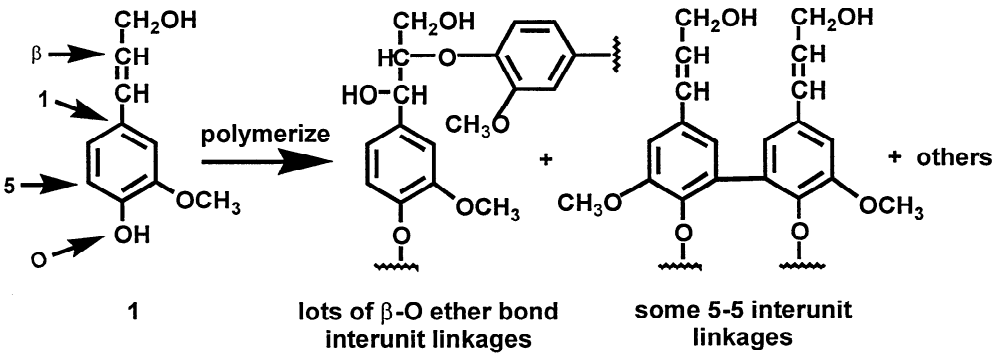

Figure 1. Polymerization of normal lignin monomer building blocks.

Coniferyl alcohol is formed in the plant by reduction of coniferaldehyde (**2**), a step that requires the enzyme cinnamyl alcohol dehydrogenase (CAD).[6,7] CAD-deficient (abbreviated CAD-) pines have been obtained through crosses of trees that have a mutant gene, the *cad-n1* allele, found in breeding stocks of loblolly pine. The *cad-n1* allele can be used in well-defined crosses to produce trees almost *totally deficient* in CAD activity,[8,9] providing trees that contain an unusual lignin without using genetic transformation techniques.

Lignins from totally CAD- trees are built up from unusual monomers. Analysis of milled wood lignins by NMR techniques indicate that coniferaldehyde (**2**), vanillin (**3**), and dihydroconiferyl alcohol (**4**) are the principal monomers (Figure 2). Relative to normal pinewood, lignin in these CAD- trees contains fewer C_β-O_4 linkages and a high number of C_5-linkages.[10] Such linkage distribution is consistent with lignin biosynthesis theory and the type of monomers available in the CAD- case.

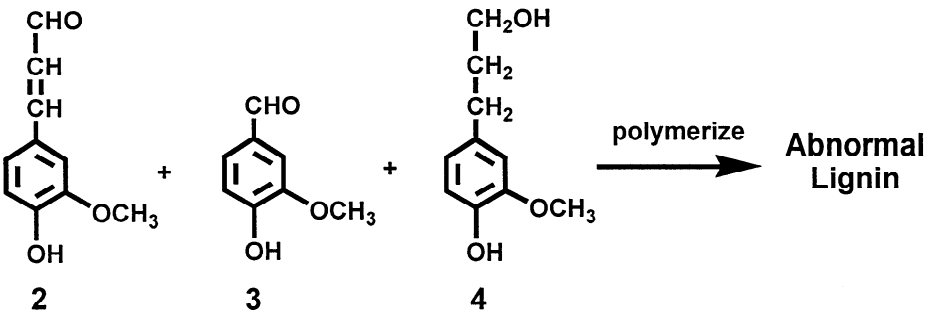

Figure 2. The unusual monomer building blocks for lignin production in CAD- trees.

Coniferyl alcohol (**1**), the building block of normal lignin, has four available radical reaction sites (O_4, C_5, C_1, and C_β). Linkages involving C_β are not possible with CAD-deficient lignin precursors **3** and **4**, and probably they are low in frequency with precursor **2**. One can speculate that C_1-link-

ages will also be more infrequent since the aldehyde group at C_1 in the precursor **3** and the saturated side-chain at C_1 in precursor **4** will not be easily lost following radical coupling to these sites. Thus, except for coniferaldehyde (**2**), the unusual CAD- precursors only have two reactive radical sites (O_4 and C_5). Because of the low number of reactive sites and the lack of active C_β-sites in the precursors, the lignin in totally CAD- pines will likely be less cross-linked, be inhibited in polymer growth, have a lower molecular weight, and, thus, be more easily dissolved in alkali. Our studies are directed at establishing this point.

RESULTS AND DISCUSSION

Pulping of CAD-Deficient Wood

Our previous studies have shown that large amounts of lignin are removed from totally CAD- wood simply by mild alkaline treatment at room temperature.[11] We have recently conducted an extensive study of soda and kraft pulping of normal and totally CAD- wood. All of the wood samples were taken from 12-year-old trees grown on the same site.

One of the first challenges that we had to address was conducting a large number of cooks with just 400 grams of dry CAD- chips. Initial studies were conducted to show that small-scale (0.5 g) cooks in 4-mL pressure vessels and mini-kappa number determinations gave results similar to 1-kg cooks and regular kappa determinations for normal loblolly pine. The dried chips were rewet and reduced in size prior to cooking. In some cases, the number of days required for the chips to sink in water under vacuum varied considerably; however, control experiments indicated that the number of days had no impact on subsequent pulping response.

The reactor size dictated that a high (7:1) liquor-to-wood ratio be employed. If a standard 4:1 liquor-to-wood ratio was used, the swelled chips in the small bombs were basically void of bulk liquor. For many of the cooks, the chemicals charged into the reactor represent the absolute amounts of NaOH and NaSH that would have been present in a 4:1 cook. Performing 7:1 liquor-to-wood ratio cooks this way meant that NaOH and NaSH *concentrations* were less than normally employed in a typical 4:1 cook. Therefore, some cooks were also done at standard concentrations, but higher absolute amounts of chemicals.

