

ACETYL DISTRIBUTION IN ACETYLATED WHOLE
WOOD AND REACTIVITY OF ISOLATED WOOD
CELL-WALL COMPONENTS TO ACETIC ANHYDRIDE

Roger M. Rowell

Project Leader
USDA Forest Service
Forest Products Laboratory
One Gifford Pinchot Drive
Madison, WI 53705-2398

Rune Simonson

Professor

and

Sabine Hess

Technician

Chalmers University of Technology
S-412 96 Goteborg, Sweden

David V. Plackett

Group Leader

Dave Cronshaw

Graduate Student

and

Elizabeth Dunningham

Graduate Student

Forest Research Institute
Private Bag 3020
Rotorua, New Zealand

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ABSTRACT

Lignin, holocellulose, cellulose, and hemicelluloses were isolated from pine wood and reacted with acetic anhydride. The order of reactivity was found to be lignin > hemicelluloses ≫ holocellulose. Cellulose did not react. At a level of bonded acetyl where all the hydroxyl groups were substituted on the lignin polymer, only about 20% of the total theoretical hydroxyl groups on the holocellulose were substituted. The data suggest that in the reaction of chloroacetic anhydride with whole wood, the initial rate-controlling step is the rate of diffusion of reactive chemical into the cell wall.

Keywords: Esterification, acetic anhydride, reactivity, lignin, holocellulose, hemicellulose, cellulose, chemical distribution, reaction rate.

INTRODUCTION

Chemical modification of wood, by acetylation, greatly improves physical and chemical properties (Rowell 1983). Dimensional stability results from bulking of the reacted acetate within the cell-wall polymer, which reduces further swelling when the modified woods come into contact with water or water vapor.

For biological resistance, however, the data to date indicate that not only is the amount of bonded acetate in the cell-wall polymers important, but also the types of cell-wall polymers that have been modified (Rowell 1992). It was therefore considered essential to determine the reactivity of isolated cell-wall polymers as well as the specific location of the bonded chemical within the reacted wood cell wall.

In the acetylation of jute, Callow (1951, 1952) found that lignin was more reactive than the holocellulose fraction. Loras (1968), however, found that little or no acetylation occurred in spruce wood lignin. A study of Loras' data suggests that a mistake was made and the isolated lignin fraction was highly substituted. Rowell found that both lignin and holocellulose were substituted in acetylated wood (Rowell 1982) and in methyl isocyanate-reacted wood (Rowell 1980). Peterson and Thomas (1978), using energy-dispersive X-ray analysis, found that bromine was distributed throughout the entire secondary wall of tribromoacetyl bromide-modified wood. Similar results were observed in studies on the chlorine distribution in epichlorohydrine-modified wood (Rowell and Gutzmer 1975).

The purpose of the research described in this paper was to determine (1) the reactivity of isolated cell-wall components from pine wood to acetic anhydride and (2) the distribution of acetyl groups in cell-wall polymers of acety-

lated whole pine wood at different levels of bonded acetyl weight gains. It is expected that these data will aid in the understanding of the mechanism of biological resistance of wood based on chemical modification.

EXPERIMENTAL

The studies on modified wood were carried out on three species of pine in three different laboratories. The experiments described in this paper were carried out on wood samples from the following pine species: southern yellow pine (SYP), Scotch pine (also known as Scots pine and Swedish pine; *Pinus sylvestris*), and Monterey pine (*Pinus radiata*). The samples of pine wood examined all responded to isolation of cell-wall polymers and acetylation in essentially the same way. For the convenience of the reader, the term *pine* is used generically in the text and particular species are described only parenthetically.

