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Title: Microdistribution and Retention of Chloropicrin in Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] Heartwood

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Quantitative and qualitative information on the retention of chlorinated residues in chloropicrin treated wood is lacking. Retention of chloropicrin and its chemical breakdown products in wood, and the effects of chloropicrin treatment of wood on invading decay fungi are explored in this thesis.

Chlorinated residues in amounts up to 1.5% (w/w, as determined by neutron activation analysis) were present in Douglas-fir wood exposed to chloropicrin vapors for 240 days. These residues could not be totally removed by aeration, acetone extraction, heating, or high vacuum treatment. Lesser amounts of chlorinated residues were detected in wood exposed to chloropicrin for shorter periods of time or treated with smaller quantities of the chemical. Analysis of vapors desorbed from chlorpicrin-treated wood suggests that chemically unaltered chloropicrin can be retained by wood up to 4 years after treatment. Decreased retention of chlorinated residues in treated wood with brown rot decay suggests that hydrogen bonding

and van der Waals forces are responsible, at least in part, for this retention.

Wood does not swell when treated with chloropicrin. Although a monomolecular layer of chloropicrin may hydrogen bond to wood at some sites, lack of swelling indicates that chloropicrin molecules are retained by forces weaker than the hydrogen bonds which hold the lignocellulose matrix of wood together.

Covalent linkages may also form between chloropicrin, or chlorinated breakdown products of chloropicrin, and wood. Energy dispersive X-ray analysis showed that chloropicrin residues were present in greater concentrations in areas of the wood rich in lignin or extractives. Covalent linkages between chloropicrin breakdown products and vanillin were detected in mass spectrographic analyses of chloropicrin/vanillin mixtures. Thin layer chromatography of extracts from chloropicrin treated wood indicated that chloropicrin may be bonding to dihydroquercetin (a phenolic wood extractive) and to other extractives in wood.

Microscopic observation of Douglas-fir wood exposed under severe laboratory decay conditions to Poria carbonica (a brown rot fungus), and to Ganoderma applanatum (a white rot fungus), showed that these fungi will attack chloropicrin treated wood, producing bore holes and erosion of cell lumen surfaces similar to that observed in untreated wood. Lysis and vacuolation of the fungal hyphae, particularly of G. applanatum, indicates that chloropicrin has an inhibitory effect on fungi in wood.

Microdistribution and Retention of Chloropicrin in Wood

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INTRODUCTION

Fumigants have been used commercially to control wood decay fungi in transmission poles since the early 1970's (20). Since that time considerble research has been done to evaluate their field effectiveness and determine their gross distribution in treated wood products. Studies at Oregon State University have shown that chloropicrin has remained effective against decay fungi in transmission poles for 12 years (21) and bioassays show that fungitoxic chloropicrin vapors are present in wood samples removed from timbers up to 5 years following treatment (21).

Research on the nature of this retention of chloropicrin by wood, the breakdown of the chemical over time, and reinvasion of treated wood by fungi is lacking. If the amounts and location of chloropicrin remaining in wood following fumigation can be determined, this may provide insight into better methods for fumigant treatment and should suggest, depending on the nature of retention, if chloropicrin can be considered for use as a long-term wood preservative.

I investigated these problems and the potential for the use of chloropicrin as a long-term preservative through experiments designed to determine:

- The means of retention of chloropicrin and its degradation products in wood.
- The location and concentration of chloropicrin and its degradation products within the wood structure.

- The amounts of chlorine retained in chloropicrin-treated, decayed and sound wood, and the effects of chloripicrin treatment on the penetration and breakdown of wood by fungi.

BACKGROUND

Retention of Chloropicrin

One of the main advantages of using fumigants to treat wood is that gas molecules can move through the pits without surface tension interference (4), allowing the gas to rapidly diffuse through the wood structure. If the fumigant is not bound to the wood, however, and forces such as surface tension--which serve to retain conventional liquid preservatives in wood--are not active, then the fumigant may also readily diffuse out of the wood. It is known that fumigants will bond to organic matter and clays in the soil (15, 19) and low levels of residue of many fumigants have been recorded in stored grains after aeration (2). Smith (37) determined that residues of propylene oxide can remain in wood after aeration, and these residues are fungitoxic to mature mycelium of Lentinus lepidius. Low levels of chloropicrin residues remaining in stored grain products after fumigation have been reported but no quantitative data is available, and the fungitoxic nature of these residues was not studied (13).

Evidence that chloropicrin residues may be binding to wood was suggested in studies where significantly smaller quantities of chloropicrin vapor diffused longitudinally in severely decayed wood than in less decayed wood (16). This suggested that more chemical was retained in the severely decayed wood because the greater surface area or micropores in the cell wall created by fungal degradation provided more sites for chemical bonding or physical entrapment of the chloropicrin molecules.

Bonding of Chloropicrin to Wood

Cowling (11) determined that at weight losses above 40%, wood decayed by the white rot fungus, Polyporous versicolor, absorbed significantly more moisture than non-decayed wood (compared to oven dry weights) when samples were exposed to various relative humidity conditions. The reverse situation occurred in samples decayed by the brown rot fungus, Poria monticola, where a general reduction in hygroscopicity occurred with increasing decay at all stages. Cowling suggested that in white rot decay, the increase in hygroscopicity may have been due to the reduction in the amount of crystalline material in the wood which he considered to be less hygroscopic; presumably this material was also converted to more hygroscopic amorphous cellulose. With brown rot decay he suggested that the amorphous cellulose may have been attacked preferentially causing a relative reduction in hygroscopicity.

Schulze-Dewitz et al. (36) also found that brown rotted woods generally had greater relative sorbtion when the wood had been incubated with fungi for 4 months. However, when sorbtion capacity was measured in woods decayed to a range of weight losses between zero and 25% it was found initially to decrease, then increase, then decrease again.

Even if no significant bonding of a fumigant to wood by hydrogen- or covalent-type bonds occurs, if greater microvoid space is created by the degradation of the wood material, this may allow greater amounts of the chemical to be retained in decayed wood than in sound wood.

Molecules of polar liquids will move into the amorphous regions of a ligno-carbohydrate matrix expanding this matrix in proportion to

the size and quantity of molecules introduced (32). Polar molecules unite with dry cell wall material by means of hydrogen bonding (32). The dipole moment of chloropicrin is approximately the same as that for water (43) and hydrogen bonding should be possible between the oxygens of the chloropicrin molecule and the hydrogen molecules in wood. However, because chloropicrin contains no hydrogen, dipole-dipole interaction of its molecules will be much weaker than the hydrogen bonding attraction (28) which occurs between water molecules and allows the buildup of multimolecular layers attached to the wood matrix (the cause of dimensional change/moisture relationships in wood). The energy of the bonds between chloropicrin molecules, or between chloropicrin and wood molecules may be too low to overcome the attractive forces holding the amorphous regions in wood together to limit swelling of wood in the presence of chloropicrin. Fumigants such as chloropicrin can be sorbed to surfaces by physical adsorbtion and held by van der Waals forces (8) or depending on environmental conditions can be chemically (covalently) bound to some extent (5). The physically bound residues can be removed far more easily than residues bound covalently by means such as prolonged aeration, or by more drastic treatments such as the use of heat, solvents, or reduced pressure.

Cooper (personal communication, P. A. Cooper, 2/1/83) has analyzed chloropicrin retention by wood, comparing its retention time on ground wood particles (used as the absorbtive phase in a gas chromatography column) to that of other solvents. He found that chloropicrin had a retention time similar to that of non-polar solvents such as benzene. Analysis of the ground wood after treatment was not made to determine if covalently bound residues were present.

The persistence of chloropicrin residues in treated grain was found to be greater when the grain was subjected to treatment for greater periods of time (48). Grain subjected to 112 hours of chloropicrin treatment retained 29.62% residue after gas sweeping but only 8.56% residue was retained after gas sweeping if the treatment was for 0.5 hours. Chloropicrin (or breakdown residue) was not completely removed after heating of the grain samples to 120°-130°. This evidence suggests that chloropicrin strongly hydrogen bonds or possibly even covalently bonds to grain; however, no information concerning this, or the fungitoxic nature of this residue was given.

The preceding evidence suggests that chloropicrin could possibly bond at least in small quantities to other organic, cellulosic materials such as wood. Since chloropicrin is known to be very susceptible to nucleophillic attack (9, 14), the likelihood of a covalent attachment of chloropicrin in wood increases if weak bases occur naturally in the extractive materials.

Chemical Breakdown of Chloropicrin

Chloropicrin in the gas-phase can be photolytically decomposed to phosgene and nitrosyl chloride (27, 3). Moilanen et al. (27) found that 20 days were required to decompose half of the chloropicrin at an initial concentration of 14 µg/ml, when exposed to sunlight wavelengths (> 290 nm). Chloropicrin was not observed to decompose in the dark over a 7 day sampling period. Hydrolysis of chloropicrin in aqueous solution at pH 7, when irradiated by a monochromatic light source at 254 nm was very rapid (9). In 10 hours greater than 95% of the chloropicrin in a 10^{-2} M solution had decomposed. Decomposition

was slower when the system was exposed to sunlight wavelengths, and "almost no hydrolysis" was detected under conditions of ambient laboratory light, or in darkness.

