DEVELOPMENT AND COMPOSITION OF THE WARTY LAYER IN BALSAM FIR. II. COMPOSITION

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ABSTRACT

From its response to various chemical, physical, fungal, and enzymatic treatments, it was concluded that the warty layer in balsam fir consisted largely of a ligninlike material that was visibly more resistant to extraction than a large fraction of other lignin in the fiber cell wall. Since the warts were the cell-wall component most accessible to the treatment solutions, it is probable that the material in the warty layer was more concentrated and condensed than lignin in other parts of the wall. Vacuum drying at 105 C appeared to condense the wart structure still further, making it even more resistant to most treatments. Gel filtration indicated that the warty layer was extracted as a high molecular weight material by certain treatments. The warty layer may act as a barrier that slows the penetration of liquids into the cell wall and thereby may cause different rates of delignification for different wood species. The basal component of individual warts and some of the accompanying encrustant on the inner surface of the cell wall were found to contain an amorphous carbohydrate, probably a pentosan or a pectic substance. Attempts at physical isolation of the warts were largely unsuccessful.

Additional keywords: Abies balsamea, warts, softwoods, extraction, fungi, enzymes, chemical treatment, lignins, cell structure, cell walls, drying, delignification.

INTRODUCTION

The chemical reactivity of the warty layer has been studied in several investigations (e.g., Wardrop et al. 1959; Scurfield and Silva 1969, 1970), and most workers agree that the warty layer is very resistant to chemical dissolution (Liese 1965). A notable exception is Tsoumis (1965), who argued that individual warts could be extracted with hot water alone or with a series of hot organic solvents over a prolonged period, while the amorphous region between the warts was more resistant. In general, however, the reported effects of different chemical treatments on wood warts have been surprisingly consistent, irrespective of the species examined. This suggests that the composition of the warty layer may be similar in all wood types.

Wardrop et al. (1959) stated that the warty layer had chemical reactivity similar to lignin, but in addition, they showed staining evidence for the presence of protein. The latter is not normally found to any extent in the secondary wall. They also found that warts are characterized by strong UV-absorbing properties while the amorphous layer displays a very weak absorbance. Scurfield and Silva (1969) concluded from UV absorption and chemical reactivity data that warts, and perhaps their covering membrane, may both have a lignin component but that the two structures differed in chemical composition. Côté et al. (1966) also implied that the warty layer contains lignin, because warts were present in "lignin skeletons" created by hydrolysis of wood with hydrofluoric acid. Côté (1972) was unsure, however, whether or not this terminal lamella contained the same kind of lignin as that in the secondary wall. Part I of this paper (Baird et al. 1974) reported that the bulk of the warty layer in developing balsam fir tissue exhibited staining properties similar to lignin; however, the basal portions of individual warts were sometimes less darkly stained than the outer portions. Dunning (1969) speculated that warts in longleaf pine may be some combination of lignin and carbohydrate since a mild oxidative treatment followed by an alkaline extraction was required for their complete removal.

An indication that wart material may be different from cell-wall lignin is that the wart structure remained more or less intact

TABLE 1. *Action of chemicals on the warty layer of balsam fir*

Reagent	Temp °C	Time hr	Effect
Benzene-ethanol (2:1)	reflux	6	
↓ Ethanol	reflux	6	
↓ Water (sequential Soxhlet extraction)	reflux	2	
Dioxane	20	144	None detected
Dioxane	60	24	
Dimethylformamide	125	27	
Phenol	90	21	
78% Phosphoric acid	20	3	
10% Chromic acid:10% nitric acid (1:1)	20	1.5	Warts reduced to low mounds, amorphous layer removed
72% H_2SO_4	20	35	Warts slightly smaller, amorphous layer present (Fig. 3)
Dimethylsulfoxide-0.1% HCl	150	3	Warts slightly smaller
	150	5	Warts smaller, removed in some areas
Dimethylsulfoxide-0.5% HCl	150	3	Warts removed, grains remain
15% H_2O_2	20	2	
5% H_2O_2-16.7% acetic acid	20	2	None detected
7.5% H_2O_2-25% acetic acid	45	2	
15% H_2O_2-50% acetic acid	45	2	Warts unchanged, amorphous layer may be attacked
7.5% H_2O_2-25% acetic acid	90	2	Warts and amorphous layer partially removed
15% H_2O_2-50% acetic acid	90	2	Warts and amorphous layer completely removed
30% H_2O_2	90	2	
Chlorite delignification, pH 4.0 (Thompson and Kaustinen 1964)	20	672	Warts reduced to low mounds, amorphous layer partly removed (Fig. 2)
Cadoxene (Henley 1961)	20	96	Warts unchanged, pit margo removed
4-Methyl morpholine-4-oxide	90	22	Cell wall indiscriminately eroded
0.1% KOH	90	2	Warts and amorphous layer slightly attacked, warts lying down
12.5% NaOH-4.1% Na_2S	60	2.5	
17.5% NaOH	60	2.5	
0.25% Digitonin in ethanol	20	264	None detected
5.0% urea	20	144	
NCS tissue solubilizer (Amersham/Searle)	50	22	
Dimethyl sulfoxide (DMSO)	150	10	(Fig. 4)
Dioxane-0.5% HCl	70	16.5	Warts removed[a]
30% H_2O_2	20	336	(Fig. 5)
3% Peracetic acid	60	25	
3% Peracetic acid	60	1.3	Warts removed from loblolly pine[a]