Isolation of wood components

Holocellulose.—Pine meal (25 g) was weighed in a 2-liter flask; and water (600 ml), 5 ml acetic acid, and 10 g NaClO₂ were added. The flask and contents were stirred manually, covered, and placed in a waterbath at 70 C. After 30 min, another 5 ml acetic acid and 10 g NaClO₂ were added, and the sample was manually stirred. Acetic acid and sodium chlorite in the same amounts were added again after 30 min and then at 1-h intervals for 6 h. This resulted in a total of nine additions (total of 45 ml acetic acid and 90 g NaClO₂). The holocellulose was filtered over a coarse glass filter, washed with distilled water, air-dried overnight, and then oven-dried at 40 C overnight. The yield was about 54%. The holocellulose was analyzed for lignin content by klason analysis (72% H₂SO₄) and was found to contain 1.3% to 1.5% lignin (Moore and Johnson 1967).

Cellulose.—Holocellulose (as described in the previous section), (20 g, air-dried only) was stirred in 1 liter of a 12% NaOH solution, and nitrogen gas was bubbled through for 1 min. The flask was securely closed and stirred gently by rotating end-over-end at about 6 r/min for

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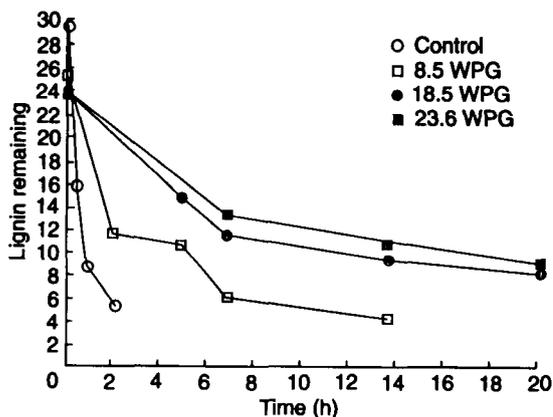


FIG. 2. Sodium chlorite delignification of acetylated southern yellow pine for various weight percent gains.

reacted anhydride and byproduct acetic acid. These two nonbonded chemicals, if present in the wood, could cause increased carbonyl absorption.

The three major changes that are observed in the IR spectra of wood or its components on acetylation are: (1) a reduction in the hydroxyl (O-H) stretching band ($3,200\text{--}3,500\text{ cm}^{-1}$); (2) an increase in the carbonyl (C=O) stretching region ($1,735\text{--}1,765\text{ cm}^{-1}$); and (3) increases at various particular frequencies in the carbon-oxygen (C-O) stretching region ($1,000\text{--}1,245\text{ cm}^{-1}$). For all component samples except cellulose, a smaller increase also occurred around $1,370\text{ cm}^{-1}$, which can be assigned to the carbon-hydrogen (C-H) bond of an $-\text{O}-(\text{C}=\text{O})-\text{CH}_3$ group.

Since the hydroxyl in water also absorbs in the region of $3,200$ to $3,500\text{ cm}^{-1}$, this peak may not decrease as a result of acetylation if the KBR pellet is not kept dry. The hydroxyl peak was nearly absent in the spectra for the highly acetylated lignin.

A very small peak occurred in the range of $1,736$ to $1,751\text{ cm}^{-1}$ for acetylated cellulose, which shows that a very low level of acetylation had taken place. The level of detected acetyl groups, however, was within the experimental error of the acetyl gas chromatographic analysis.

The biggest increase in carbonyl stretching resulting from addition of acetyl groups was

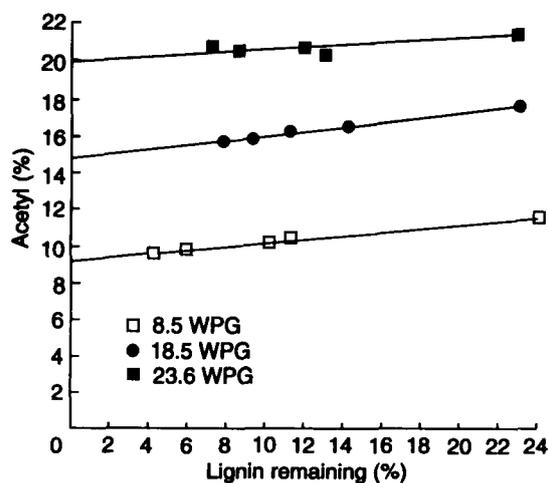


FIG. 3. Lignin remaining in partially delignified acetylated holocellulose from southern yellow pine (dotted line extrapolates actual data to 0% lignin).

observed in the isolated acetylated lignin and hemicelluloses.