Data for the soda pulping of CAD- and normal wood with 18% active alkali at several different H-factors (cook severities) are shown in Figure 4. It is obvious that CAD- pines are much more easily pulped under this soda condition. The relatively low response of the normal wood to

changes in the H-factor is probably related to the lower concentration of NaOH available in the 7:1 liquor-to-wood ratio cooks. Only small decreases in delignification were observed when the active alkali levels were lowered to 16.5 and 15% for CAD- cooks (done at 1575 H-factor).

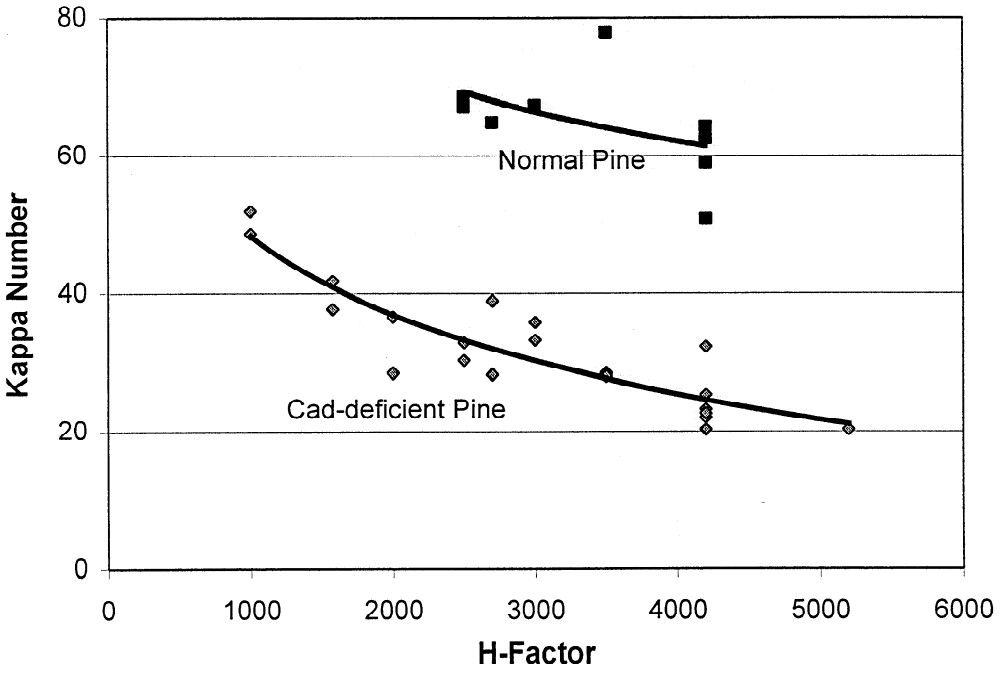

Figure 4. Relationship between kappa number and H-factor (energy input) for pulps obtained from 18% active alkali cooks of CAD- and normal loblolly pine chips, using a 7:1 liquor-to-wood ratio.

For kraft pulping, we observed that the CAD- wood delignified somewhat more easily than normal wood when the chemical concentrations of NaOH and NaSH were moderate; but, if the concentrations were high, there was little difference in the degree of delignification (Figure 5). Delignification occurs in three stages: initial, bulk, and residual. We interpret our results to mean that, in the CAD- case, the initial and bulk phase reaction rates are quite fast, but the residual phase rate is slow, similar to normal wood. For harsh cooks, each wood delignifies down to roughly the same level of residual lignin.

Figure 6 shows the data from a large number of high-chemical kraft cooks. There were two curves observed for the CAD- case. The upper curve, which used the same CAD- chips as in the soda cooks, indicates that CAD- wood is only slightly easier to pulp than control wood. However, at this point, we had exhausted our first set of chips and turned to another set.

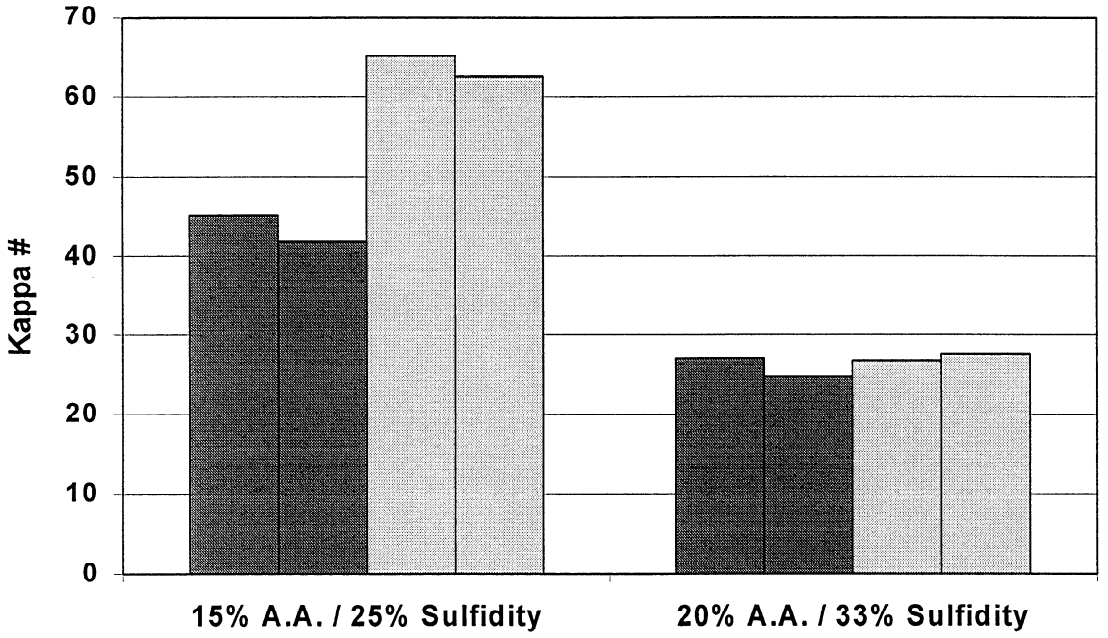

Figure 5. Relationship between kappa number and cook chemical composition for duplicate CAD- (dark-colored bars) and normal (light-colored bars) loblolly pine pulping, using a 7:1 liquor-to-wood ratio and a 2000 H-factor in each case.