[a]These treatments studied more extensively - see text.

during some relatively mild treatments, which removed a major portion of the middle lamella and cell-wall lignin. Côté and Day (1962) showed that for both hardwoods and softwoods, a 4-h digestion in acidified sodium chlorite at 75 C dissolved the middle lamella lignin but left the warty layer unchanged. Jayme and Azzola (1966) reported no trace of damage to the wart structure of beech vessel members in a neutral sulfite semichemical pulp, while the accompanying amorphous layer was noticeably attacked. Berenzon and Bogomolov (1970), however, found that, under the harsher conditions of high-temperature alkali delignification (kraft and soda pulping), the warty layer was chemically unstable and dissolved.

In addition to chemical treatments, the effects of a few wood-degrading fungi on the warty layer have been explored (Liese and Schmid 1962; Liese 1970). Warts appeared to be more readily decomposed than the amorphous layer in that small craters sometimes occurred during the first stages of enzymatic dissolution.

The objective of this work was to monitor the effects of various chemical, physical, fungal, and enzymatic treatments on the warty layer of balsam fir in an effort to probe the chemical composition of this enigmatic structure and to note the comparative reactivities of individual warts, the accompanying amorphous layer, and the cell-wall structure as a whole.

EXPERIMENTAL

Radial sections (100 μm) of never-dried balsam fir [*Abies balsamea* (L.) Mill.] sapwood were subjected to various chemical, enzymatic, fungal, and physical treatments. Similar sections of loblolly pine (*Pinus taeda* L.) were chemically treated only with peracetic acid. Details of all chemical treatments are recorded in Table 1.

The following enzymes were used to treat balsam fir sections: lipase 448, hemicellulase, peroxidase, and pectinase (Nutritional Biochemical Corp.); ribonuclease and pepsin (Worthington Biochemical Corp.); trypsin and polyphenol oxidase (P-L Biochemicals, Inc.). The peroxidase and polyphenol oxidase were also used in combination with an alkali pre- or post-treatment (0.1*N* KOH, 90 C, 2 h). The pectinase was also used on: (1) balsam fir sections previously infected for 8 weeks with the white-rot fungus, *Trametes suaveolens*, (2) balsam fir chlorite-holocellulose prepared according to Thompson and Kaustinen (1964), (3) loblolly pine sections. All enzymes were used as supplied except pectinase, which was first dialyzed to remove glucose known to be present in the preparation (Timell 1962). The activity of each enzyme was confirmed and the enzyme used under conditions suggested by the supplier.

The following white-rot fungi purchased from the American Type Culture Collection were cultured aseptically in asparagine-glucose medium (Bartnicki-Garcia 1966) before introduction of balsam fir sections: *Daedalea unicolor*, *Polyporus anceps*, *Polyporus versicolor*, *Poria subacida*, *Schizophyllum commune*, *Trametes suaveolens*. After 2–26 weeks of treatment, the sections were removed and gently scraped with a dissecting needle to remove the fungus from the sample surfaces.

Three different attempts were made to physically isolate the warty layer from balsam fir wood: (1) both transverse and radial sections were ultrasonicated in water, in 0.1*N* KOH, and in pectinase at 300 kc/sec for 2 h at 30 C with a GE ultrasonic generator; (2) wood was ground to a fine powder, suspended in water, and fractionated by differential centrifugation; (3) surfaces of radial sections were embedded with molten polystyrene and the wood was scraped away with a dissecting needle, hopefully leaving only the protruding warts embedded in the polystyrene. To determine the thermoplastic behavior of the warty layer, balsam fir sections were heated in an autoclave at 121 C (15 psi steam) for 2.5 h. The results of all treatments were monitored with a transmission electron microscope according to the procedures outlined in Part I of this work (Baird et al. 1974).

In conjunction with selected chemical treatments that were found to dissolve the

warts, further analyses were made in an effort to identify residual as well as extracted wood material. While the initial treatments were made on never-dried wood, for these more detailed analyses sections were vacuum-dried for 2 h at 60 C before and after treatment to enable yield calculations. Klason lignin determinations were made on the treated sections using ½0th proportions of those specified in Tappi Standard T 13 m-54. Apparent carbohydrate portion was determined by difference. Also, some wood samples were vacuum-dried at 105 C before chemical treatment to determine any effect of the higher temperature. The UV absorbance spectra of selected extracts were determined with a Cary 15 spectrophotometer. DMSO extracts were ultracentrifuged to determine whether warts were dissolved by the treatment or freed intact from the cell wall.