Distribution of acetyl content

The results in this section are given for SYP only, but similar results were also found for Scotch and Monterey pine.

The distribution of acetyl groups in acetylated whole wood holocellulose and lignin cell-wall components was determined by removing the lignin from wood with sodium chlorite. The sodium chlorite isolation procedure did not hydrolyze the acetyl groups. In Fig. 2, the percentage of lignin in a sample is plotted against delignification time. As the level of acetylation increased, longer sodium chlorite treatment time was needed to remove the acetylated lignin. Even continuing the procedure for several days did not remove all the lignin. However, part of the carbohydrate polymers were lost as a result of the extended extraction procedure.

A straight-line plot was obtained when acetyl content was plotted against the lignin remaining in the chlorite-isolated holocellulose samples. (Fig. 3). Extrapolating each line to 0% lignin remaining gave the acetyl content of the holocellulose at that WPG.

TABLE 1. *Distribution of acetyl groups in southern yellow pine.*

WPG	Acetyl content (percent)		
	Whole wood ^a	Holocellulose ^b	Lignin ^c
8.5	11.6	9.0	20.0
18.5	17.7	14.6	28.2
23.6	21.6	19.8	29.6

^a From analytical determination.

^b Value from Fig. 3 projected to 0% lignin.

^c Calculated from percentage of acetyl content in whole wood: (0.67)(% acetyl content in holocellulose) + (0.28)(% acetyl content in lignin).

TABLE 2. *Degree of substitution (DS) of hydroxyl groups in southern yellow pine with acetyl groups.*

WPG (percent)	DS in lignin	DS in holocellulose	
		Total accessibility ^a	Limited accessibility ^b
8.5	0.78	0.12	0.21
18.5	1.10	0.19	0.35
23.6	1.15	0.26	0.47

^a Assuming accessibility of all cell wall hydroxyl groups.

^b Assuming 100% accessibility of hemicellulose and lignin hydroxyl groups but only 35% accessibility of cellulose hydroxyl groups.

Table 1 shows the acetyl content of the holocellulose derived from Fig. 3 and the calculated value of the acetyl content of the lignin. With a theoretical acetyl content of 25.7% in the lignin, the lignin component was nearly completely reacted at the low (8.5) WPG. This is in keeping with the earlier result of lignin as the fastest reacting component. The theoretical acetyl content for each component was calculated using equations reported previously (Rowell 1980, 1982).

Using the derived values for the acetyl content of holocellulose and lignin, the degree of substitution (DS) of each component was calculated (Table 2). Assuming that all hydroxyl groups were accessible for acetyl substitution, the table shows that at 8.5 WPG, about 80% of the lignin hydroxyls were substituted but only 12% of the holocellulose hydroxyls. The lignin hydroxyls were completely substituted at about 18 WPG.

Sumi et al. (1964) showed that only 60% of the total hydroxyl groups were accessible to tritiated water in spruce wood. Stamm (1964) estimated that 65% of the cellulose in wood was crystalline and therefore probably not accessible for reactions involving these hydroxyl groups. Assuming that only 35% of the cellulose hydroxyls are accessible for acetylation, Table 2 shows the DS in the holocellulose fraction was about double that value calculated, assuming 100% cellulose hydroxyl accessibility. Since very little, if any, reaction of cellulose occurs during uncatalyzed acetylation, the DS in the hemicellulose fraction was probably much higher than expected by these calculations. It is hard to imagine, however, that none

of the surface hydroxyl groups in the amorphous regions of native cell-wall cellulose reacts with acetic anhydride. Experiments such as these show more clearly the differences between native cellulose and the cellulose we isolated.

