tem offered excellent reproducibility as indicated by the low standard errors of injection for each of the monomers quantified.

ACKNOWLEDGMENT

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Registry No. Vanillin, 121-33-5; *cis*-*p*-coumaric acid, 4501-31-9; *trans*-*p*-coumaric acid, 501-98-4; *cis*-ferulic acid, 1014-83-1; *trans*-ferulic acid, 537-98-4; *trans*-sinapic acid, 7362-37-0; *p*-hydroxybenzaldehyde, 123-08-0; syringaldehyde, 134-96-3; vanillic acid, 121-34-6; *p*-hydroxybenzoic acid, 99-96-7; syringic acid, 530-57-4; acetovanillone, 498-02-2; *p*-hydroxyacetophenone, 99-93-4; acetosyringone, 2478-38-8; *p*-chlorobenzaldehyde, 104-88-1; sinapic acid, 530-59-6.

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Scanning Electron Microscopy of Mixed Hardwoods Subjected to Various Pretreatment Processes

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Mixed southern hardwood chips were subjected to rapid steaming, steam explosion, autohydrolysis, and wet oxidation pretreatments. Chips from each of these pretreatments were examined by scanning electron microscopy to help determine what had occurred in the components and morphology of these chips. Steam explosion was the most effective pretreatment for altering the morphology of the wood by causing separation of fibers at the middle lamella. Previous work has shown that all of the pretreatments altered the chemical components of wood, though in different ways, which was reflected in the electron micrographs of this work. Lignin tended to form beads on the surface of some of the fibers. Autohydrolysis and wet oxidation caused splitting of the cell walls parallel to the orientation of the microfibrils of the S-2 cell wall layer, while rapid steaming caused some splitting of the cell walls perpendicular to the long axis of the fibers.

Scanning electron microscopy is a valuable tool when studying changes in morphology of wood treated by processes such as pressure and heat refining (Short and Lyon, 1978; Koran, 1970; Murmanis et al., 1986). Considerable information may be obtained by scanning electron microscopy regarding the separation of fibers, the condition of the surface of the fibers, and separation of cell wall layers. It is now widely accepted that enzymatic hydrolysis of lignocellulosic materials depends on reducing the crystallinity of cellulose and removing the lignin sheath surrounding the cellulose fibers (Gencer and Mutharasan, 1979; Ryu et al., 1982; Saddler et al., 1982). Marchessault et al. (1980) showed that there was no detected loss of crystallinity as a result of steam explosion of aspen chips, while Tanahashi et al. (1983) even observed an increase in crystallinity after steam explosion of some species of wood; consequently, in pretreatment processes separation of the lignin from the cellulose is responsible for the increased rate of cellulase hydrolysis.

This study was undertaken to investigate the changes in morphology of wood induced by the pretreatments rapid steam hydrolysis, steam explosion, autohydrolysis, and wet oxidation. Although wood was the substrate for this study, the results should be useful for other substrates for biomass conversion such as sugar cane bagasse and corn stalks, which have compositions roughly the same as wood (Schultz et al., 1984).

EXPERIMENTAL SECTION

Mixed southern hardwood chips, predominantly oak and gum species, were pretreated according to previous methods: rapid steam hydrolysis (Biermann et al., 1984), steam explosion (Schultz et al., 1983), wet oxidation (McGinnis et al., 1983), and autohydrolysis (Biermann, 1983). In the case of rapid steam hydrolysis, wood chips

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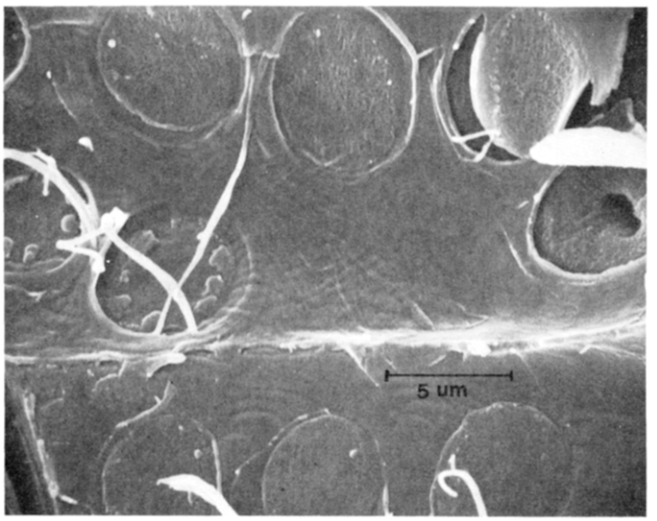

Figure 1. Wood fiber steam exploded for 1 min at 200 °C.