Materials in the pectinase hydrolyzate of chlorite-delignified balsam fir were fractionated by thin-layer chromatography. The concentrated hydrolyzate was spotted on precoated Kieselguhr plates buffered with 0.05 *M* sodium acetate and then developed with 4 repetitions of ethyl acetate-isopropanol-water (8:2:1). Chromatograms were detected by spraying with anisaldehyde reagent (Waldi 1965).

Both low molecular weight carbohydrate and lignin materials in treatment solutions were fractionated by descending paper chromatography using Whatman No. 1 paper. Carbohydrate chromatograms were developed with one of two different solvent systems, either ethyl acetate-acetic acid-formic acid-water (18:3:1:4) alone, or ethyl acetate-pyridine-water (8:2:1) to move the neutral sugars away from the acids, followed by the (18:3:1:4) solvent. Carbohydrates were then detected with *p*-anisidine hydrochloride (Hough et al. 1950). For chromatography of lignin fragments, butanol-pyridine-water (10:3:3) was used as a development solvent and 2,4-dinitrophenylhydrazine (Bland 1949) or diazotized *p*-nitroaniline (Pearl and McCoy 1960) as a detection reagent.

Gas-liquid chromatography was employed in an attempt to isolate semivolatile components extracted from wood in the DMSO and dioxane-HCl treatments. A Varian Aerograph Model 1400 fitted with a 6-ft column of 10% Carbowax 20M on Chromosorb W was used under the following operating conditions: injection temperature, 210 C; column temperature, 150 C for 16 min, then programmed to increase at 10 C/min to 210 C where the temperature was maintained; detector temperature, 270 C; helium carrier gas flow, 75 ml/min.

An estimate of the molecular weight distribution of extracted materials was obtained by using gel filtration chromatography with Sephadex G-25 and DMSO as the gel-eluent system (Lundquist and Wesslen 1971). DMSO and dioxane-HCl extracts were freeze-dried and redissolved in a small amount of DMSO before column loading. The elution was monitored continuously by recording absorbance at 280 nm using a 1-mm flow cell.

Controls and known reference compounds were chromatographed with all fractionation samples.

RESULTS AND DISCUSSION

Chemical treatments

The results of numerous chemical treatments on the warty layer of balsam fir (Fig. 1) are summarized in Table 1. With few exceptions, the results of these treatments were in agreement with those of other workers. Treatments reported in the literature to remove the warty layer but that had no effect on balsam fir warts were phosphoric acid (Scurfield and Silva 1969) and neutral solvent extraction (Tsoumis 1965). Generally, however, balsam fir warts were found to exhibit the same reactivity as the warts of other species, both softwoods and hardwoods.

General observations. Several deductions can be made concerning the chemical composition of the warty layer, based upon the response of this structure to different reagents as visualized by electron microscopy before and after treatment.

Neutral solvents did not remove the warty

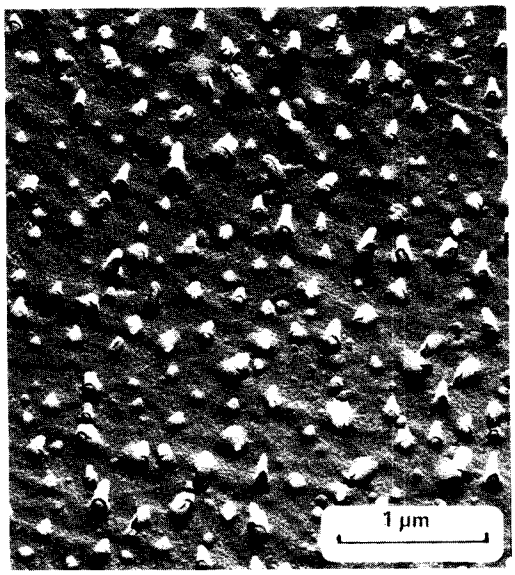

FIG. 1. Warty layer on the inner cell-wall surface of a mature tracheid in balsam fir.

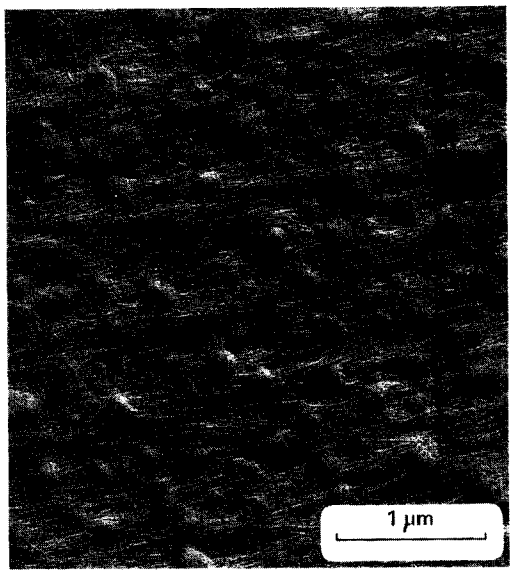

FIG. 2. Warty layer of balsam fir after treatment with 4.5% sodium chlorite, pH 4.0, at room temperature for 4 weeks.

layer, so it is not likely that wart structure consists of resinous deposits or other extractives. Digitonin, a membrane-dispersing agent, had no effect whatsoever. The NCS tissue solubilizer (Amersham/Searle), a quaternary ammonium base, also had no effect. Therefore, lipoprotein membranes seemed not to be involved or were inaccessible.