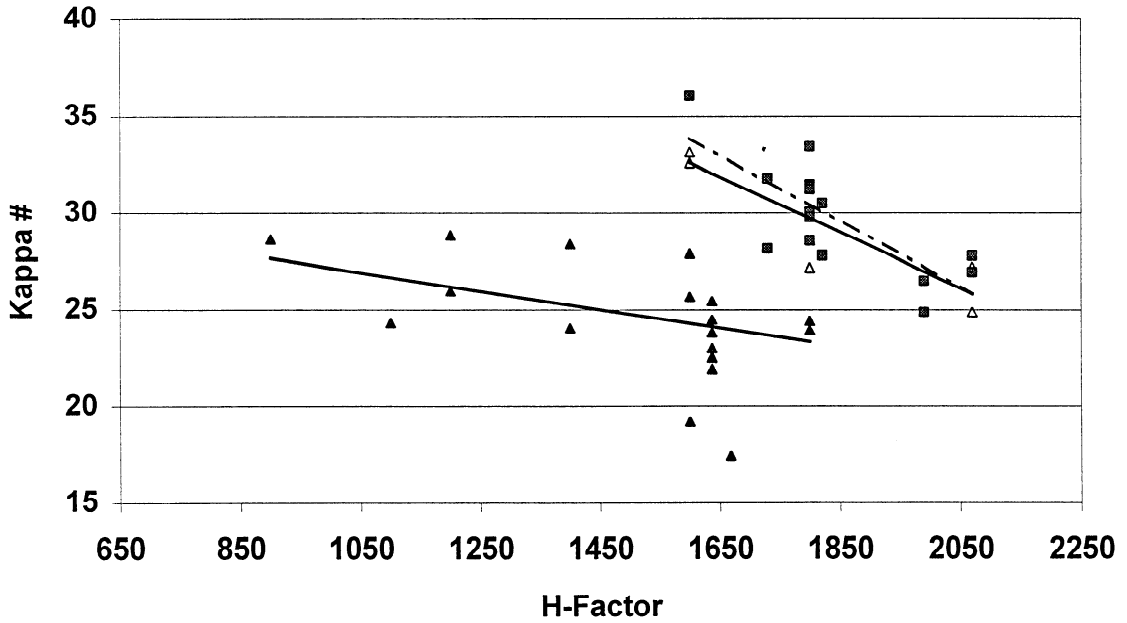

Figure 6. Comparison of delignification results from kraft cooks (20% AA and 33% sulfidity, 7:1 liquor-to-wood ratio) of different sets of CAD-deficient wood (▲, Δ, —) and control wood (■, ----).

The bottom curve represents data obtained from CAD- chips that were rewet for different lengths of time. The new chips were considerably more reactive than the first batch, further verifying the special nature of CAD- wood. The reasons for the pulping differences between the first and subsequent CAD- chip batches are not clear; we suspect that the first batch contained one or more uncharacteristic chips. (Only 12 grams of chips made up the first chip batch; one or two bad chips could have a large impact.) It should be pointed out that our soda cooks employed first-batch CAD- chips; consequently, the observed ease of pulping would likely have been even greater with other batches of chips. Different batches of normal chips behaved identically.

There is a much lower pulp yield in the CAD- case. Typically the yield is 15% lower than control pine for all types of cooks; we attribute this result to the poor growth rate for the CAD- tree and, thus, its juvenile state.

Are the Kappa Numbers Valid?

The comparison of kappa numbers from the pine cooks could be a problem if the CAD- and control lignins had different reactivities towards $KMnO_4$. Since CAD- lignin presumably contains high amounts of aldehyde, we might expect less consumption of $KMnO_4$. This concern was dispelled when it was shown that there were no significant differences in $KMnO_4$ consumption tests[12] of CAD- and control isolated milled wood lignins (Table 1).

Table 1. Potassium permanganate consumption data for MWL lignins.

Sample	Amount (mg)	$KMnO_4$ consumed (mL)	% $KMnO_4$ consumed	Equivalent (mL $KMnO_4$ / mg)
CAD-deficient MWL	5.72	3.40	6.8	0.594
	17.16	10.20	20.4	0.594
	34.32	20.78	41.6	0.605
	45.76	27.60	55.2	0.603
Control MWL	5.78	3.00	6.0	0.519
	17.33	10.15	20.3	0.585
	34.65	20.67	41.3	0.596
	46.20	26.79	53.6	0.579

Isolating the lignins from the pulping liquors also provided support for greater lignin removal in the CAD- case. The CAD- liquors contained higher amounts of dissolved lignins. At the same H-factor, there were ~50% more dissolved lignins in the CAD- case. Lignins isolated from CAD-

and control pine cooking liquors displayed nearly the same molecular weights, regardless of the cooking time. It should be noted, however, that the isolated acetylated lignins displayed variable solubility in THF, the solvent for the molecular weight studies, raising doubts as to whether the molecular weights were representative.

Bleachability

To learn more about the relationship between lignin structure and reactivity, we compared the bleachability of CAD- and normal pulps that had been produced in a similar manner and had identical 29-kappa numbers. Both pulps were produced using 7:1 liquor-to-wood ratio, 20% active alkali, and 33% sulfidity; however, the CAD- wood was cooked at *less than half* the H-factor (790 vs. 1800) of the control wood.

The unbleached CAD-deficient and normal pulps were treated in an identical manner with a $D_oE_1D_1E_2D_2$ bleach sequence and the brightness values determined. The bleaching was conducted on 2 g of starting pulp; by the time the last stage had been reached and several samples removed for analysis, less than 0.5 g of pulp was treated. Bleaching on such low scale presents several challenges; great care was taken to try to mimic the exact conditions, such as exiting pH values, used in typical pulp bleaching. Roughly 3 cm-diameter pads of bleached pulp were prepared for brightness determinations (see the Experimental Section for exact details). The brightness readings for the micro-pads were very close to the normal TAPPI pad readings for a sample pulp when the basis weights were above 200 g/m^2.