Since the environment for chloropicrin in a treated timber would be largely protected from light, no photolysis, and little photohydrolysis would be expected to occur except perhaps in the outer 2.5 mm of wood exposed to sunlight (7). Over a period of years however, small amounts of phosgene (Cl_2CO) and nitrosyl chloride (NOCl), and their photolysis and hydrolysis products NO, NO_2, N_2O_4, Cl_2, and H^+ would possibly accumulate if sufficient moisture and ambient light were present. It is possible that the rate of the hydrolysis reaction may be increased if the acidity is lower than pH 7, as occurs in Douglas-fir heartwood.

One of the principle products of the photolysis and photohydrolysis reactions, phosgene, is a highly toxic gas. Phosgene will undergo a strong nucleophyllic substitution reaction with alcohols producing transition chlorocarbonates and yielding alkyl carbonates (28). It is possible that phosgene could react with the free hydroxyl sites of wood to form covalent bridges between various wood components, which may confer some resistance to fungal attack. The predominant location for this reaction (if it occurs) would be in the outer shell of wood exposed to sunlight; however, even if limited to this site it may provide a thin protective barrier to protect against biological invasion and degradation.

Hydrolysis of chloropicrin has also been investigated by researchers (33) working with wine products where chloropicrin was used to sterilize wine barrels. The degradation products $ClCH_2NO_2$ and

Cl_2CHNO_2 were isolated from wine, both of which had low toxic and sterilizing potential. Neither of these products had been reported in previous literature concerning hydrolysis of chloropicrin.

Biological breakdown of chloropicrin by microorganisms has recently been studied by Castro (personal communication, C. Castro, 2/10/83). His preliminary findings are that a bacterium, Pseudomonas sp. is capable of degrading chloropicrin. This may have implications in wood treatments with chloropicrin since species of Pseudomonas have been isolated from wood products (30, 24) some of which may have the ability to chemically degrade creosote.

Other Chemical Reactions of Chloropicrin

Chloropicrin is very susceptible to nucleophyllic attack by weak bases (9) and produces ortho esters (14), carbonate esters, Cl, NO_2 and other products when reacted with alkoxides. Although heartwood is generally acidic, localized components may act as weak bases to participate in the reaction, and water within wood may also act as a weak base to produce small amounts of the breakdown products.

Fungal Attack of Chloropicrin-Treated Wood

The attack of wood by brown rot and white rot fungi has been well documented by microscopic studies (45, 25). In general, these studies show that white rot fungi produce a progressive thinning of the secondary cell wall, produce radial cracks or checks in this wall in early stages of decay, and may cause separation to occur within the wall structure or in the adjacent middle lamellae. In brown rot degraded wood, no progressive thinning has been observed and the wall retains

its original sound dimensions during early decay stages; collapse of the wall caused by uniform degradation throughout the wall will occur in later decay stages. The attack of the cell wall in brown rots is likely to be irregular even within single tracheids. Cracking or checking of the secondary cell wall also is apparent in later decay stages. Bore holes are produced by both types of fungi; however, bore hole production has been observed to occur more slowly and with smaller holes in white rot decay than in brown rot decay (42).

There is less information concerning the attack of preservative treated wood by these Basidiomycetes. Bravery et al. (6) studied the inhibition of attack of Polyporous versicolor in tri-n-butyl tin oxide treated wood. The hyphae of this fungus were able to colonize wood treated with low retentions of the chemical and to start enzymatic dissolution of the cell walls. Inhibition of the fungal action and lysis of the hyphae (denatured, collapsed cytoplasm) would occur after degradation had freed enough of the bound chemical from the wood cell walls. With increasing exposure to the decay fungus the preservative deposit in the walls was stripped away and fungal attack proceeded as in untreated wood.

Wilcox et al. (46) studied the action of brown rot fungi in wood treated with low levels of pentachlorophenol (PCP). The fungi were able to degrade PCP treated cell walls similarly to those of untreated wood, up to retention levels of 3%; however, the authors indicate that less shrinkage may have occurred in the decayed PCP treated wood. Their data suggest that enzymatic degradation, to release the cell wall bound PCP, is necessary before fungal action is inhibited; but, they conclude that extracellular enzyme inhibition may also occur.

Schmidt et al. (35) have studied the germination of brown and white rot basidiospores on woods treated with low concentrations of chromated copper arsenate (CCA) and PCP. They found that germination was inhibited at lower concentrations of the chemicals than that which inhibits mycelial growth. Ungerminated spores on treated wood had degenerated or had become highly vacuolated. Where spores had germinated (at the lowest treatment concentrations used in the study--0.0001% W/W) the germ tubes were much shorter in the treated woods than on control samples.

In nucleophilic substitution reactions, chloropicrin can be attacked by any group with an unshared pair of electrons. Such groups might include the SH, OH, NH_2, NH, COOH, aromatic OH and NH_2 contained in all living organisms (8). Chloropicrin reacts with free thiol groups, oxidizing them to disulfide groups, and thus should react with proteins containing these groups and inhibit enzyme systems whose activity depends on the presence of free-SH groups (13). It can be concluded from this information that chloropicrin would act as a multisite inhibitor of cellular fungal activity, and would also interfere with the reactions of some extracellular enzymes. If a sufficient amount of free chloropicrin is retained by the wood it is likely that fungal growth will be inhibited before extensive colonization and enzymatic degradation can occur. Breakdown of chloropicrin may also occur in wood, and if so, some of the chloronated breakdown products, notably phosgene, if present in sufficient quantity should also inhibit fungal growth.

If appreciable amounts of chloropicrin do break down in wood and these products covalently link to the wood structure, then fungal

degradation of the wood cell wall may have to proceed to release the fungicidal moiety before inhibition of fungal growth occurs. However, at present it is not known if these types of reactions occur in wood, and if they do, if the products of these reactions are fungitoxic.

PROCEDURES

Technology is not yet available to perform a detailed chemical analysis of the individual chemical components in intact wood. In addition, many of the techniques and analytical instruments that I used in the research were designed only for elemental analysis, and I was frequently unable to distinguish between intact chloropicrin and chlorinated breakdown residues from chloropicrin. Despite these limitations, this work does explore the problem of chloropicrin residue retention and distribution in wood, in greater detail than previously reported and should provide a base for further research in this area.

I. Sorbtion

A. Dimensional Change Occurring in Wood Treated With Chloropicrin

Twelve Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] blocks, 10 x 10 x 150 mm long were exposed to Poria placenta for 2 to 18 weeks in modified soil block chambers (Appendix I; personal communication, T. C. Scheffer, 1982). After equilibrating the decayed blocks to about 10% moisture content, weight loss values were calculated. The blocks were grouped into three weight loss classes designated as: low, 1-8% weight loss; moderate, 8-16% weight loss; and high 16-30% weight loss. Three blocks from each group were placed in a dessicator containing a saturated atmosphere of chloropicrin; the remaining blocks were left untreated as controls. Periodically, over a 28 day period, the blocks were removed from the dessicator and measured across their tangential face to determine dimensional changes. The

dimensional change of the control blocks was also measured and the percentage change of the controls was subtracted from that of the treated blocks. An analysis of variance test was used to determine if significant dimensional change had occurred due to the adsorbtion of chloropicrin.

B. Non-Steady-State Desorbtion and Residue Analysis

Cooper (10) reported on the non-steady state diffusion of chloropicrin from wood. The purpose of my work in this study was to extend the test period for desorbtion beyond that indicated in Cooper's work in an attempt to determine if total desorbtion could be achieved under ambient laboratory conditions in wafers that were about one tracheid length in thickness.

Douglas-fir wafers, 45 x 90 x 5 mm thick were cut to expose the end grain along the broadest surface. Four sets of ten wafers were equilibrated in an Aminco[1] controlled environment chamber set at 22°C and 50% R.H. The air flow rate in the chamber was 4.2 m^3/minute. Following equilibration, two sets of wafers (designated set A_t and B_t) were placed above a liquid reservoir of chloropicrin and sealed in a dessicator within the Aminco chamber. The remaining two sets of wafers (control sets designated A_c and B_c) were sealed in a dessicator containing no chloropicrin. Weights of the wafer sets were taken periodically by briefly removing them from the dessicators and weighing on a balance sealed within the Aminco chamber. After 5,767 hours the treated wafers were removed from the dessicators and allowed

[1]Aminco-Aire. American Instrument Company. Silver Spring, MD.

to desorb chloropicrin. The control wafers were removed from their dessicator 267 hours after the treated wafers when the amount of chloropicrin being desorbed was low enough that accumulation within the chamber atmosphere would not appreciably affect the weight of the control wafers. Desorbtion was continued for 3,191 hours with periodic weighings of each wafer set. The percent weight change of the control wafers, due to moisture fluctuations, was subtracted from the treated wafers and the percent weight change of these wafers calculated based on their original untreated weight.