Energy-dispersive X-ray analysis

Using chlorine-tagged bonded acetyl groups generated by reacting wood with chloroacetic anhydride, it is possible to determine the distribution of the chloroacetyl groups throughout the wood cell wall. The X-ray maps generated from SEM-EDX analysis were prepared by scanning across both the radial and tangential walls of Monterey pine (see Fig. 4). No difference was observed in the pattern of chlorine distribution between the radial and tangential wall scans. Spot analyses were also done, using a 1-sec acquisition time, to confirm the increase in chlorine concentration with increasing WPG.

Figure 4 shows the chlorine concentration across the cell wall in Monterey pine with increasing amounts of chloroacetyl substitution. At low substitution (B, 4.3 WPG), more chlorine was in the secondary cell wall and less in the middle lamella. At 10.2 WPG (C), the chlorine distribution across the cell wall and middle lamella was almost uniform. At high substitution (D, 27.7 WPG), the concentration of chlorine was higher in the middle lamella than in the cell wall.

This pattern was repeated many times in all samples tested by both line-scans and EDX maps. The data showed little or no chlorine in the middle lamella at low levels of reaction

24 h at 24 C. The sample was then filtered by suction over a coarse glass filter. The residue was then extracted three times with 1 liter of 7.1% NaOH. After the final extraction, the residue was washed with 5% NaOH followed by cold water. The residue was then stirred briefly in the funnel with 10% acetic acid and allowed to stand for 10 min. The mixture was filtered, and the cellulose washed thoroughly with water and air-dried.

Hemicellulose.—The first two extracts from the cellulose preparation (holocellulose extracted with 12% and 7.1% NaOH) were mixed, and the solution was neutralized with hydrogen chloride and evaporated. The extract was then dissolved in 2 liter of distilled water. The solution was adjusted to pH 4 with acetic acid and the hemicellulose was precipitated with ethanol. Isolation was done by centrifugation. After drying at 40 C, a fine, light-yellow powder was obtained.

Lignin.—A commercial lignin, Indulin-A¹ (kraft lignin), obtained from Westvaco Corporation (Charleston, SC) (10 g), was stirred in 1 liter of 0.5 M acetic acid and then recovered by filtration on a glass filter. The lignin was then thoroughly washed with distilled water until the pH of the filtrate was about 4, then dried at 40 C.

Acetylation

Whole SYP, Scotch pine, and Monterey pine and isolated wood components were acetylated using acetic anhydride at 120 C as previously described (Rowell et al. 1986). The reaction was carried out for various lengths of time to give increasing amounts of bonded acetyl. The degree of acetylation is reported as either weight percent gain (WPG) or actual analytically determined acetyl content. The WPG was determined using wood oven-dry weight before and after acetylation. Acetyl content was

determined analytically using a gas chromatographic method after saponification of the bonded acetyl with sodium hydroxide by acidification (Moore and Johnson 1967).

Chloroacetylation

Whole Monterey pine wood specimens were reacted with chloroacetic anhydride in refluxing xylene. After being reacted for various lengths of time, the wood specimens were removed from the reactor. They were then extracted for 2 h with xylene in a Soxhlet extractor, air-dried, and finally oven-dried at 105 C overnight. Specimens with WPG of 4.3, 10.2, and 27.7 were produced.

Infrared spectroscopy

Whole SYP, Scotch pine, and Monterey pine and isolated wood components, both acetylated and control, were ground to 420 μm and pressed into potassium bromide (KBR) pellets. Care was taken to keep the KBR powder dry before and after pelletizing.