The cellulose solvent, cadoxene, had no apparent effect on the warty layer, though radiating microfibrils in the margo of bordered pit membranes were dissolved. 4-Methylmorpholine-4-oxide eroded the entire wood structure, including the warty layer, but this chemical is not a specific solvent since it reportedly dissolves cellulose, hemicellulose, and lignin (Johnson 1969).

Treatments with concentrated alkali had no effect on the warty layer, but the temperature, 60 C, was probably not high enough for them to be effective delignifying agents. At higher temperature (90 C) and low alkali concentrations (0.1*N*), the warts were slightly affected, but perhaps the major attack under these conditions was on the less resistant carbohydrates.

The following reagents dissolve but are not completely specific for lignin: DMSO-HCl, chromic-nitric acid, H_2O_2-acetic acid, and H_2O_2 alone. Under mild conditions these reagents can dissolve a portion of the warty layer, leaving small, flat mounds. When harsher conditions are used, the entire structure is removed (Baird 1974).

Chlorite treatment is known to extract nearly all the lignin portion of wood while leaving essentially the total carbohydrate fraction (Thompson and Kaustinen 1964). In balsam fir chlorite-holocellulose (Fig. 2), only flat wart remnants about the same diameter as the bases of untreated warts were found. The S3 layer here remained slightly encrusted. Interestingly, there is a similarity between the residual warts of balsam fir holocellulose (Fig. 2) and the developing warts in differentiating tissue (see Fig. 8F, Baird et al. 1974).

Sulfuric acid shrunk the warts but the amorphous layer remained (Fig. 3). Treatment with this reagent is known to hydrolyze polysaccharides, leaving "Klason lignin" (Brauns 1952). Some carbohydrate component from the interior of the wart structure may have been removed, or else the lignin portion may have been condensed by

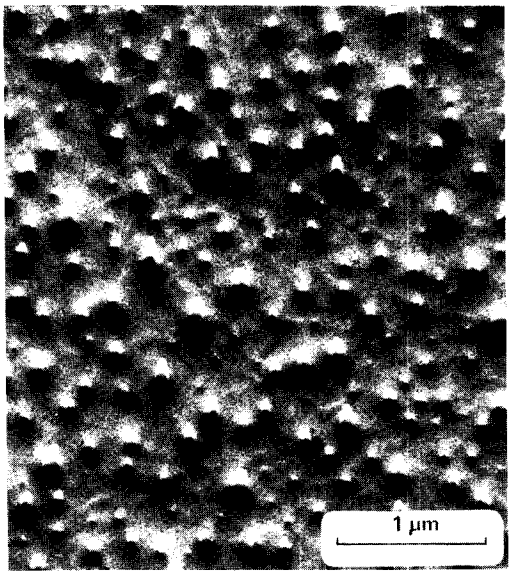

FIG. 3. Appearance of balsam fir warts after 35 h of exposure to 72% sulfuric acid at room temperature.

the acid to cause the shrinkage. In any case, most of the wart structure and amorphous layer are clearly ligninlike in composition, and it is therefore difficult to agree with Wardrop et al. (1959) that warts are unchanged upon treatment with sulfuric acid as some shrinkage definitely occurs (Fig. 3).

Detailed observations. Four chemical treatments were studied more thoroughly to obtain information on the nature of those reactions that dissolve warts. The actions of dimethylsulfoxide (DMSO) at 150 C, dioxane-0.5% HCl at 70 C, 30% H_2O_2 at 20 C, and 3% peracetic acid at 60 C on the warty layer of balsam fir were monitored by electron microscopy over a series of treatment times. For example, Fig. 4 shows the condition of the inner surface of the cell wall at different stages of DMSO extraction. Warts disappear in the period around 10 h, being first reduced to granular patches and then completely removed.

Amounts of total wood, lignin component, and apparent carbohydrate component dissolved before the warty layer was removed are reported for all four treatments in Table 2. An important observation here was that while the warty layer was the cell-wall component most accessible to a treatment solution, it did not disappear until after removal of significant amounts of other cell-wall constituents. Therefore, while the major part of the warty layer is similar to lignin in its response to chemical treatments, warts are more persistent than at least some of this other wood lignin. If this persistence reflects a difference in reactivity, it could stem from a higher degree of condensation of the lignin molecule in the wart structure as compared to that in the rest of the cell wall. On the other hand, it is conceivable

TABLE 2. *Amounts of wood components removed before the warty layer is removed by various chemical treatments*

Treatment	Total wood removed %	Original lignin removed %	Original apparent carbohydrate removed %
Balsam fir			
DMSO, 150 C, 10 hr	32	46	25
Dioxane-0.5% HCl, 70 C, 16.5 hr	46	65	38
30% H_2O_2, 20 C, 14 days	20	16	22
3% Peracetic acid, 60 C, 25 hr	26	55	12
Loblolly pine			
3% Peracetic acid 60 C, 80 min	2	ND[a]	ND

[a] Not determined.