Following the desorbtion weighings, one control and one treated wafer set (sets A) were placed in a 115°C oven for 5 hours. The other two wafer sets (sets B) were extracted with acetone (3-1 hour changes, under 150 torr vacuum). All wafers were then reequilibrated to 22°C and 50% relative humidity and their weights taken for determination of residue retention in the treated wafers.

Samples of the acetone extracts were analyzed by thin layer chromatography on silica gel plates sequentially developed in 96:4, chloroform/methanol, and 50:40:10, chloroform/ethyl acetate/formic acid solvent systems. Chlorinated residues on the TLC plates were detected with a silver nitrate bromophenol-blue spray reagent (40).

II. Neutron Activation Analysis

Methodology - All analyses were carried out in a TRIGA[2] atomic research reactor located on the Oregon State University campus. Eight

[2]TRIGA Reactor Division, General Atomic Company, P.O. Box 81608, San Diego, CA 92138.

samples could be analyzed along with a standard solution of ammonium chloride in each reactor run. All samples were sealed in high density polyethylene vials and were irradiated in the reactor's rotating rack. Initial experiments were undertaken to determine the appropriate flux, activation time, and decay time needed for analysis of chlorine in 1 g wood samples. The following conditions were found to be suitable for the analysis and were used throughout the NAA experiments:

Reactor power - 100 KW (neutron flux approximately 3×10^{11} $\frac{n}{cm^2 - sec}$)
Activation time - 15 minutes
Decay time - 45 to 160 minutes

Samples were counted on a multichannel pulse height analyzer with a lithium-drifted germanium detector. A low level discriminator was normally used to suppress the 0.845 and 1.81 MeV peaks of ^{56}Mn and other lower energy peaks which may have been present in the spectra, allowing the 2.167 MeV peak of chlorine to be easily analyzed. Live-count time for all samples was 200 seconds. Standard statistical analysis of the data was done on a WANG 500 computer using program 4165 provided by the Radiation Chemistry Laboratory, OSU.[3]

In recent years activation analysis has been used in the field of Forest Products to perform basic elemental analyses of wood (47, 30, 26) and charcoals (12), and for determination of chlorine in spent bleaching pulp liquors (22). A good description of activation analysis and gamma ray spectroscopy may be found in Chapters 7 and 17 in the text of Wang et al. (41).

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[3]R. Schmitt, project leader.

A. Preliminary Analysis of Background Chlorine in Wood

At the time this analysis was initiated (July 1980) accurate analyses for chlorine levels present in Douglas-fir wood were not available. Since that time, however, Cutter et al. (12) have reported finding of 67 ppm Cl in Douglas-fir wood. This amount was an average from an analysis of two replicate samples split from a 1-cm cube of Douglas-fir (personal communication, Bruce E. Cutter, 8/10/80).

The purpose of my analysis was to determine if the amount of naturally occurring chlorine in Douglas-fir wood would interfere with my analysis of chloropicrin contributed residues.

Seven 9 x 9 x 25 mm wood blocks were cut from random locations along a Douglas-fir board which was large enough to provide adequate test specimens for future NAA experiments. The blade of the saw, table and fence were wiped down with water, 95% ethanol, and acetone before cutting, and all samples were handled with clean polyethylene gloves to prevent chlorine contamination of the samples. The samples were sealed in clean polyethylene vials and the analysis was carried out as described in the previous section.

B. Analysis of Treated Samples

1. Sorbtion Samples

Drill shavings from the heat treated or acetone extracted wafers (from part I.B.) were analyzed by NAA as previously described.

2. Non-decayed Wood

Wood blocks - Thirty 9 x 9 x 150 mm long blocks of Douglas-fir, cut with the long axis oriented with the wood grain, were equilibrated at 22°C and 50% R.H. until a moisture content of approximately 11% was attained. One end of each block was sealed with paraffin film and paraffin wax, and a 5 mm diameter, 7 mm deep injection hole was bored into one side, 15 mm from the sealed end of each block. Each hole was then sealed with paraffin film and paraffin wax. Fifty µl of chloropicrin was injected through the film into each hole and the hole then resealed. The samples were placed in polyethylene bags and incubated at ambient laboratory temperatures (21-23°C) within an exhaust hood for 3 weeks. A 25 mm segment was then cut 55 mm from the coated block end, immediately heat sealed in a high density polyethylene vial, and analyzed[4] as previously described.

Following analysis the samples were stored to allow the radioactivity to decay to background levels. All of the samples were then removed from their vials. Ten of the samples were allowed to aerate under ambient conditions within an exhaust hood for 24 hours. The remaining samples were ground in a Wiley mill (washed with water, alcohol, and acetone prior to grinding each sample) through #20 mesh screening and also aerated for 1 day.

The unground samples were sequentially reanalyzed after additional aeration periods of 72 hours and 360 hours. Four of these

[4]Due to difficulty in scheduling reactor time the samples would remain sealed within the vials from one day to two weeks before analysis could be performed.

samples were then evacuated under a 10 torr (29.5 inches of mercury) vacuum for 24 hours and reanalyzed. Two of the samples were minced with a razor blade and aerated for 3 hours, before NAA.

Ground wood - Because of the chlorine contamination of the ground wood reported in the previous section, procedures had to be altered so that contaminant chlorine could be accounted for in the analysis.

Approximately 300 g of Douglas-fir was ground in a Wiley mill (washed as previously described) through a #20-mesh screen. This wood was then thoroughly mixed to distribute contaminant material evenly throughout, and four 0.5 g samples were taken for NAA. The average ppm chlorine in these samples was 36 with a standard deviation of 5.

The remainder of the ground wood was treated with chloropicrin at a rate of 50 µl chloropicrin/g wood and then mixed in a sealed container for one hour. Four 0.5 g samples were then sealed in polyethylene vials for later NAA. The treated ground wood was aerated in an exhaust hood and occasionally mixed. Four replicate samples were then taken at 24 and 1,176 hours, and additional samples were taken after 480 and 816 hours after treatment.

Similarly treated ground wood was sampled immediately after mixing and after 24, 96, 480, and 1176 hours of aeration. Four duplicate samples were analyzed either by neutron activation or by a modification of a closed-tube bioassay (34) using approximately 0.5 g of the ground wood rather than an increment core.

3. Decayed Samples

Weight loss vs. chlorine retention. - Forty eight 9 x 9 x 150 mm long blocks of Douglas-fir were decayed to a range of weight loss from

0-33% in decay chambers--1 liter canning jars containing malt agar--inoculated with Poria carbonica. The blocks were supported above the agar by plastic screening and glass rods in an attempt to limit transfer of soluble material from the agar to the wood. After incubation, blocks were equilibrated to approximately 11% moisture content. Treatment, incubation, and preparation of the blocks for NAA were as described previously in this section. The average chlorine content of four control samples (decayed but not treated) was subtracted from the treated samples to correct for chlorine contributed to the samples by the medium or the fungus. Percent weight loss in each sample was regressed against the total µg of chlorine in each after treatment.

Alkali solubility vs. chlorine retention. - After the radioactivity had decayed to background levels, samples were ground through #20 mesh screening and the percentage of alkali soluble material in each (1)[5] determined. The percentage of alkali soluble material in the four control samples was regressed against their total chlorine content and this regression factor was used to correct for the amount of chlorine present in the treated samples. Percent alkali soluble material in each treated sample was regressed against the corrected µg of chlorine retained.

III. Energy Dispersive X-ray (EDX) Analysis to Determine the Microdistribution of Chlorinated Residues

Three 9 x 9 x 150 mm long Douglas-fir blocks were treated by submerging one end of the blocks in liquid chloropicrin for a 2 week

[5]Procedures suggest using duplicate 2 g samples but because of limited sample quantities, I assayed smaller amounts without replication.

period. The blocks were then razor sectioned to expose transverse surfaces and split to expose the radial surfaces. Immediately after sectioning, the blocks were placed in a high vacuum and coated with gold and palladium for analysis of chlorine by EDX analysis under the scanning electron microscope.

Radially split and razor cross sectioned samples of treated and control wafers from section I.B., and other thin wafers of Douglas-fir heartwood that had been treated in a saturated atmosphere of chloropicrin vapor for 24 months were also prepared and analyzed by EDX analysis. Surfacing of these blocks was done both on dry blocks and on blocks which had been wet with distilled water. Coating was with carbon rather than gold/palladium. Analysis was carried out on a Cambridge Stereo Scan - Mark IIA scanning electron microscope[6] with an EDAX[7] X-ray analyzer.

IV. Infrared and Mass Spectrographic Analysis

A. Analysis of Chloropicrin and Derivitized Wood Components

Derivitives or synthesized components representative of the monomer or polymer units of the cellulosic, lignin, and extractive fractions of wood were mixed with chloropicrin and analyzed to determine if chemical bonding could occur during treatment.