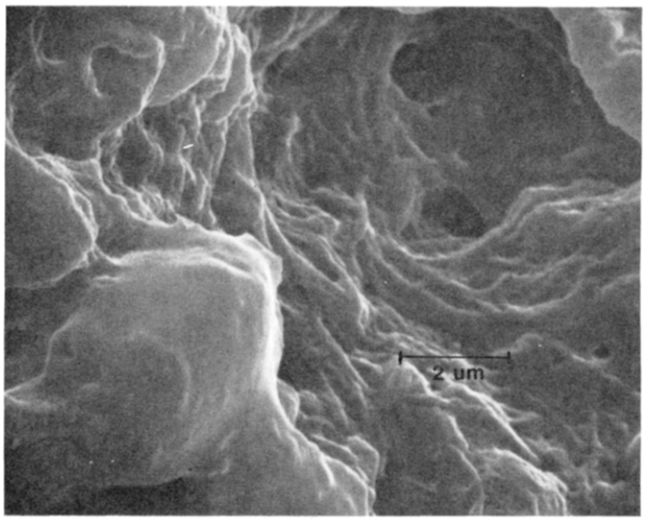

Figure 2. Wood fiber (from a cell fragment cluster) steam exploded for 1 min at 233 °C.

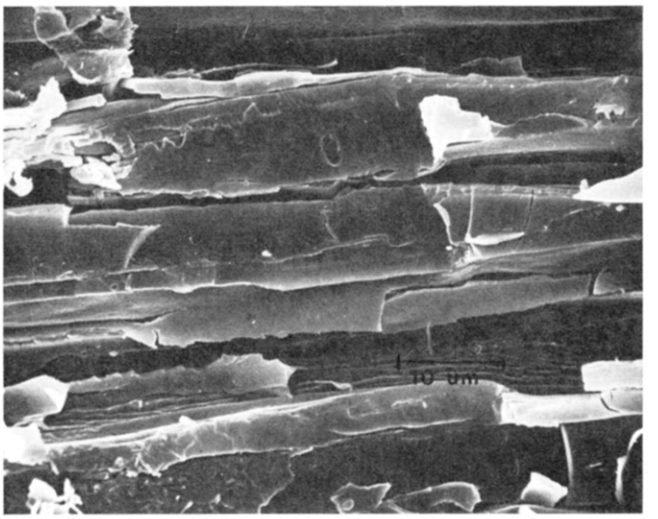

Figure 3. Wood chip steamed at 260 °C for 1 min.

inside a reactor were subjected to a continuous flow of steam for 1 min with the continuous removal of steam and steam condensate from a tube going from the bottom of the reactor to an external condenser. Steam explosion exposed the chips to steam in a pressurized reactor for 0.5–2.0 min followed by a sudden decompression. Wet oxidation was carried out with excess water in a sealed, stirred reactor with 240 psi oxygen for 30 min after a 15-min heat-up period. Autohydrolysis was carried out in the same manner as wet oxidation except no oxygen was added and the reaction time was 5 min after a 15-min heat-up time. In all of the pretreatments, the temperature was measured inside the reactor in close proximity to the wood chips.

Samples for electron microscopy were air-dried (to prevent extraction of material) and mounted on aluminum specimen stubs with silver, conductive paint. Fresh surfaces had been exposed by cutting the chips with a razor blade for all materials except the steam-exploded material, which already was in the form of individual fibers. Samples from all the pretreatments were extracted with 9:1 dioxane–0.025 M HCl to remove low molecular weight lignin molecules that may have formed during their pretreatment. This extraction was carried out by refluxing for 1 h. All of the samples were coated with a gold–palladium alloy in high vacuum on a cool-stage, diode sputter coater and examined on a Hitachi Model HHS-2R scanning electron microscope. A total of 50 specimens of 5–10 small chips each were examined, including four untreated controls. Over 200 exposures were taken.