Delignification of acetylated specimens

Acetylated SYP, Scotch pine, and Monterey pine were delignified with sodium chlorite as described in the section on holocellulose isolation, except that the procedure was carried out for 21 h (Rowell 1980). After delignification, the klason lignin (72% H_2SO_4) was determined on each sample. Acetyl content was also determined on each delignified sample.

Scanning electron microscopy and energy-dispersive X-ray analysis

Chloroacetylated samples and an untreated control were carbon-coated in an Edwards vacuum carbon-coating machine and then placed in a Cambridge Stereoscan 240 scanning electron microscope (SEM), where they were photographed and examined using a Tracor Northern Energy Dispersive X-ray analyzer (EDX). The X-ray maps and line-scans were taken of the samples. The accelerating voltage was 15 keV, and X-ray map point acquisition time was 0.5 sec.

¹ The use of trade names or company names is for the convenience of the reader and does not constitute official endorsement by the U.S. Department of Agriculture.

RESULTS AND DISCUSSION

Rate of acetylation

Cellulose isolated from the three pine species, filter paper cellulose, and decoated cotton cellulose were reacted separately with acetic anhydride. In all cases, no acetylation took place in the 4-h reaction time using acetic anhydride.

The most reactive isolated cell-wall component was lignin (Fig. 1). An acetyl content of almost 10% was achieved within 15 min of reaction with a maximum acetyl content of about 18% after 4 h. The theoretical maximum acetyl content for lignin would be approximately 26%, assuming 1.2 hydroxyl groups per nine-carbon unit (Kirk 1975). Some possible reactive sites may have been lost due to the isolation procedure.

Isolated pine hemicelluloses were the next most reactive components. A maximum acetyl content of 30% was achieved after 3 h. The theoretical maximum acetyl content for hemicellulose would be approximately 24%, assuming that the wood contained 67% holocellulose (45% cellulose + 12% pentosans + 10% hexosans) and 28% lignin (Rowell 1982).

Whole pine wood was the next most reactive component. The rate of reaction for whole wood may be controlled by the rate of diffusion of chemical into the cell wall (as discussed later). Diffusion of chemical was not a problem for the isolated cell-wall components.

The most interesting rate of reaction was the rate for isolated holocellulose. Since the reactivity of isolated cellulose was almost zero, this curve must represent mainly hemicellulose reactivity. Because isolated hemicelluloses react much faster and to a greater extent than holocellulose, there may be a problem with accessibility of the hemicellulose in the holocellulose to the anhydride. Repeated attempts to acetylate the holocellulose from the pine species examined always showed a slower rate of reaction with the uncatalyzed procedure used.

For many years, researchers have suspected that the properties of isolated cell-wall poly-

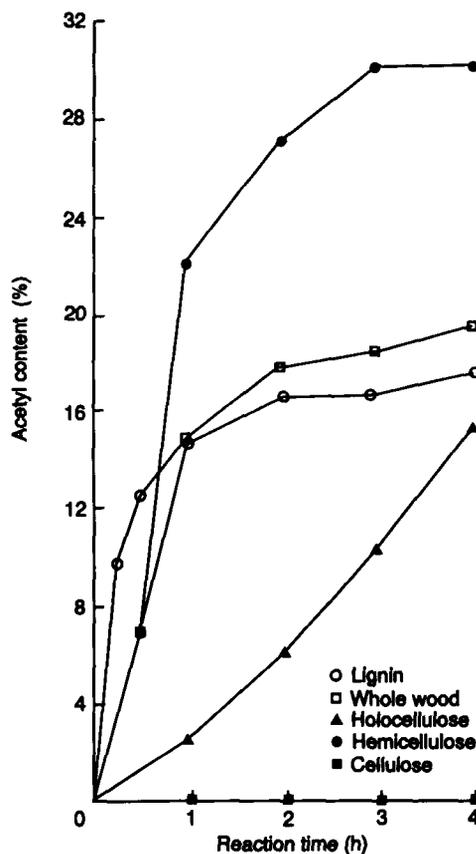


FIG. 1. Rate of acetylation of pine and isolated pine cell-wall polymers.

mers are different from those present in the native whole wood. Accessibility, structure, degree of polymerization, and reactivity are different for isolated lignin, hemicellulose, and cellulose samples than for the corresponding polymers in native whole wood. Perhaps questions of reactivity of individual cell-wall polymers cannot be answered until their reactivity can be determined in whole wood. Solid-state nuclear magnetic resonance (NMR) may eventually prove useful in answering these questions.