Infrared analysis. - Individual samples (~ 0.25 g) of vanillin, acetyl ferullic acid, (+)-catechin, tannic acid, cellobiose, and solka

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[6]Kent Cambridge Scientific, Inc., Morton Grove, IL.

[7]EDAX International, Inc., Prairieview, IL.

flok were saturated with 250 µl of liquid chloropicrin and then aerated for 5 minutes. Samples of the mixtures (~ 5 mg) were then mixed with potassium bromide and pelletized for infrared analysis. Each sample was again analyzed after approximately 45 and 90 minutes of aeration. Infrared scans of chloropicrin and untreated wood derivatives were also made for comparison.

Mass spectroscopy. - Approximately 5 mg of each of the components listed in the previous section were individually mixed with 2 ml of chloropicrin. Gas chromatographic (GC) analysis of these mixtures indicated that only the vanillin/chloropicrin mixture contained volatile components other than chloropicrin that would elute through the chromatograph column. Therefore, this was the only sample analyzed by mass spectroscopy (MS). Vanillin is a derivative of the lignin molecule in wood, and differs only in that the propyl side chain of the lignin monomer has been replaced by an aldehyde group. Because of chemical similarity between vanillin and the lignin monomer, many chemical reactions that lignin would undergo should be adequately represented by the vanillin molecule. Chromatographic separation of the vanillin/chloropicrin components for the MS analysis was performed with a 10 foot by 2 mm I.D. pyrex column with a 7% OV 101 liquid phase coated on a 100/120 supelcoport support phase. The instrument used for this analysis was a Finnigan model 4023 GC-MS quadrupole mass spectrometer system.[8]

B. Analysis of Desorbed Gases From Chloropicrin Treated Wood

Matched 25 mm long increment core segments from a southern pine laminated timber that had been treated 4 years earlier (18) were

[8]Finnigan Co., San Jose, CA.

sealed in separate vials for a closed-tube assay (34) and for analysis of desorbed vapors by GC/MS. Douglas-fir wafers, 1 mm thick x 25 m long, that had been exposed in a saturated atmosphere of chloropicrin for 7 months and then aerated for 1 to 5 hours were also analyzed similarly. Mass spectroscope conditions were the same as those cited in the previous section.

V. Microscopy of Treated Wood Samples Infected With Decay Fungi

Douglas-fir wafers 1 x 10 x 130 mm long were exposed to a saturated atmosphere of chloropicrin for 35 weeks. Five wafers were then aerated for 1 hour and were immersed for 15 minutes in sterile water. The treated wafers and 5 untreated, sterilized, control wafers were then individually implanted in 2 week old malt agar cultures of Ganoderma applanatum (Pers. ex S.F. Gray) Pat., Madison isolate FP-57035-5 contained in 25 x 185 mm culture tubes. Tubes were loosely sealed with cotton plugs and incubated at 26°C. All cultures containing treated wafers were killed, and after 50 days these wafers were reimplanted in fresh cultures. Wafers were observed periodically under the light and electron microscopes to compare differences in fungal attack of treated and control wafers (see Appendix 1 for sample preparation for microscopy). The presence and type of bore holes produced by the fungi and erosion of the lumen surface were the primary characteristics used to compare differences in attack. Observations of the hyphae were also made to determine if treatment may have effected morphological changes.

Similar experiments were also performed with the fungus Poria carbonica Overh., Madison isolate FP-105585.

RESULTS AND DISCUSSION

I. Sorbtion

A. Dimensional Change Occurring in Wood Treated with Chloropicrin

No significant dimensional change (F statistic, $\alpha = .05$) in the blocks treated with chloropicrin was indicated by the analysis of variance. The lack of swelling suggests that if chloropicrin does hydrogen bond to wood, or to water molecules sorbed to the wood, the bonding sites are not located where the added bulk of the chloropicrin molecule would force the wood polymer chains to separate to any significant extent. Dipole attraction between chloropicrin molecules may provide a means for retention of the chemical in void spaces, but this attraction is not strong enough to act as a bulking agent and cause swelling by overcoming the attractive forces of wood.

B. Non-Steady-State Desorbtion and Residue Analysis

The percent weight change of the chloropicrin treated wafers (sets A and B) during the adsorption and desorbtion of the wafers is plotted in Figures 1 and 2, respectively. With both wafer sets a hysteresis-type curve was produced; however, the desorption curves--if extrapolated-- would appear to stabilize at weights approximately 1 to 1.5 percent greater than the original weights of the samples. This suggests that residual chloropicrin, in some form, may be retained in wood for relatively long periods of time before total desorbtion (if this occurs) is achieved.

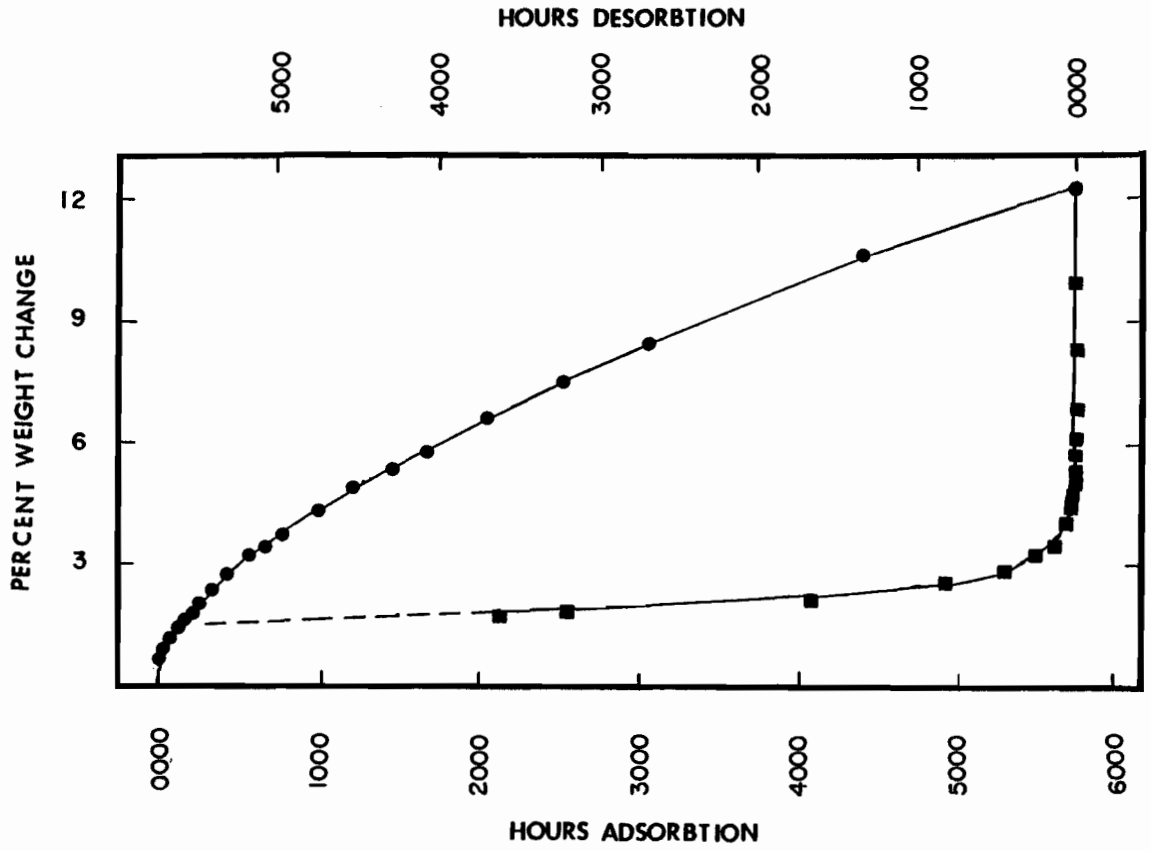

Figure 1. Sorbtion (-●-) and desorbtion (-■-) of chloropicrin from Douglas-fir wafers (A) over time.