There was no difference in the D_1-brightness values for the CAD- and normal pulps (Table 2). However, the measured brightness values after the D_2 stage were quite different; the CAD- pulp displayed a lower value than the normal pulp. The reason for this difference is related to differences in pad characteristics. The CAD- pad has a relatively low opacity, which allows light to penetrate through the pad and reflect off the black background, giving the appearance that the pulp itself is dark colored (Figure 7). The CAD- brightness pad was *as visibly bright as* (if not more than) the control pulp pad.

Table 2. Brightness results for CAD-/Control bleaching comparison.

Pulp Type	D1 Brightness	D2 Brightness
CAD-	65.44	67.65
Control	65.24	76.04

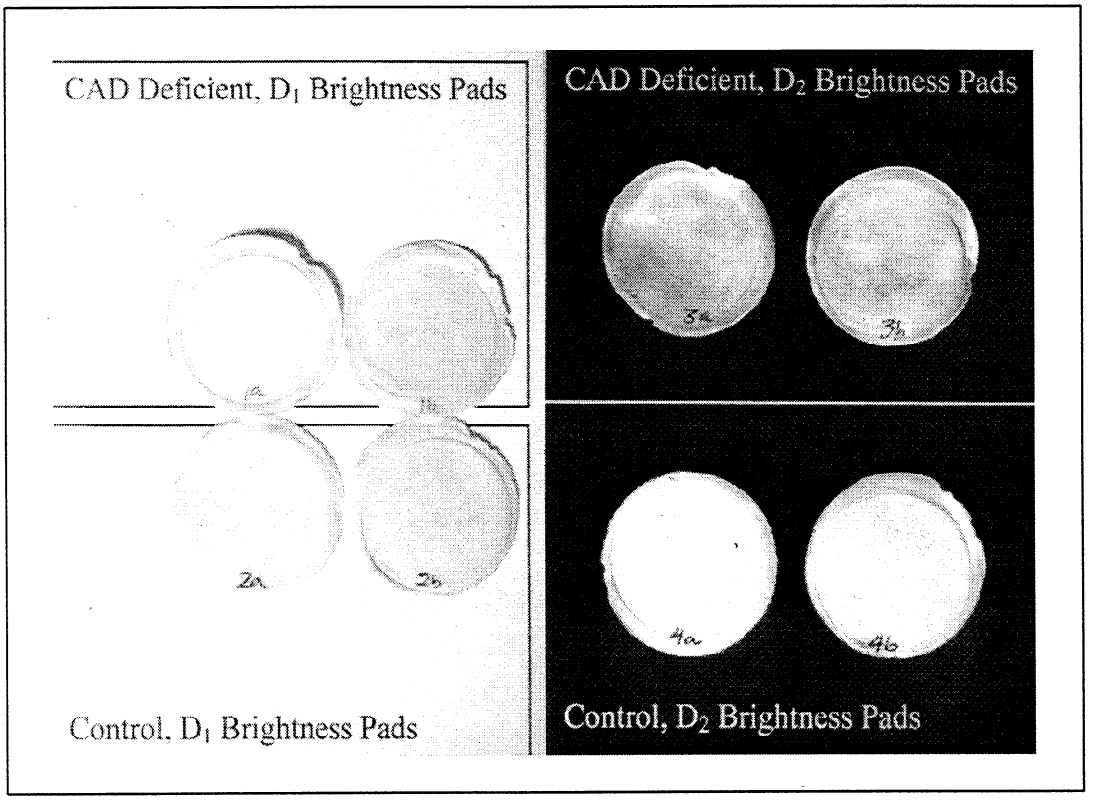

Figure 7. Photographs of (A) partially bleached pulps against a white background and (B) fully bleached pulps against a black background for CAD- and normal pulps.

Some physical measurements were performed on the fully bleached and unbleached CAD- and normal pulps. The data in Table 3 indicate that CAD- pulps are denser than control pulps, but the differences are not large. The small size of the pads and the physical state of the fully bleached CAD- pad may have influenced the measurements in this case. Here, the pad is not flat, but rather warped somewhat by the pressing and drying that was conducted.

Table 3. Density values (g/cm^3, average of 3-5 measurements) determined by two methods for CAD- and control unbleached and bleached pulps.

Pulp Type	**Soft Platen Density**	**Hard Platen Density**
CAD- Unbleached	0.630	0.579
Control Unbleached	0.568	0.533
CAD- Bleached	0.999	1.023
Control Bleached	0.987	0.935

In addition to the two standard methods of determining density, we also examined the pulps using scanning electron microscopy (SEM). The pulp samples were embedded in a resin, etched to remove the top surface of the resin and then polished with grinding paper to exposed the pulp. White streaks of rubbery-like material formed on the surface of the resin-embedded, fully bleached CAD- sample, but not the other samples. The streaks can be seen on close examination of the SEM cross-sectional pictures (Figure 8B). It is also apparent from the SEM cross-sectional pictures that the fully bleached CAD- pulp is more dense than the fully bleached control pulp. This same density difference was observed in the cross-sectional SEM pictures of the unbleached pulps. SEM surface views of the pulp fibers revealed that the CAD- fibers were flatter and more thin-walled than the control pulps, both in the fully bleached and unbleached cases. These characteristics are common for early-wood fibers.