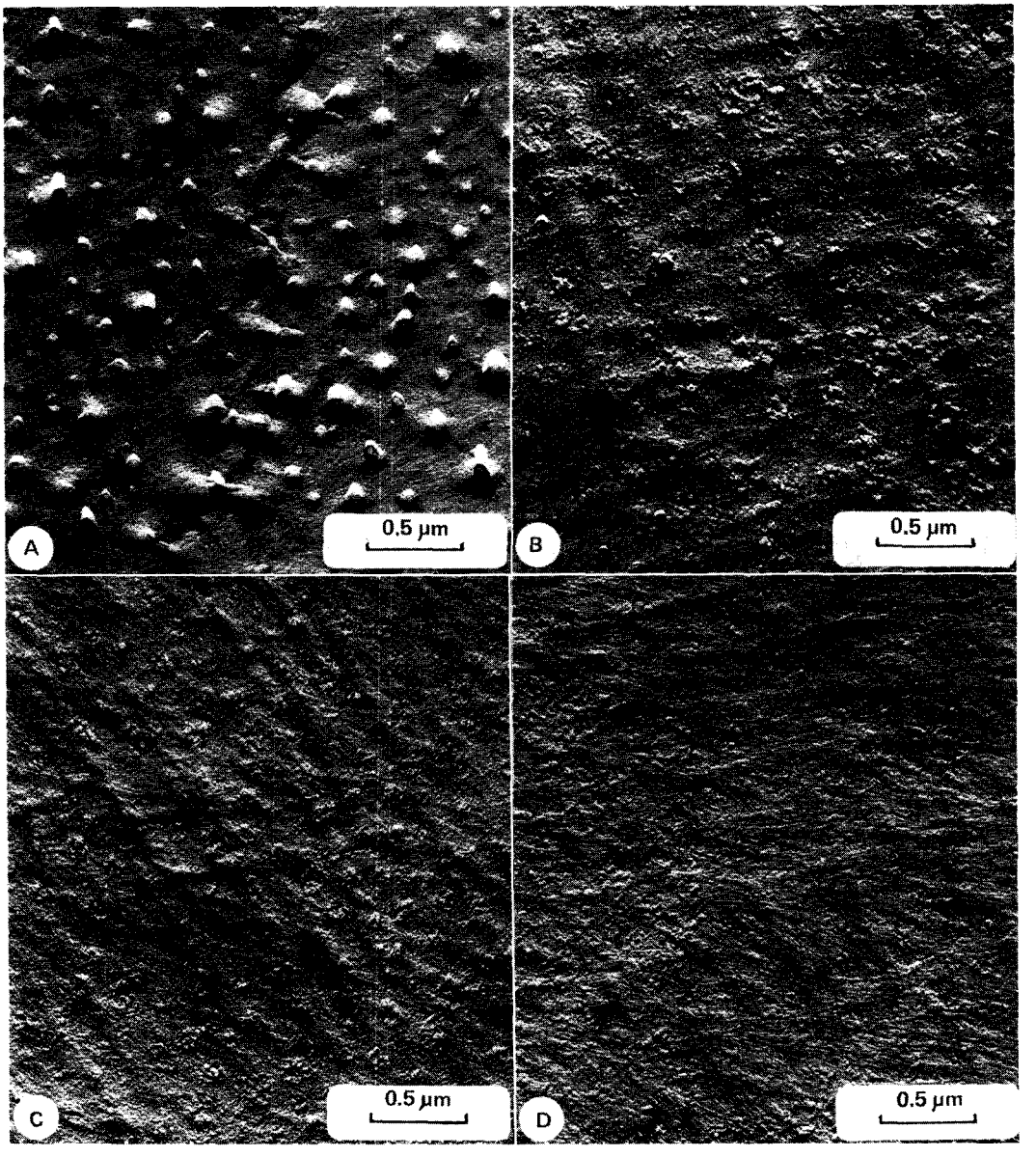

FIG. 4. Stages in treatment of balsam fir warty layer with DMSO at 150 C: A. 5 h, B. 10 h, C. 13.5 h, D. 15 h.

that some lignin can be removed from the warts without inducing much change in wart appearance.

The warts on wood vacuum-dried at 105 C before treatment had the same response to DMSO treatment as those dried by the normal procedure (vacuum – 60 C). For the dioxane-HCl, H_2O_2, and peracetic acid treatments, however, warts and other cell-wall lignin were highly resistant to dissolution after the higher temperature. This resistance suggests that high-temperature vacuum drying condenses the wart structure to the point of preventing oxidative attack while not affecting high-temperature extraction with DMSO.