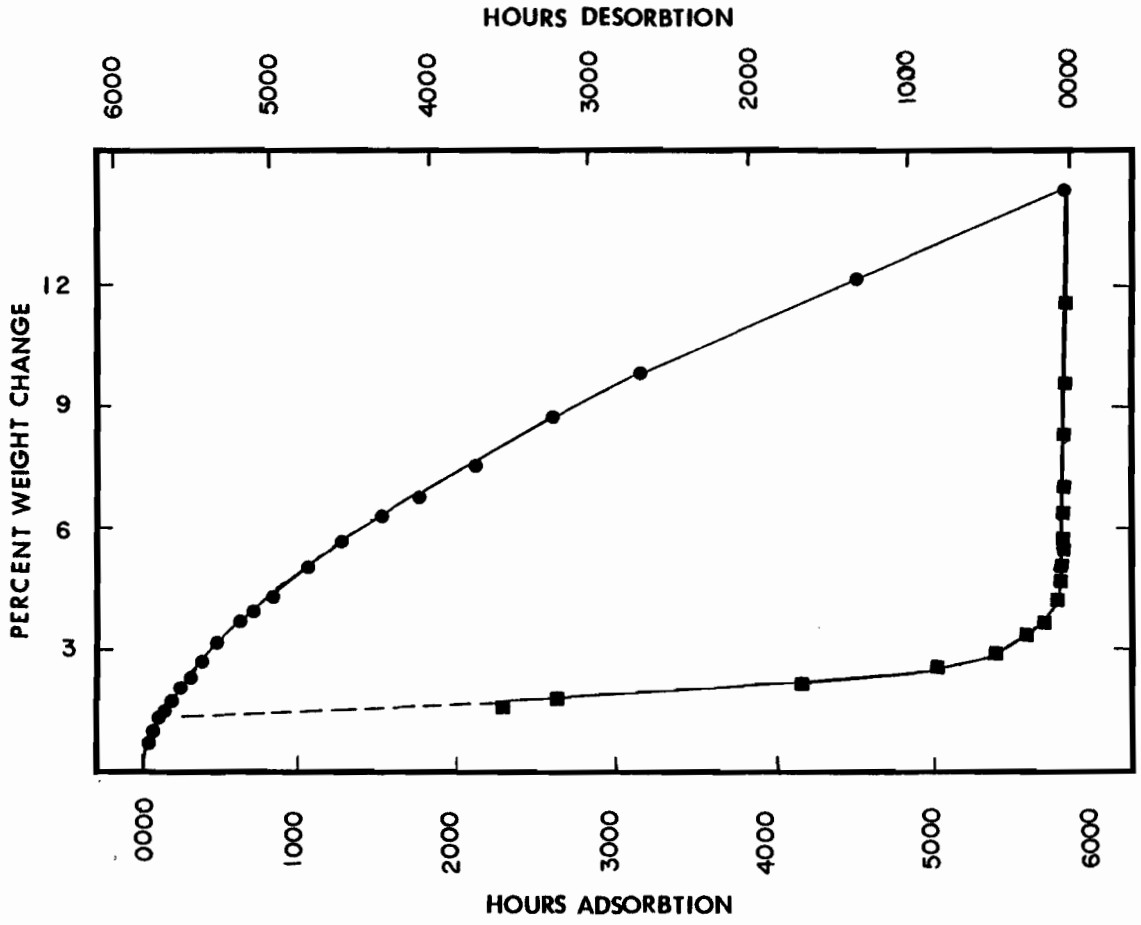

Figure 2. Sorbtion (-●-) and desorbtion (-■-) of chloropicrin from Douglas-fir wafers (B) over time.

Treated and control wafers that were heated to 115°C and then reequilibrated to 22°C and 50% relative humidity lost 2.68% and 4.38% of their original untreated weights, respectively, indicating that some residue resulting from the treatment still remained in the wood. Treated and control wafers that were acetone extracted and then reequilibrated lost 0.87% and 0.34% of their original weight, respectively, showing that somewhat more material was removed from the treated wafers than the controls. This finding was also supported by the darker color of the acetone extracts from the treated wood and suggests that chloropicrin treatment could possibly be solubilizing some fraction of the wood, allowing it to be more easily extracted.

TLC of the acetone extracts from the wafers in the chloroform/methanol solvent showed that two substances (R_f values of 0.10 and 0.44) eluted from the control extract whereas only one substance (R_f value of 0.10) eluted from the treated extract. The eluted materials were not identified but both could be distinguished from dihydroquercetin (an extractive commonly isolated from Douglas-fir wood), which did not move in this solvent system. When the TLC plates were developed in the second solvent system (chloroform/ethyl acetate/formic acid) a substance having the same R_f value as dihydroquercetin was eluted from the control but only a faint trace was eluted from the treated extract. The chlorine spray detector showed that none of the eluted materials were chlorinated; however, chlorine was indicated at the baseline of the treated extract chromatogram and to a lesser extent at the baseline of the control chromatogram. The data suggest that chloropicrin may be binding to one of the unidentified extractives eluted from the acetone extract of the control

sample (R_f value of 0.44 when developed in chloroform/methanol) and probably to a large portion of the dihydroquercetin in the wood, inhibiting these extractives' mobilities in the solvent systems tried on the TLC plates.

II. Neutron Activation Studies to Determine the Amount of Chlorinated Residues Retained by Wood After Treatment

A. Preliminary Analysis for Background Chlorine in Wood

The average background ppm chlorine in my untreated Douglas-fir samples was 2.14 with a standard deviation of 0.8. This was low enough so that it would not interfere with later analyses.

B. Analysis of Treated Wood Samples

1. Sorbtion samples

The amount of chlorinated residue remaining in the chloropicrin treated and control wafers after extraction with acetone or heating to 115°C for 5 hours is shown in Table 1. The amount of chlorinated material in the control samples was slightly higher than that obtained in the untreated samples from section II.A., possibly because of chlorine contamination on the drill bit used for sampling. However, this amount of chlorine is much smaller than the approximate 1% w/w (9355 ppm) of chlorine residue in the aerated/heat treated samples or the 0.5% w/w (4950 ppm) chlorine residue in the aerated/extracted treated samples (Table 1). The data from the heat treated samples confirms that of the weight measurements of the samples (section I.B.) in showing that a small residue is present, but further indicates that

Table 1. Chlorine content of control and chloropicrin treated wafers which were aerated and heated to 115°C for 5 hours or extracted with acetone.

	PPM chlorine	
	Treated	Control
Heated to 115°C	9355 ± 106	22 ± 3
Extracted with acetone	4950 ± 126	21 ± 2

this residue is directly contributed to the wood by chloropicrin or chlorinated breakdown residues of chloropicrin. Analysis of the acetone extracted samples indicates that a significant amount of these residues also re-mains in the wood which should have produced a net gain in weight of the treated samples. As a net loss in weight was observed in the treated samples this offers further evidence that chloropicrin may be altering some component in the wood, making it more soluble in acetone.

2. Non-decayed wood

Wood blocks and ground wood.--The data in Tables 2 and 3 show that amounts of chlorine in excess of 50 ppm can remain in chloropicrin treated wood after aeration and vacuum treatment. The smaller amounts retained in these samples compared to the sorbtion samples (Table 1) reflects the different method of treatment; a single small treatment and short incubation as opposed to constant exposure to the chemical over a relatively long period. Zakharenko et al. (48) determined that chloropicrin residues were retained in greater amounts after aeration of treated grain if the grain were exposed to longer fumigation periods. This suggests that the differences in residue retentions in my samples may be caused by the length of exposure to chloropicrin as well as its vapor concentration. The data do suggest, however, that even when small dosages are applied, a persistent chloronated residue can remain in the wood long after treatment.

The slow desorbtion of chloropicrin from treated wood is also demonstrated in the closed-tube assay experiment conducted over a 1176 hour desorbtion period. If it is assumed that all the chlorinated

TABLE 2. Chlorine content of wood samples initially injected with 50 µl of chloropicrin.

Sample #	No aeration ppm Cl	24 Hour aeration ppm Cl	72 Hour aeration ppm Cl	360 Hour aeration ppm Cl	Evacuated samples ppm Cl
1	116 ± 4	98 ± 9	110 ± 4	105 ± 4	89 ± 8
2	146 ± 5	121 ± 8	118 ± 5	119 ± 6	129 ± 10
3	147 ± 5	99 ± 8	128 ± 5	105 ± 6	121 ± 10
4	160 ± 5	125 ± 9	145 ± 6	120 ± 5	127 ± 10
5	250 ± 8	174 ± 6	240 ± 9	203 ± 7	
6	603 ± 16	498 ± 14	532 ± 16	569 ± 17	
7	154 ± 7	130 ± 6	134 ± 7	143 ± 6	Minced samples
8	211 ± 8	196 ± 7	211 ± 10	160 ± 6	
9	289 ± 8	295 ± 10	287 ± 8	250 ± 7	270 ± 16
10	95 ± 4	79 ± 5	91 ± 4	85 ± 4	93 ± 6
11	86 ± 3	364 ± 26			
12	346 ± 9	458 ± 29			
13	75 ± 4	158 ± 16			
14	122 ± 5	218 ± 21			
15	142 ± 6	229 ± 11			
16	569 ± 16	280 ± 15			
17	119 ± 4				
18	98 ± 4	Due to excess chlorine			
19	195 ± 6	introduced by grinding			
20	103 ± 5	these samples could			
21	174 ± 6	not be used and were			
22	545 ± 15	not analysed			
23	242 ± 9				
24	224 ± 9				
25	117 ± 4				
26	52 ± 3				
27	137 ± 5				
28	171 ± 6				
29	85 ± 4				
30	216 ± 9				

TABLE 3. Chlorine content of ground wood treated with chloropicrin, then aerated for various periods of time.