Infrared spectra analysis

The infrared (IR) spectra were run on whole wood or wood components that had been thoroughly extracted with benzene:ethanol (2/1, v/v) and water to remove any remaining un-

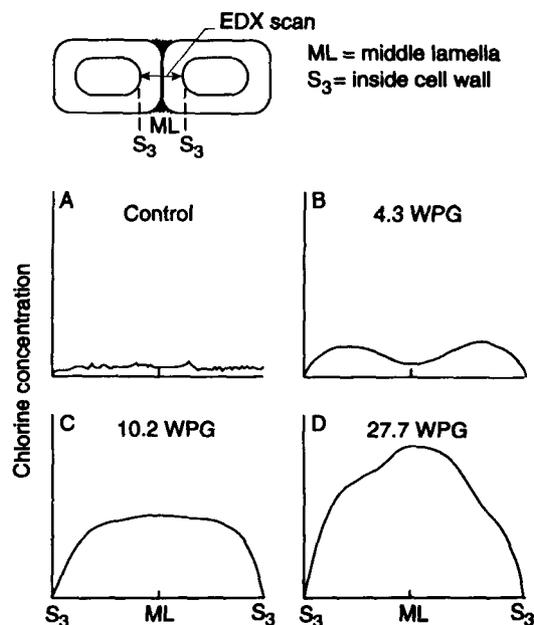


FIG. 4. EDX analysis of chlorine across two cell walls.

with chloroacetic anhydride. The chlorine concentration increased in the middle lamella as the reaction time increased until, finally, the middle lamella contained the highest concentration of chlorine.

CONCLUSIONS

Of the isolated cell-wall components, lignin reacted at a faster rate with acetic anhydride than the hemicelluloses, which reacted faster than the holocellulose fraction. Isolated cellulose did not react with acetic anhydride in the absence of a catalyst. The native cellulose may be modified in the cellulose isolation procedure, so this result does not necessarily mean that no cellulose modification takes place during the uncatalyzed acetylation of whole wood.

At a bonded chemical weight gain where resistance to biological attack is obtained (about 18 weight % gain, Rowell 1992), all the lignin hydroxyl groups were substituted, but only about 20% of the holocellulose hydroxyls were substituted. This is based on assuming 100% accessibility of all hydroxyl groups in the holocellulose polymers. The actual degree of substitution in the hemicellulose portion of the

holocellulose was much higher since most cellulose was not reactive under the reaction conditions used in these experiments.

At a bonded WPG of 8.5, where biological attack still occurs (Rowell 1992), 80% of the lignin hydroxyl groups were substituted. This may indicate that modification of the lignin hydroxyls does not play a major role in the mechanism of resistance to biological attack.

Data from energy-dispersive X-ray analysis indicate that the initial rate of reaction of whole wood with chloroacetic anhydride is controlled by diffusion. Because isolated lignin was found to be the most reactive to acetic anhydride, it might be expected that acetylation would occur preferably in the high-lignin-content middle lamella region at the low weight gains. Just the opposite was found. This suggests that the initial distribution of chloroacetic anhydride to the reactive sites in the cell wall was governed by the rate of diffusion of the reactant into the cell wall rather than the rate of the chemical reaction. This may not be true for reactions with acetic anhydride since the chloroacetic anhydride molecule is larger and more hydrophilic than acetic anhydride.

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