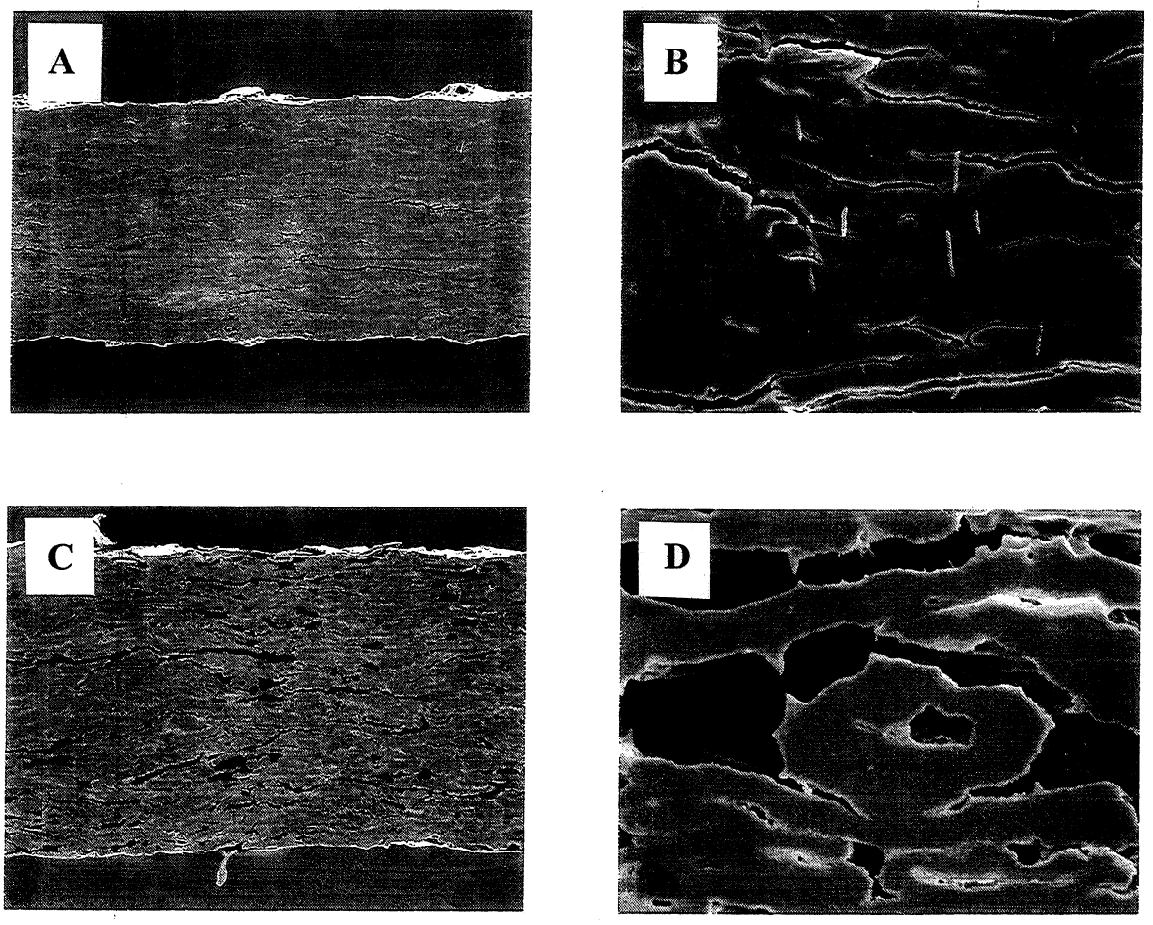

Figure 8. Scanning electron micrographs of fully bleached pulps: (A) CAD- at 150 magnification, (B), CAD- at 1500x, (C) normal at 150x, and (D) normal at 1500x.

In summary, the bleachability of CAD- pulps appears to be about the same as a normal pulp. The pad formation is very different in the CAD- case; this leads to a more dense, low opacity sheet that gives a false brightness value. We are uncertain what causes this unusual sheet formation property; however, we speculate that it may be related to increased levels of hemicelluloses and/ or other cell wall components that may be produced by the tree in response to, and in compensation for, the altered lignin structure.

Lignin Molecular Weight Characterization

Milled wood lignin obtained from CAD- wood is ~50% lower in molecular weight than that obtained from normal pine; this difference was also confirmed by another laboratory (Figure 9).

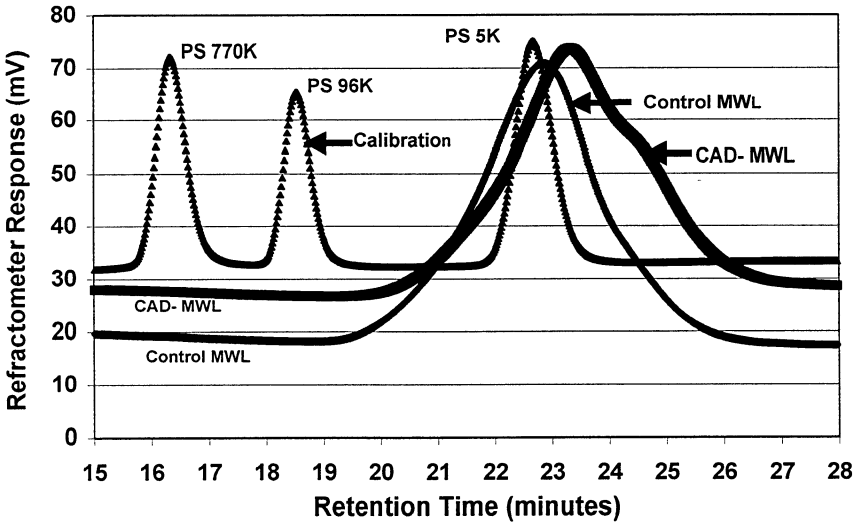

Figure 9. Comparison of the molecular distributions of CAD-deficient and normal milled wood lignins with polystyrene (PS) standards.

CONCLUSIONS

With a higher amount of 5-5 lignin linkages, CAD- wood should be more difficult to delignify than normal wood. But just the opposite is found. Totally CAD- loblolly wood is much more easily delignified in a pulping stage than normal pine. The relatively low molecular weight of the totally CAD- lignin is likely the principal reason for the easy lignin removal; it takes fewer cleavages to get a water-soluble piece. Another factor could be that the lignin is less cross-linked. Interestingly, even though there are probably a higher number of C_5-linkages in the lignin present in the unbleached CAD- pulp, the bleachability of this pulp was similar to that of normal wood.