Chlorine content of treated and untreated wood (ppm Cl)						
Untreated control	Treated wood					
	No aeration	24 Hrs. aeration	72 Hrs. aeration	480 Hrs. aeration	816 Hrs. aeration	1176 Hrs. aeration
36 ± 3	14,800 ± 1,900	79 ± 3	77 ± 4	64 ± 2	73 ± 3	57 ± 3
27 ± 3	7,800 ± 1,000	85 ± 4	88 ± 5	76 ± 2	74 ± 3	67 ± 4
37 ± 3	7,450 ± 1,000	84 ± 4	84 ± 5	72 ± 3	--	70 ± 4
44 ± 4	7,350 ± 1,000	83 ± 4	75 ± 5	--	--	72 ± 3

material volatilized from the wood is in the form of chloropicrin, in the last sampling period of 480 to 1176 hours (Table 4) an average of approximately 1 ppm of chloropicrin was desorbed from the 0.5 g samples into the 6 ml void space of the closed-tubes. If the desorbtion rate over this period was constant, a concentration of 0.003 µg/ml of chloropicrin in the first 24 hours of incubation would have been present in the closed tubes. This concentration is apparently capable of inhibiting the growth of P. carbonica but it is very low compared to the 0.06 µg/ml minimum lethal dosages of chloropicrin determined previously for other decay fungi over a 24 hour period (17). This assay was not continued long enough to determine if a chlorinated residue would be left in the wood when desorbtion was low enough to allow growth of the fungus in the closed-tube assay.

3. Decayed samples

The amount of chlorine (3 moles per mole of chloropicrin) retained in decayed and nondecayed blocks treated with chloropicrin is regressed against weight loss of the samples in Figure 3, and against the percentage of alkali soluble material in Figure 4. A weak negative correlation (R^2 = -0.56) exists in the regression against weight loss indicating that with increasing decay, less chlorine is retained by the samples. A similar and somewhat stronger correlation (R^2 = -0.62) is apparent in the regression against percentage of alkali soluble material.

The three data points in these plots that appear to skew the regressions (> 100 µg chlorine) are from non-decayed samples that were analyzed in separate reactor runs from the decayed samples.

TABLE 4. Comparison of the chloropicrin content of treated ground wood after aeration with closed-tube assay data of the same wood.

	% Growth[1]		ppm Chloropicrin in samples
	Not activated	Activated[2]	
No aeration	0	0	79-528
24 hour aeration	0	0	18-21
96 hour aeration	0	2	13-19
480 hour aeration	0	--	10-13
1176 hour aeration	0	--	8-13

[1]% growth calculated by dividing the total mm of growth in the 4 treated tubes by that in 4 control tubes containing either activated or unactivated, untreated ground wood.

[2]Activated samples analyzed by neutron activation analysis prior to closed-tube analysis.

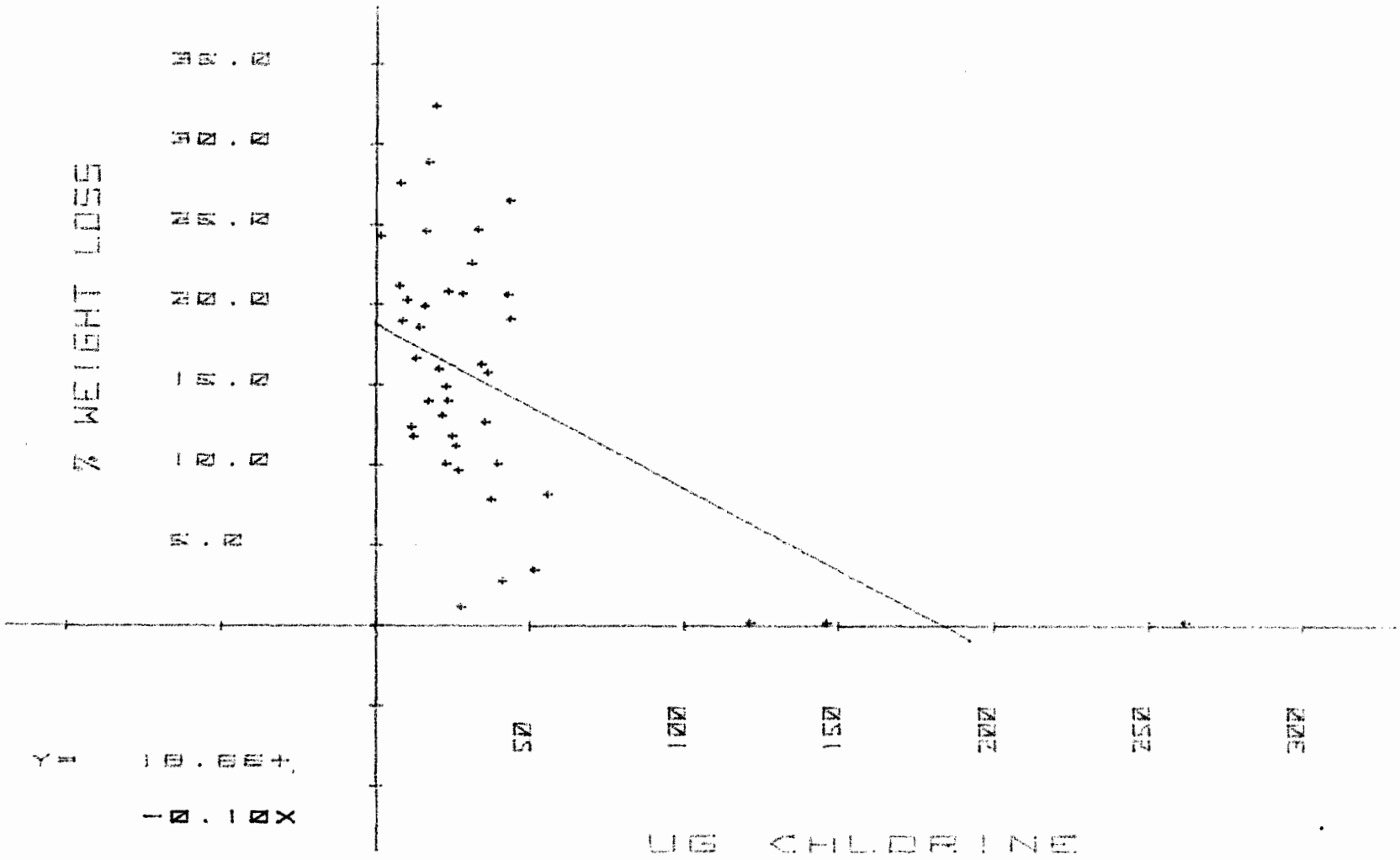

Figure 3. Chlorine retention in samples decayed to a range of weight losses by Poria carbonica prior to treatment with chloropicrin.