RESULTS AND DISCUSSION

Steam Explosion. As previously reported (Schultz et al., 1983) steam explosion at 190–200 °C gives a product typical of material normally used to make fiberboard. The three features recognized by Koran (1970) in his study of jack pine were present: fiber bundles, fully separated fibers, and fiber fragments. Tanahashi et al. (1983) found only small fiber fragments and fiber bundles after steam explosion of a Karamatsu (from the larch family) but all three features of steam-exploded Shirakaba (a member of the birch family). There is agreement among these authors that the fiber bundles are caused by the crossing of longitudinal fibers with ray fibers that are relatively difficult to separate. That 190–200 °C were mild conditions is indicated by Figure 1, which shows most of the pit membranes to be intact. Separation of fibers occurs at the compound middle lamella, due to the softening of lignin above 135 °C especially in the presence of water (Goring, 1963). Tanahashi et al. (1983) confirmed this by their work with differential thermomechanical analysis. As steaming time (at 28 kg/cm^2 steam pressure) increased to 2 min, softening increased, but as steaming time increased from 2 to 16 min, softening decreased. This phenomenon was attributed by the authors to repolymerization of the hydrolyzed lignin fragments. Spalt (1977) showed that thermoplastic fusion of lignin on the surfaces of the fibers resulted in the high wet strength of hardboard.

At higher temperatures, 220–230 °C, fiber fragments tend to form clusters (Schultz et al., 1983) partly due to thermoplastic melting and subsequent fusion of lignin, giving a glassy appearance to the surface of the fibers at high magnification (Figure 2). Also adhesion of the fiber fragments may have been assisted by condensation of furfural with lignin. Furfural is produced from the 5-carbon sugars of hemicellulose; the reaction is catalyzed by acetic acid, which is produced by cleavage of the acetyl groups of hemicellulose (Spalt, 1977).

Steaming. Steaming, autohydrolysis, and wet oxidation did not separate the fibers from each other to the extent that steam explosion did. The reason is that there was no sudden decompression or other physical treatment as in the case of steam explosion. Rapid steaming at temperatures above 240 °C led to the formation of a few splits that are almost perpendicular to the main axis of the fibers (Figure 3). The surface of some of the steamed fibers was

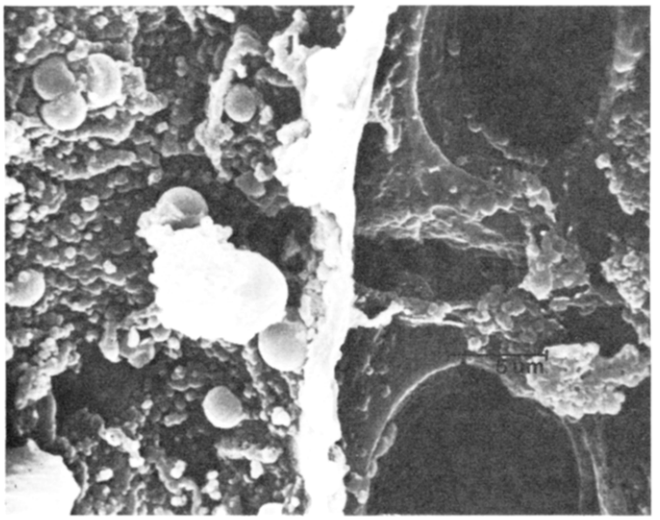

Figure 4. Surface of wood pretreated by autohydrolysis at 260 °C for 5 min.

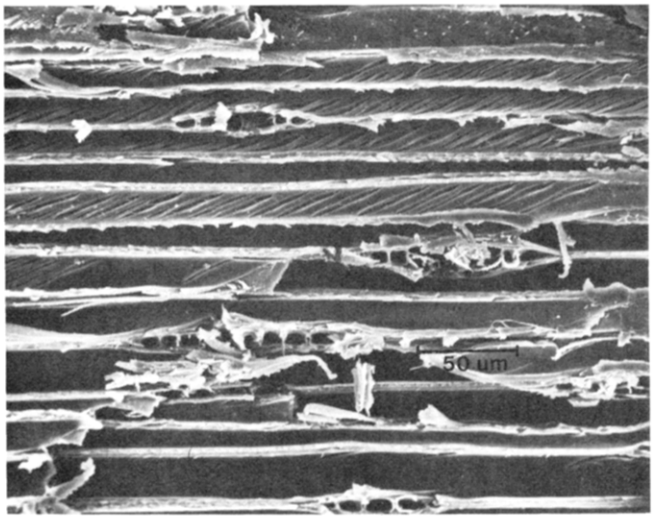

Figure 5. Wood chip pretreated by autohydrolysis at 240 °C for 5 min.