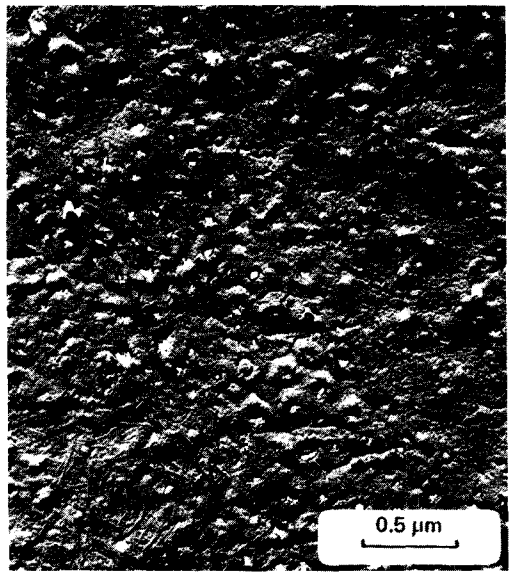

FIG. 5. Intermediate stage of balsam fir wart degradation by 30% hydrogen peroxide (at room temperature for 7 days). Note the "crater" morphology of residual wart material.

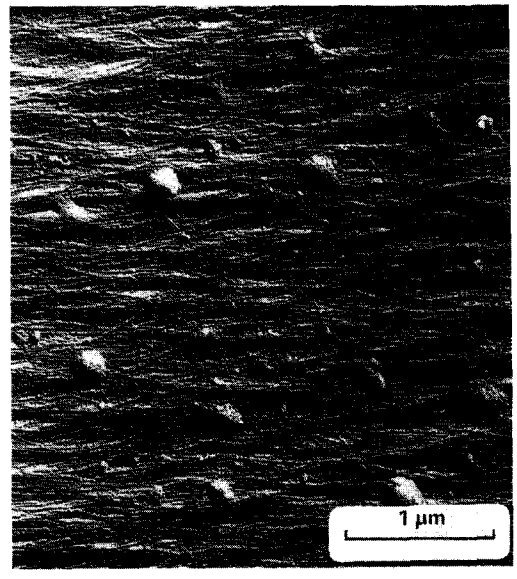

FIG. 6. Warty layer of untreated loblolly pine. Note that the S3 microfibrils are clearly visible.

The warty layer makes up only about 2% of mature balsam fir wood (Baird 1974). No chemical treatment used in the present study was found to be completely wart-specific, and isolated wart substance represented only a small fraction of the total material extracted by any given treatment. Therefore, what was sought in subsequent analyses were any differences in the composition of the extraction solutions just before and just after visualized wart removal. Only with the DMSO and dioxane-HCl treatments were the warts almost completely removed over a short time range, making this analysis-by-difference potentially meaningful. However, to reiterate, it is possible that wart dissolution was actually quite gradual and not reflected morphologically until after a minimum time of treatment.

Ultracentrifugation of DMSO extracts produced no sediment, indicating that warts were in fact dissolved and not merely released from the cell wall during treatment. The UV absorbance spectra of all DMSO extracts examined were indicative of lignin. The warty layer, therefore, has a UV absorbance similar to lignin; or, if different from lignin, it is masked by lignin extracted from other portions of the wood.

Paper and gas chromatography failed to reveal any component of carbohydrate or lignin origin associated exclusively with the warty layer of balsam fir. Gel filtration chromatography showed that the warty layer was extracted by dioxane-HCl (and probably by DMSO) as a material of high molecular weight.

With the H_2O_2 treatment, wart dissolution was not uniform over a given wood section for a given treatment time; therefore, further analyses of extraction solutions were not attempted. Small oxygen bubbles that formed on the wood sections probably protected some surface areas and caused the heterogeneous response. One unique aspect of the H_2O_2 treatment was that partially dissolved warts occasionally appeared as small craters (Fig. 5), possibly indicating that the central core of the warts was attacked first by the reagent.

Peracetic acid was used also to treat a second species, loblolly pine. Warts were removed from pine after only 80 min, while in balsam fir 25 h were required. Peracetic acid has been shown to be highly specific

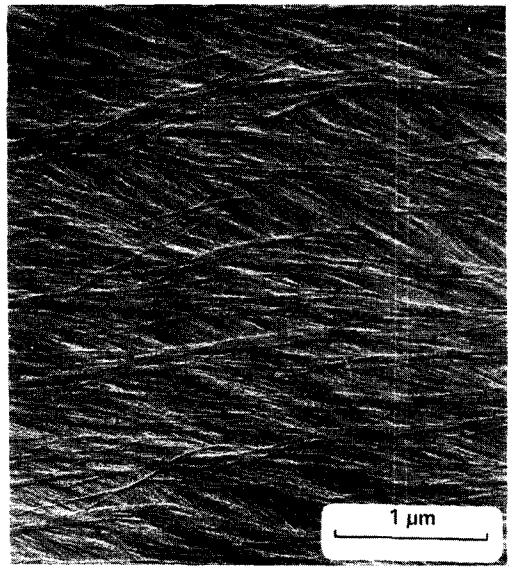

FIG. 7. Tracheid lumen surface of balsam fir after treatment with sodium chlorite followed by pectinase. Compare to Fig. 2 without pectinase treatment.