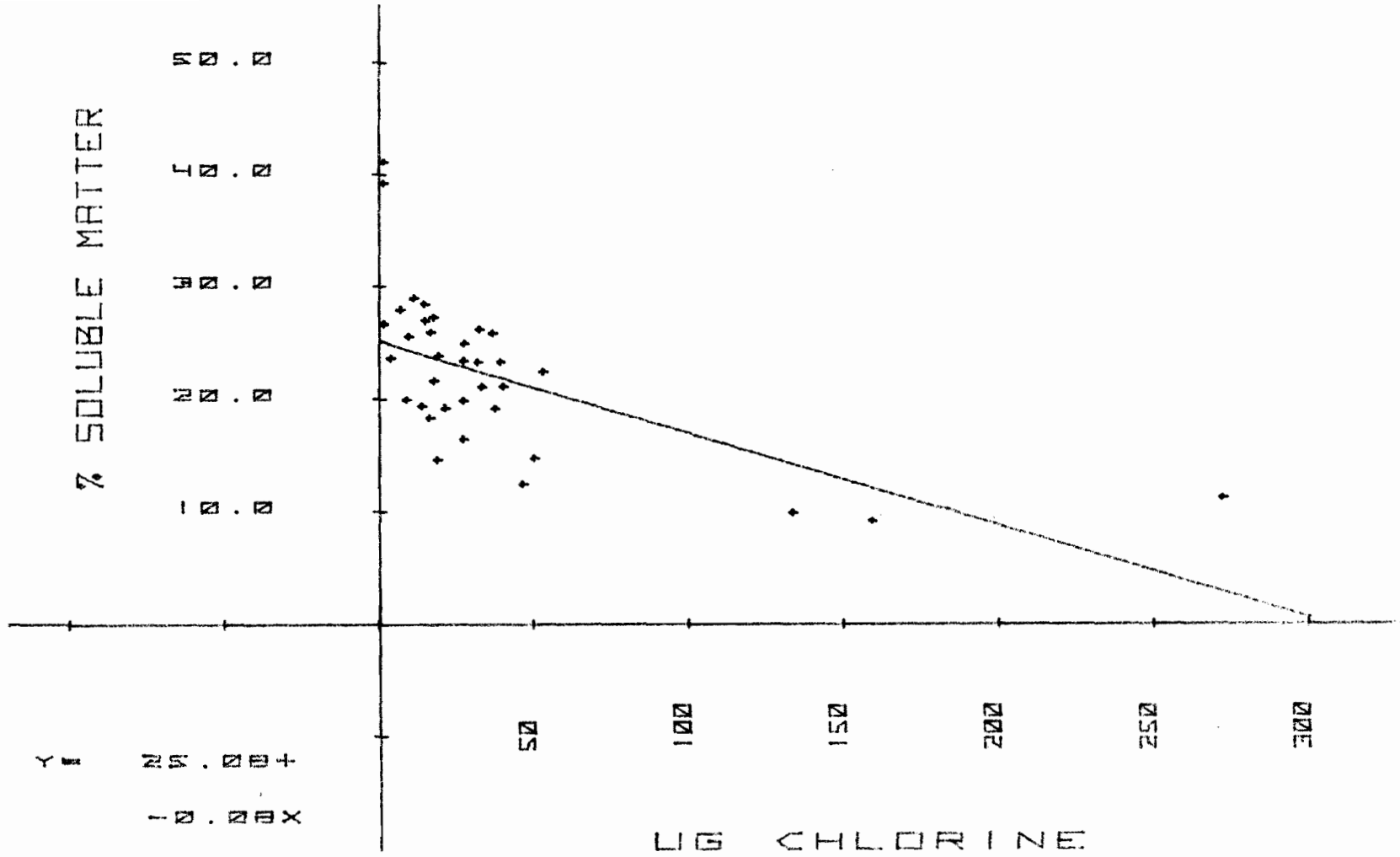

Figure 4. Chlorine retention in samples decayed by Poria carbonica prior to treatment with chloropicrin. Amount of decay in the samples determined by the percentage of alkali soluble material.

Regressing the amount of chlorine against the percent alkali soluble matter in the samples without these data points (Figure 5) yields a significant regression (F-statistic = 14.7, F-significant α.05 = 4.2) with an R^2 value of -0.57.

These data show a trend that generally conforms to the findings of researchers studying water sorbtion in brown rotted wood (11, 36), and suggest that hydrogen bonds may be important in retaining chemically unaltered chloropicrin in wood. However, the data could also be interpreted to support the hypothesis that brown rot fungi alter, or selectively remove some wood component to which chloropicrin or its breakdown products may chemically link.

Both weight loss and alkali solubility were used as measures of the amount of fungal degradation present in the samples. Less chlorinated residues were retained in samples with greater amounts of decay; however, a stronger correlation between decay and chlorine retention was apparent when the alkali solubility test was used to estimate decay. The alkali solubility data were generated from the same samples analyzed for chlorine content; these samples were cut from the larger blocks used for determining weight loss. Since the amount of decay can vary considerably within a sample of decayed wood, alkali solubility data should give a more accurate measure of decay in the samples analyzed for chlorine content in this study than the weight loss data.

III. Energy Dispersive X-ray (EDX) Analysis to Determine the Microdistribution of Chlorinated Residues

Representative micrographs and EDX spectra of the wood blocks treated with liquid chloropicrin for 2 weeks (Figures 6 and 7) show

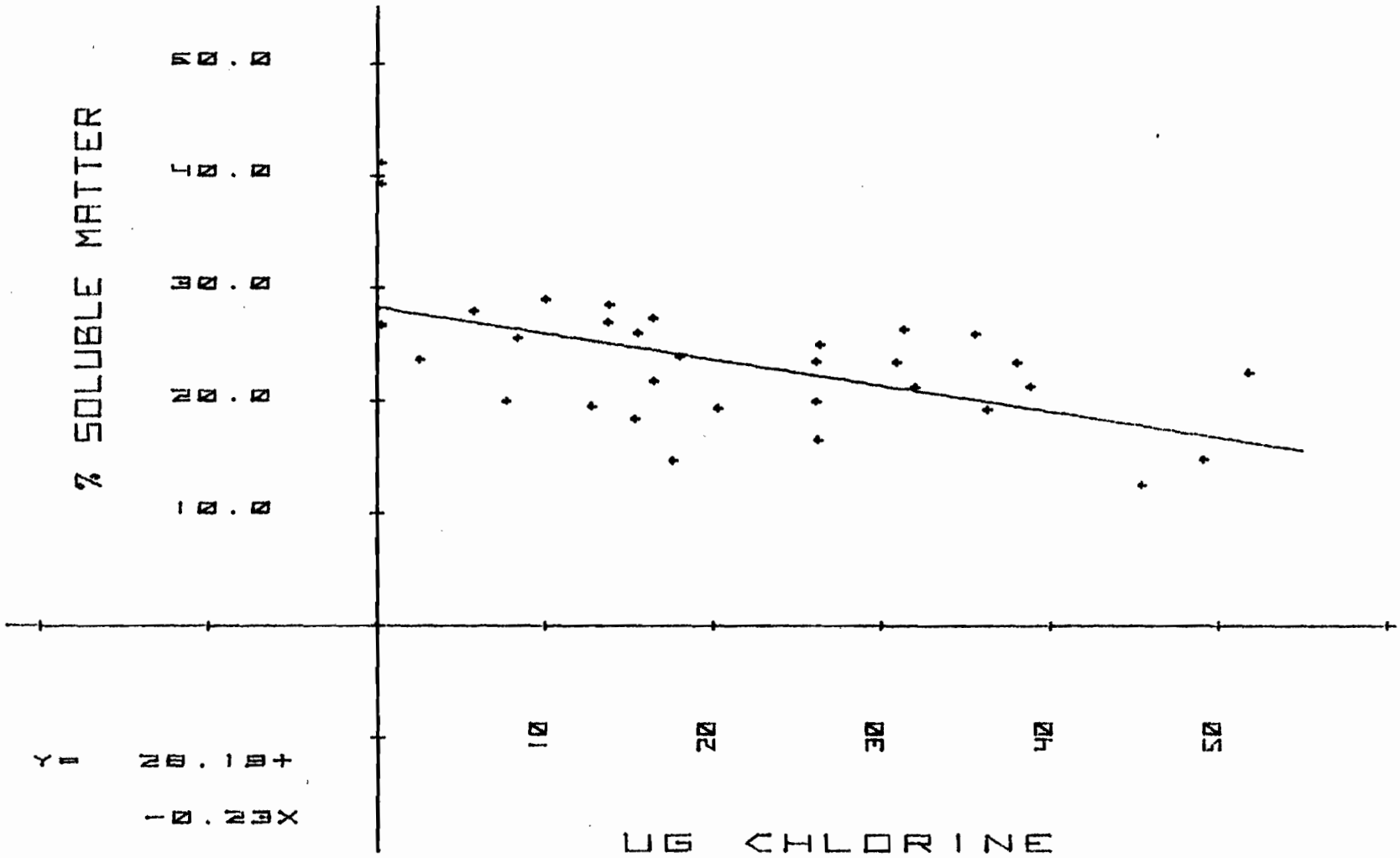

Figure 5. Same regression as shown in Figure 4 minus three data points (> 100 µg chlorine).