The significant variability of lignin structure in plant species is well-established knowledge;[13,14] however, it appears that lignin composition and structure may be manipulated beyond what was previously anticipated.[9,10] We are only beginning to evaluate the impact of these changes in sub-unit composition and linkages; however, they point to promising opportunities to manipulate the reactivity wood in pulping and bleaching.

EXPERIMENTAL

Chip Preparation. The supplied[11] air-dried CAD- and normal pine chips were re wetted by placing the chips in a desiccator containing sufficient water to cover the chips once they sank. The chips sank into the water after approximately 2 days of reduced pressure provided by a water aspirator. The wet chips were reduced in size using a Wiley mill, screen size 1, and then conditioned in sealed bags for at least 24 hours. The consistencies of the chips were determined, in duplicate, by weighing the chips after oven drying at 105°C in a tarred pan for 2+ hours.

When the first batch of CAD- chips ran out, more chips were re wetted for continued studies. The re wetting procedure in this case took longer than normal, 5 vs. 2 days. The chips used from this re wetted batch had a much lower than expected kappa number after pulping. However, new batches of CAD- that were re wet for different lengths of time and then pulped gave similar kappa numbers; the same was observed for control chips. The re wetting procedure did not extract much lignin. This fact became apparent by examining the water extract by UV at 280 nm. The UV samples were prepared by evaporation of the re wetting liquor to about 20 mL and then dilution to 50.00 mL with water. A 1.00 mL-aliquot of the 50-mL sample was then diluted to 10.00 mL with 4 mL of water and 5 mL of *p*-dioxane. The UV spectra (200-500 nm) of 50% aq. dioxane solutions of CAD- and control pine re wetting liquors were taken using a Shimadzu UV160-U spectrophotometer. The absorption of each sample at 280 nm was inserted as x into the calibration equation determined from analysis of 4 different concentrations of Repap lignin in 50% aq. dioxane (Figure 10) in order to determine the amount of lignin in each sample.

Using the calibration equation, we calculated the CAD- extraction lignin amount to be 38.5 mg, which represented just 0.25% of the 14.4 g of CAD- chips extracted. The lignin content of a control pine water extraction was 0.05% of treated chips (6.5 mg from 14.2 g). Obviously, very little material was extracted in either case.

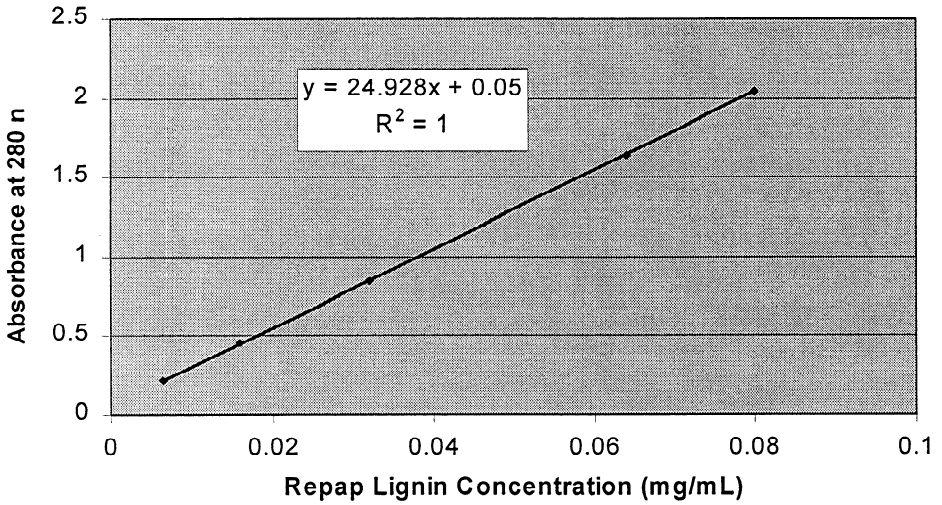

Figure 10. Calibration curve for UV absorption vs Repap lignin concentration.

Using the calibration equation, we calculated the CAD- extraction lignin amount to be 38.5 mg, which represented just 0.25% of the 14.4 g of CAD- chips extracted. The lignin content of a control pine water extraction was 0.05% of treated chips (6.5 mg from 14.2 g). Obviously, very little material was extracted in either case.

Micro-Pulping Reactions. Cooking with 4.5 mL pressure vessels (bombs), sealed with Teflon O-rings, has previously been described for model studies.[15] A Techne SBL-1 fluidized sand bath was used as the heat source, which was controlled by a Techne TC-AD temperature controller. To a 4.5 mL bomb was added 0.5000 g of o. d. chips and the pulping liquor (by syringe). A 7:1 liquor-to-wood ratio was used to provide enough residual black liquor for post-run lignin analyses. The active alkali and sulfidity levels in the liquor were determined by the ABC titration method.[16]

The cook temperature profile consisted of a 100-170°C temperature rise over a 60-min time period and 170°C for the time needed to achieve the desired H-factor. At the conclusion of the run, the bomb was removed from the hot sand bath, cooled with ice water, opened, and emptied (with rinsing) into a Waring blender. Enough water was added to the blender to allow good mixing; the solution was blended to achieve good pulp disintegration. Because the product yield was typically ~200 mg, there was no attempt to separate shives that were typically present in higher kappa number pulps. Consequently, this introduced possible errors in the kappa numbers of such pulps.

The kappa of each sample was performed according to TAPPI UM-246,[17] with two variations: the sample volume was diluted by two in order to allow good sample disintegration in the blender, and the samples were oven-dried, rather than used wet. The wet method requires the determination of pulp consistencies. The sample yield was too low to allow both consistency and kappa determinations.