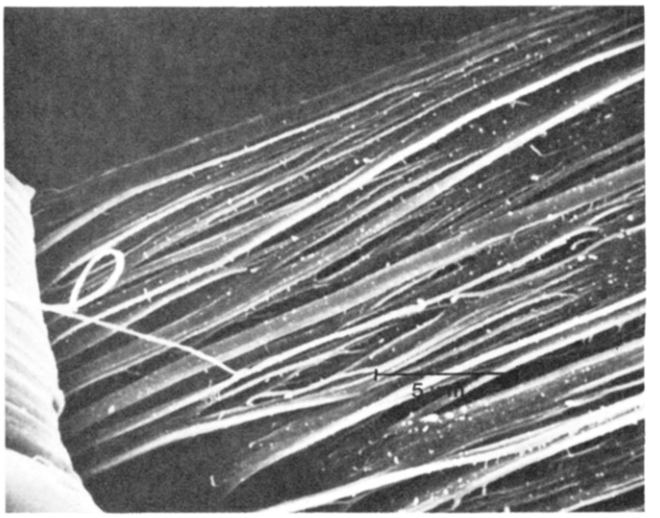

Figure 6. Wood chip pretreated by autohydrolysis at 240 °C for 5 min.

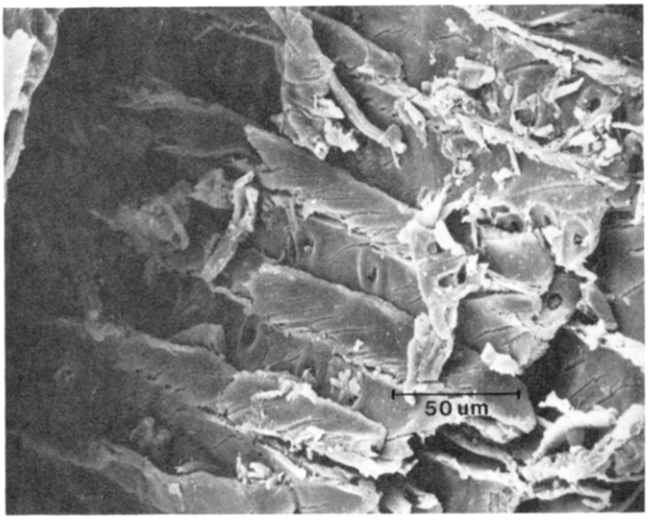

Figure 7. Wood chip pretreated by wet oxidation at 160 °C for 30 min.

roughened by what was apparently lignin (Biermann et al., 1984). (The surface was similar to the surface shown in Figure 4 for autohydrolysis.) The material is assumed to be lignin based on the following arguments. An insoluble residue formed in the steam condensate of wood steamed at 275 °C. This residue, corresponding to 10% of the dry wood weight, was completely soluble in acetone, yielded 90% Klason lignin, and had an IR spectrum typical of lignin. Because of its properties and high yield, this precipitate must be primarily from the lignin. This same precipitate could also have been left on the surface of the fibers. This conclusion is substantiated by other studies, as indicated next.

Numerous works in the literature show that under steaming conditions lignin is depolymerized. Spalt (1977) reviewed depolymerization of lignin during steam explosion, while Marchessault et al. (1980) isolated low molecular weight lignin fractions (700 g/mol, average) from steam-exploded aspen, which could even be extruded (Marchessault and St-Pierre, 1980). Tanahashi et al. (1983) described a brown oily substance insoluble in water but soluble in methanol that appeared in steam-exploded wood but was hardly detected in thermomechanical pulp or ground pulp. This evidence indicates that hydrolysis of lignin in the presence of steam at these temperatures depolymerizes and weakens lignin and allows it to flow.

Autohydrolysis and Wet Oxidation. As shown in Figure 4, the surface of wood pretreated by autohydrolysis is rough. These beads are most likely lignin as dioxane–water extraction removes them from material pretreated at lower temperatures. They are not removed in the case of material pretreated by autohydrolysis at high temperatures, probably because the lignin is in a high molecular weight form due to condensation reactions that tend to occur at high temperatures (Spalt, 1977) or long reaction periods (Tanahashi, 1983).