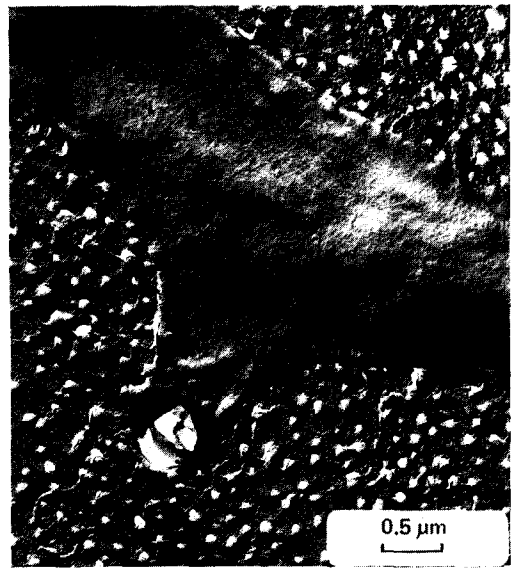

FIG. 8. *Schizophyllum commune* fungal hypha on warty layer of balsam fir wood treated 1 week.

in its attack upon pine lignin (Leopold 1961), and the observed difference in wart reactivity between these two species might be reconciled if the lignin portion of the warty layer in balsam fir were more condensed than the lignin in pine warts, other factors being constant. Total wood delignification was also much slower for balsam fir than for loblolly pine. After a 10.7-h treatment with peracetic acid at 60 C, there was a 23% weight loss for pine compared to only 11% for balsam fir. The nature of the inner cell-wall layer may be responsible for this difference, since wood structure is most accessible to treatment solutions through the empty cell lumina. The inner layer of pine is an open, microfibrillar structure with flat warts directly on the S3 (Fig. 6). In balsam fir the S3 layer is completely covered by a chemically resistant layer of amorphous material in addition to the warts (Fig. 1). Here it is reasonable to propose that this amorphous covering may act as a barrier to the inward penetration of peracetic acid, thereby contributing to a reduced rate of delignification.

Enzymatic treatments

None of the commercial enzymes used as probes in this work had any noticeable effect on the warty layer of balsam fir. Peroxidase or tyrosinase in combination with an alkali pre- or post-treatment was also ineffective.

Pectinase had no visible influence on the warts of untreated loblolly pine or balsam fir. However, the patchlike wart remnants and remaining S3 encrustant on the chlorite-delignified balsam fir (Fig. 2) were completely removed by pectinase (Fig. 7). Timell (1962) reported that this particular pectinase preparation could hydrolyze several noncellulosic polysaccharides with the formation of mono- or oligosaccharide products. Thin-layer and paper chromatography revealed the following materials to be present in the pectinase hydrolyzate of chlorite-delignified balsam fir: xylose, arabinose, mannose, glucose, galactose, and three components whose mobility and color reaction indicated that they were likely oligomers of pentose and uronic acid units. This finding concurs with the results of Meier (1964) who found that the glucurono-arabinoxylan content is high in the S3 layer of softwoods.

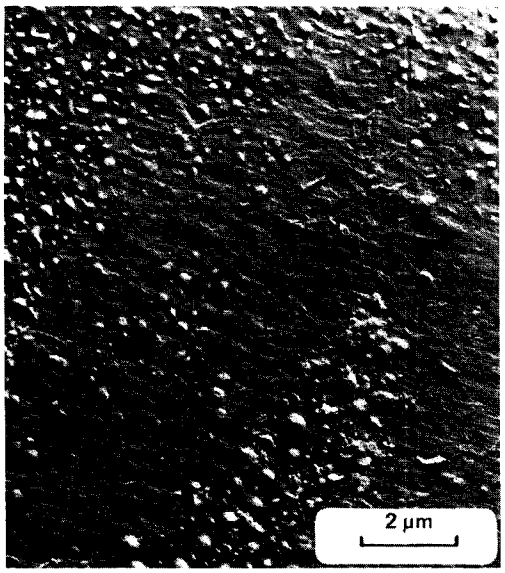

FIG. 9. Warty layer of balsam fir after exposure to *Polyporus versicolor* fungus for 10 weeks.