Lignin Isolation from Pulping Liquor. Lignins were isolated from the liquors obtained from the micro-pulping runs. After disintegration of the cooked chips, the pulp was washed with approximately 500 mL water. This volume of liquor was reduced to approximately 200 mL by evaporation. Approximately 2 mL of 0.025 M diethylenetriamine pentaacetic acid (DTPA) was added to each liquor sample for chelation of metals. After stirring for 1 hour at room temperature, the liquor was acidified to pH 2 with 1N HCl. This caused the lignin to precipitate. Freezing, thawing, and centrifuging the sample facilitated further precipitation. The supernate was decanted and put through the acidification, freezing, thawing, and centrifugation cycle two more times. The lignin samples were then transferred to vials with water, frozen, and then freeze dried. The purity of the collected lignins was determined from their UV absorbance at 280 nm compared to a calibration chart of Repap lignin concentration vs. absorbance (Figure 10).

In general, considerably more lignin was isolated from CAD- liquors than from control liquors; for example, 126 mg of lignin was isolated from the black liquor of a CAD- 4200 H-factor soda cook, while 84 mg was isolated from a comparable control run, employing the same amount of starting o.d. wood.

Micro-Brightness Determination. A set of micro-brightness pads of varying basis weights was prepared from a standard bleached pulp. An ~1% consistency slurry of pulp was acidified with ~0.5 N H_2SO_4 to a pH of 4.8-5, divided in half, and two pads were prepared by pouring the slurries into a pad-forming stand tube.[18] After drainage and aspiration for 1-2 minutes, the pad was removed from the centrifuge tube and pressed under 50 psi to force excess water from the sample. The pad was then conditioned in a constant humidity room overnight and read for directional brightness using standard TAPPI method 452.[19]The brightness readings for the micro-pads were very close to the normal TAPPI pad readings for the same pulp when the basis weights were above 200 g/m^2 (Figure 11).

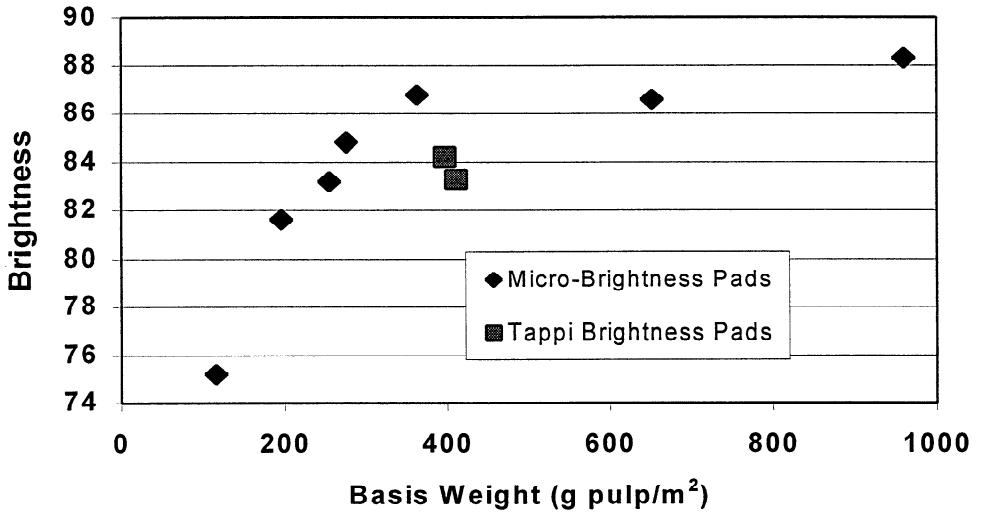

Figure 11. Directional brightness of normal and micro-pads at different basis weights.

Bleaching Procedures. The concentration of the ClO_2 solution to be used was determined by titration. Exactly 10 mL of ClO_2 solution was added to an Erlenmeyer flask containing 50 mL of water and 20 mL of 1 N KI. This was immediately titrated with 0.05 N sodium thiosulfate to a colorless endpoint; the titrant volume was recorded as "A". To the flask was added 20 mL 4 N H_2SO_4. The solution was titrated with the sodium thiosulfate (without re-zeroing) until a colorless endpoint was reached, using a starch indicator (titrant volume = "B"). The concentration of the ClO_2 was calculated with the equation below:

$$[ClO2] = \frac{(B-A)\times 0.169}{2}$$

Bleaching was done in tarred Kapak pouches using a syringe to deliver water, ClO_2 solution, and NaOH volumes required to achieve the conditions outlined in Table 4. After addition of the required reagents, the bag was heat-sealed and its contents massaged to achieve good mixing. The bag was then put in a Brookfield constant temperature bath, model TC-200, to achieve the 70°C-reaction temperature. At intervals, the bag was removed from the bath and further massaged. At the conclusion of the reaction time, the bag was removed from the water bath, the pouch was cut open, and the pH of the reaction mixture was at a recorded. The pulp was then filtered and washed with copious amounts of water.

Table 4. Bleaching conditions; all stages were done at 10% CSY and 70°C.

Stage	Pulp o.d. wt. (g)	ClO_2 Level[a]	% NaOH	Time (min)
D_0	2.00	0.25 KF	-------	45
E_1	2.00	-------	[b]	60
D_1	1.50	1.00%	0.5%[c]	90
E_2	1.00	-------	0.5%	60
D_2	0.50	0.40%	-------	180

[a] For D_o stage, $\%ClO_2$ = [Kappa Factor (KF) x kappa #]/2.63
[b] %NaOH = KF x kappa # of brownstock x 0.55
[c] %NaOH in D_1 stage determined to provide an appropriate pH_{out}

Lignin Molecular Weight Determination. Isolated black liquor (BL) lignins of several of the CAD- and control pine cooks, as well as milled wood lignins prepared at NC State University,[20] were examined by gel phase chromatography (GPC). The lignins were acetylated prior to analysis.[20] Immediately before injection into the liquid chromatograph (LC), the sample was diluted to 25 mL with LC grade THF and then filtered through a 0.2 μm pore Whatman syringe filter. The GPC analysis was performed on a Hewlett-Packard 1090 Liquid Chromatograph using a photodiode array UV detector, Waters Stryagel 7.8 x 300 mm HR6, HR4, and HR3 columns, and LC grade THF as the eluent at 1 mL/min. The data were collected on a Chemstation, converted to an Excel file, and compared to a calibration plot of log MW vs. time for polystyrene standards, which allowed a calculation of molecular weight and polydispersity.