With autohydrolysis temperatures above 220 °C, regular splits appeared in the fibers at an angle of 20–30° with respect to the longitudinal axis of the fibers (Figure 5). This is the same angle as microfibrils in the S-2 cell wall layer, the thickest layer of the fiber wall. Extensive fraying of the cell wall was also observed (Figure 6).

Wet oxidation showed changes in morphology similar to those encountered with autohydrolysis. Much lower temperatures were required with wet oxidation due to the presence of oxygen and a slightly longer reaction time. Splitting of the cell wall parallel to the S-2 layer orientation was observed (Figure 7). Dioxane–water extraction of some material treated by wet oxidation caused the chips to clump together (Figure 8), a result not obtained with any of the other pretreatments. The splitting of the cell

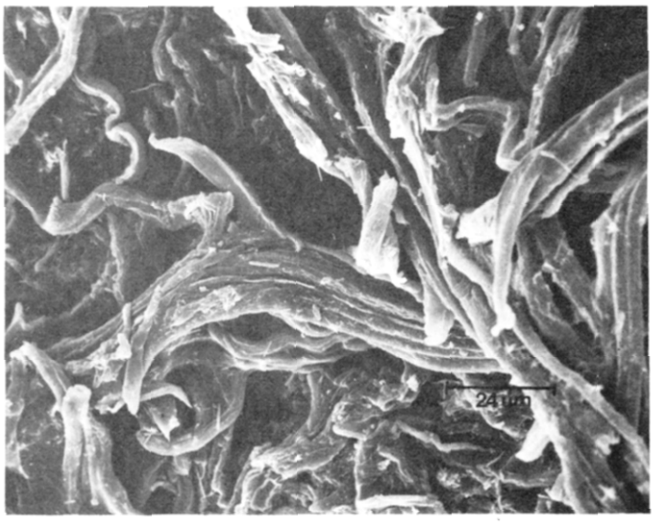

Figure 8. Dioxane–water extraction of material wet oxidized at 200 °C for 30 min.

walls in material pretreated by autohydrolysis and wet oxidation appeared to depend more on the removal of hemicellulose than cellulose (Biermann, 1984).

Registry No. Lignin, 9005-53-2.

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Epicuticular Leaf Waxes of *Citrus halimii* Stone

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Epicuticular leaf wax from *Citrus halimii* Stone was analyzed and found to contain *n*-alkanes, wax esters, primary alcohols, fatty acids, and large amounts of triterpenols and their derivatives. Benzoic acid esters and sterols were also isolated. The main components of this wax were the free triterpenols β-amyrin, α-amyrin, and lupenol. β-Amyrin and α-amyrin were also found esterified with very long chain fatty acids. Triterpenol ketones lupen-3-one and friedelan-3-one could be identified. The sterols isolated were identified as cholesterol, campesterol, stigmasterol, and β-sitosterol.

Citrus halimii is a wild species that has been reported from only a few places of the primary hill forests of southern Thailand and peninsular Malaysia. It has been described and placed into the subgenus *Citrus* in the orange subfamily Aurantioideae (Stone et al., 1973). Its potential for rootstock use, breeding, and conservation purposes has been explored neither in its native areas nor in research stations of other countries (Jones and Ibrahim, 1984). Phytochemical investigations of *C. halimii* so far have been carried out on the fruit flavanones, cations and major rind oil components (Stone et al., 1973), on essential leaf oil composition, oxidase browning of young leaf shoots, and on amylase, catalase and peroxidase isozymes of leaves (Scora et al., 1976). The *n*-alkanes from leaf tissues of various *Citrus* and *Fortunella* fruits were investigated by Nordby et al. (1979); those for 71 citrus biotypes were also reported by Scora et al. (1982). The potential usefulness of *Citrus* long-chain leaf hydrocarbons for taxonomic purposes was reported by Nordby and Nagy (1974), as well as the possibility to separate zygotic from nucellar seedlings so important in *Citrus* breeding (Norby et al., 1975). None of these reports, however, included *C. halimii*, which is a new and taxonomically still imperfectly understood taxon. We therefore report on the soluble cuticular lipids of *C. halimii*, which influence the adherence of agricultural sprays, act as a barrier to pathogen infection, and also play a role in the water economy of the plants.

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EXPERIMENTAL PROCEDURES

Healthy, mature leaves taken from the 1985 spring flush of 14-year-old seedlings of *C. halimii* (Voucher: Scora 3174

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