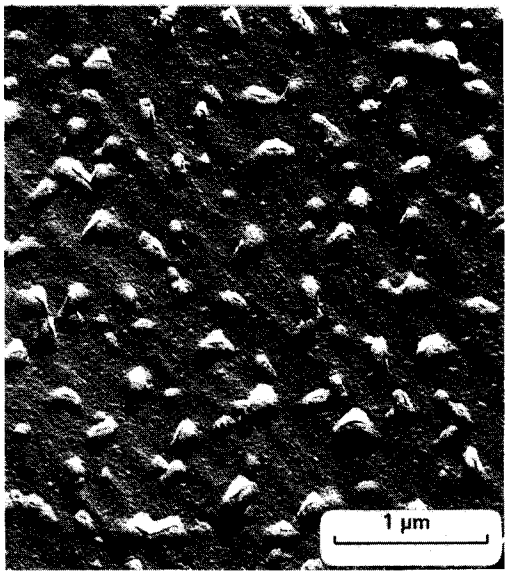

FIG. 10. Balsam fir warts steam-treated for 2.5 h at 121 C.

By visual comparison with chromatographic standards, it was estimated that each fractionated component above except glucose amounted to 0.5–1.0% of the original holocellulose before pectinase treatment; glucose was somewhat less. The pectinase hydrolyzate gave a negative test for characteristic lignin groups with 2,4-dinitrophenylhydrazine and diazotized *p*-nitroaniline.

Fungal treatments

White-rot fungi degrade lignin, hemicellulose, and sometimes, at a later stage, cellulose (Liese 1970). All 6 fungal species monitored in this study had at least some effect on the micromorphology of the cell wall. The attack had two general forms: bore hole formation and surface dissolution. Of the 6 species, all but *Trametes* formed bore holes. Boring (including warty layer dissolution) is probably accomplished by enzymatic action localized at the tips of advancing hyphae (Fig. 8). Only *Trametes* and *Polyporus versicolor* caused a general surface dissolution of the warty layer (Fig. 9). Here also the action probably resulted from enzyme systems associated with the fungi. Therefore, the white-rot fungi were able to dissolve the warty layer, but no conclusions can be drawn regarding the wart composition until the specificity of the enzymes involved is determined by further research.

Physical treatments

The warty layer underwent thermal softening at 121 C. Warts fell over from their natural protruding position and appeared to coalesce with the amorphous layer and with each other (Fig. 10). The warty layer showed signs of similar thermal softening during the early stages of the 150 C-DMSO treatment (Fig. 4A). Goring (1963) has determined the softening temperature for moist isolated components of the cell wall as follows: lignin, 90–100 C; hemicellulose, 50–60 C; cellulose, over 270 C. Atack (1972) reported that the glass transition temperature of lignin in moist wood chips is 120–135 C. The physical behavior of warts when heated appeared to be in line with ligninlike composition.

A successful method of physical isolation is apparently the only way to obtain uncontaminated, unaltered wart material for a definitive analysis. In the present work,

several attempts at this goal (described under Experimental) were largely unsuccessful.

SUMMARY AND CONCLUSIONS

It was concluded from its response to many different treatments that the warty layer in balsam fir consists mostly of a ligninlike material. The basal component of the individual warts and some of the encrusting layer contain a noncellulosic carbohydrate, probably a pentosan or a pectic substance.

On the basis of persistent wart structural integrity, the bulk of the warty layer appears more chemically resistant than, and thus chemically different from, at least some of the other lignin in the cell wall. Depending on the treatment, from 16 to 65% of the lignin in wood was extracted before the disappearance of the warty layer, even though the warty layer was the cell-wall component most accessible to the treatment solutions. From these results, one possible interpretation is that lignin in the warty layer is more concentrated and more condensed than the lignin in the rest of the cell wall.

Balsam fir tracheids, which have a relatively heavy and resistant amorphous layer lining their lumina, were delignified with peracetic acid at a slower rate than loblolly pine tracheids, which have flat warts on an exposed S3 layer. A complete warty layer, therefore, may act as a barrier to the penetration of delignifying agents into the cell wall.

No chemical treatment tested was found to remove the warty layer exclusively, and analyses of the solutions in the various extraction series yielded little additional information. Different chromatographic methods detected no unique phenolic unit associated exclusively with the lignin portion of the warty layer, which was extracted by some treatments as a polymeric substance, indicating that warts probably contain high molecular weight material *in vivo*. No significant change in UV absorbance was detected in the extraction solutions at the point that the warts disappeared, implying that warts either contained no unique chromophores in the UV region or that total lignin absorbance overwhelmed any wart contribution. The chemical reactivity of the warty layer to most reagents, as well as that of other cell-wall components, was reduced by vacuum drying at 105 C. Under these conditions, irreversible dehydration and further condensation may occur to tighten the molecular structure, thereby diminishing accessibility and eliminating reactive sites.

Commercial enzymes had no effect on mature, untreated warts. Several white-rot fungi attacked the warty layer by localized bore hole formation or general cell-wall dissolution, but no conclusion could be drawn from these observations as to the specific composition of the warty layer.

Thermal softening of the warty layer during steam heating was observed and should be expected for the largely lignin composition of warts. Attempts at physical isolation of the warty layer were not successful in producing material pure enough and in high enough yields for a definitive analysis.

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