The GPC analyses of black liquor-isolated lignins were problematic. Select samples were submitted to Prof. Richard Helm's lab at Virginia Tech for molecular weight determinations. The VA Tech lab used polystyrene standards and an RI (concentration) detector in conjunction with a viscosity (universal calibration) detector. They had difficulties dissolving the acetylated lignin samples in THF, the mobile phase used for their GPC analysis. We did not experience noticeable solubility problems, but the GPC baseline varied from sample to sample. This could be related to non-specific interactions between the sample and the column. However, we assumed that it was a simple baseline drift and applied a correction to provide a Gaussian-shaped curve between two time periods for which the majority of the sample eluted and represented weight average molecular weight (M_w) range of 162-100,000. Using this integration method, we observed roughly the same M_w of 19-21,000 for every black liquor-isolated lignin examined, regardless of the duration of the cook, the cook type, or wood sample.

The MLW lignin samples were much easier to deal with; no baseline corrections were required. Our results are shown in Table 5. The same samples were also analyzed at Virginia Tech; the data is shown in Table 5, as well as graphically in Figure 9. While our numbers are not in good agreement with one another, we always observe that the M_w and number average molecular weights, M_n, of the CAD- MWL are roughly one-half that of the control pine.

Table 5. Milled wood lignin molecular weight determinations by IPST and VA Tech.

Lignin Type	IPST M_w	VA Tech M_w	IPST M_n	VA Tech M_n
Control MWL	15,304	11,000	6,849	5,690
CAD- MWL	10,130	7,060	4,576	3,310

ACKNOWLEDGEMENTS

We are grateful to Dr. Jiebing Li for performing the KMO_4 consumption tests and to Dr. Hiroki Nanko and Ms. Georgeta Maghiari for providing the STEM results. Support for this work came from a research grant from the US Department of Agriculture, grant number 97-35103-4796.

REFERENCES

1. Baucher, M., Chabbert, B., Pilate, G., van Doorsselaere, J., Tollier, M.-T., Petit-Conil, M., Cornu, D., Monties, B., van Montagu, M., Inzé, D., Jouanin, L., and Boerjan, W., *Plant Physiol.*, 112, 1479 (1996).
2. Lapierre, C., Pollet, B., Petit-Conil, M., Toval, G., Romero, J., Pilate, G., Leple, J.-C., Boerjan, W., Ferret, V., De Nadai, V., and Jouanin, L., *Plant Physiol.*, 119, 152 (1999).
3. Monties, B. in Methods in Plant Biochemistry, Editors, Dey, P. M., and Harborne, J. B., Academic Press, London, Vol. 1, pp. 113-158 (1989).
4. Adler, E., *Wood Sci. Technol.*, 11, 169 (1977).
5. Sjostrom, E., Wood Chemistry Fundamentals and Applications, 2nd Edition, Academic Press, San Diego, CA, 1993, Chapter 4.
6. MacKay, J. J., Liu, W., Whetten, R. W., Sederoff, R. R., and O'Malley, D. M., *Molecular and General Genetics*, 247, 537 (1995).
7. O'Malley, D., Porter, S., and Sederoff, R. R., *Plant Physiol.*, 98, 1364 (1992).
8. MacKay, J. J., O'Malley, D. M., Presnell, T., Booker, F. L., Campbell, M. M., Whetten, R. W., and Sederoff, R. R., *Proc. Natl. Acad. Sci. USA*, 94, 8255 (1997).
9. Ralph, J., MacKay, J. J., Hatfield, R. D., O'Malley, D. M., Whetten, R. W., and Sederoff, R. R., *Science*, 277, 235 (1997).
10. Lapierre, C., Pollet, B., MacKay, J. J., Dimmel, D. R., and Sederoff, R. R., *Proceed. Intern. Symp. Wood and Pulping Chem.*, Yokohama, Japan, June 7-10, 1999, Vol. I, p. 520.
11. MacKay, J. J., Presnell, T., Jameel, H., Taneda, H., O'Malley, D. M., and Sederoff, R. R., *Holzforschung,* 53; 403-410

12. Li, J. and Gellerstedt, G., *Proceed. Intern. Symp. Wood and Pulping Chem.*, Montreal, Canada, June 7-12, 1997, p. G1-1.
13. Whetten, R. W. and Sederoff, R. R., *Plant Cell*, 7, 1001 (1995); Whetten, R. W., MacKay, J. J., and Sederoff, R. R., *Ann. Rev. Plant Physiol. & Plant Mol. Biol.*, 49, 585 (1998).
14. Boudet, A.M., Lapierre, C., and Grima-Pettenati, Tansley Review No. 80, *New Phytol.*, 129, 203 (1995).
15. Brogdon, B. N. and Dimmel, D. R., *J. Wood Chem. Technol.*, 16(3), 261 (1996).
16. Libby, C. E. ed., Pulp and Paper Science and Technology, Vol. 1, McGraw-Hill, New York, NY, 1962, p. 229.
17. Method UM-246, TAPPI Test Methods, TAPPI PRESS, Atlanta, GA, 1992.
18. Thode, E. F., Bergomi, Jr., J. G., and Unson, R. E., *Tappi,* 43(5), 505 (1960).
19. Method 452, TAPPI Test Methods, TAPPI PRESS, Atlanta, GA, 1992.
20. Gellerstedt, G., in Lignin Chemistry, Springer Series in Wood Science, Editors, Lin, S. Y. and Dence, C. W., Springer-Verlag, Berlin, 1992, p. 491.