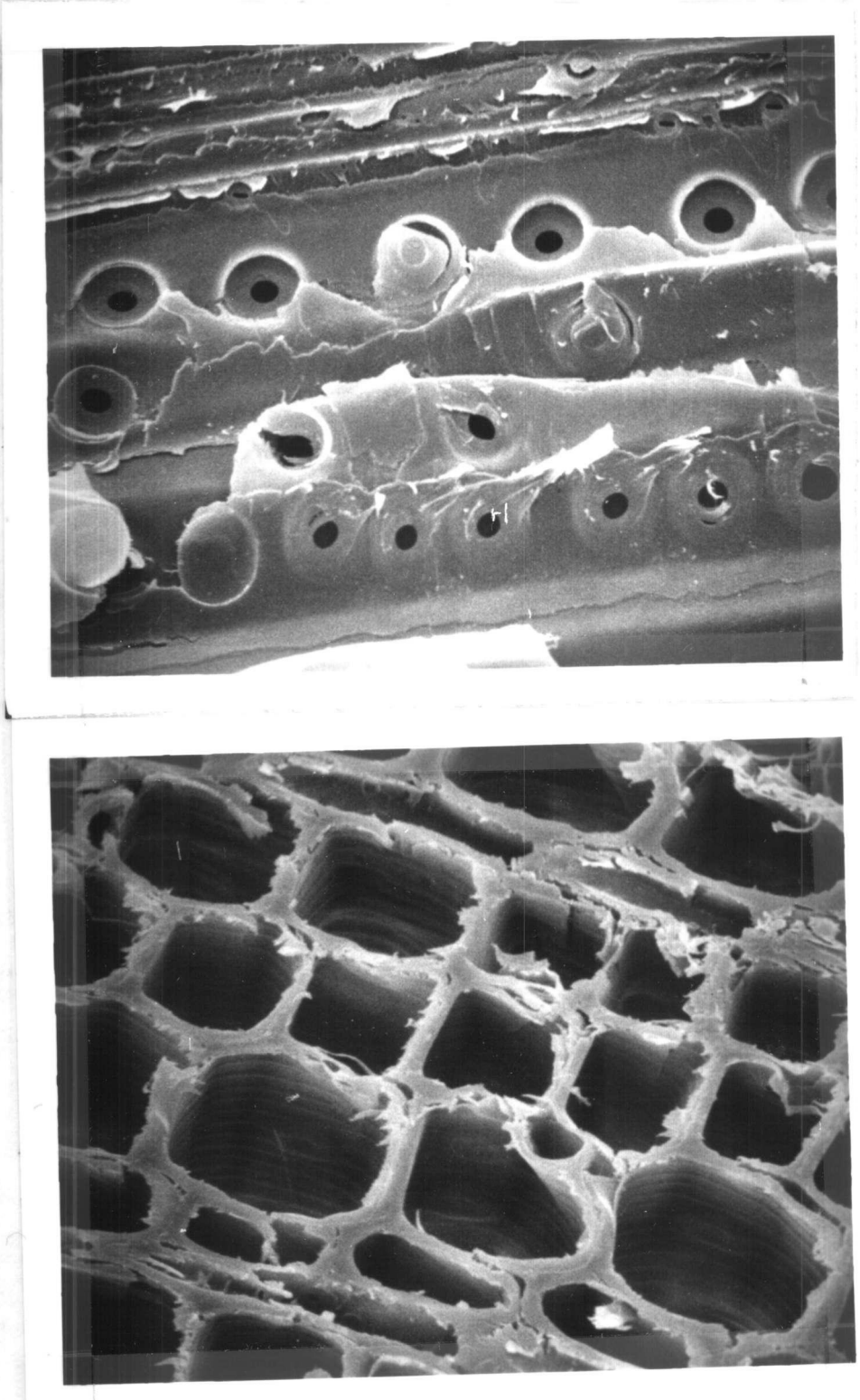

Figure 6. Scanning electron micrographs of Douglas-fir heartwood treated for two weeks with liquid chloropicrin. Above, split radial surface, 500X; below, razored cross section, 500X.

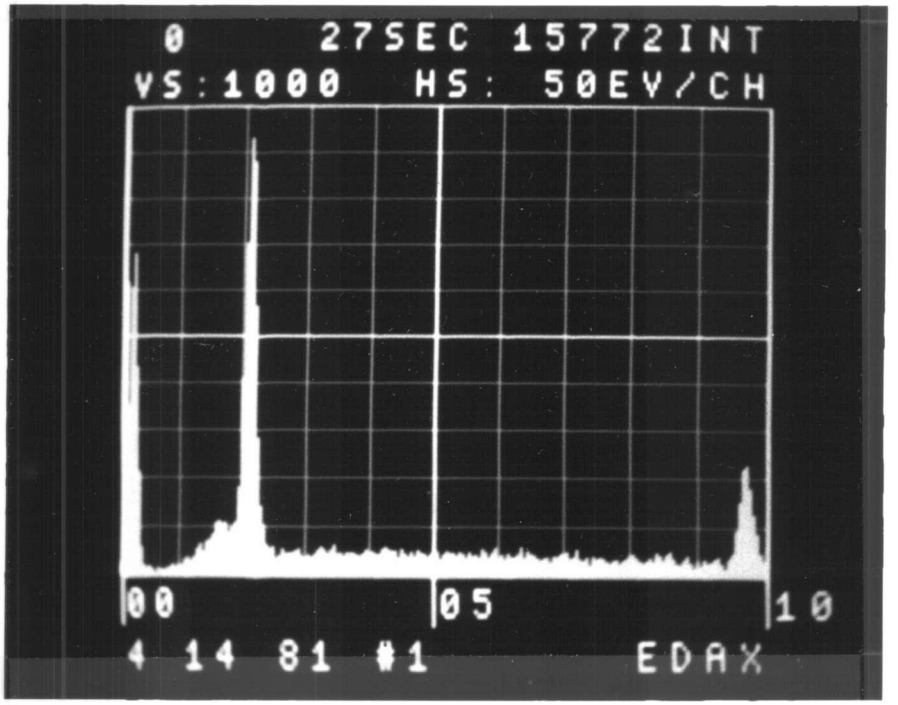

Figure 7. Typical energy dispersive X-ray analysis scan across chloropicrin-treated wood showing the absence of chlorinated material in the sample. Peaks for gold and palladium from the coating material are present in the scan.

that any chlorine present in the samples was below the detection limit of the instrumentation. EDX spectral scans included analyses of both lumen surfaces and interior cell wall material.

Spectral scans for chlorine in wood samples taken from untreated (control wafers) that had been acetone extracted or heated (section I.B.) showed that no detectable chlorine was present (Figure 8). This was confirmed by a line scan across one cell wall in a heated, control wafer (Figure 9) which showed only the presence of background chlorine. Spectral scans for chlorine in extracted or heated, chloropicrin-treated wafers (Figure 10) showed that a significant amount of chlorine was present in the samples confirming NAA data (Table 1). Line scans along the cross sectional surface of treated samples that were sectioned dry (Figure 11) and after wetting (Figure 12) show the presence of chlorine in the cell walls. Since wet sectioned wood produced a better surface for microscopy and this method did not appear to affect retention of chlorine residues in the wood, it was used for preparing the remainder of the treated samples. Scans across cell walls of treated, extracted or heated samples (Figure 13) showed that the entire cell wall contained detectable amounts of chlorinated materials but that concentration of chlorine tended to be greater in the middle lamella region. Since the middle lamella region is composed mostly of lignin, the data suggest that chloropicrin residues may be binding preferentially to lignin materials.

Line scans across ray tissue in chloropicrin treated, extracted (Figures 14 and 15) and heated (Figure 16) samples were also made. All scans indicate that greater amounts of chlorinated materials may be present in the ray tissues. A spectral scan through a section of

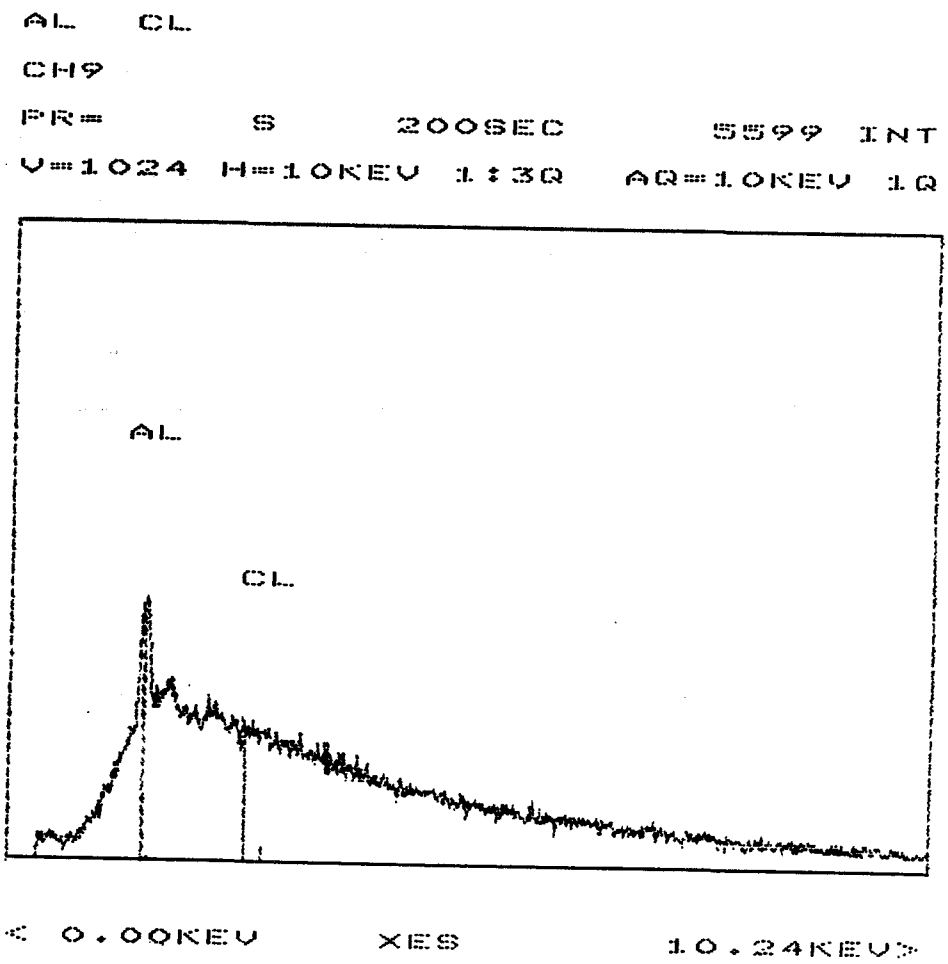

Figure 8. Representative energy dispersive X-ray spectral scan across acetone extracted or heated control wafers. No detectable chlorine peak is present.

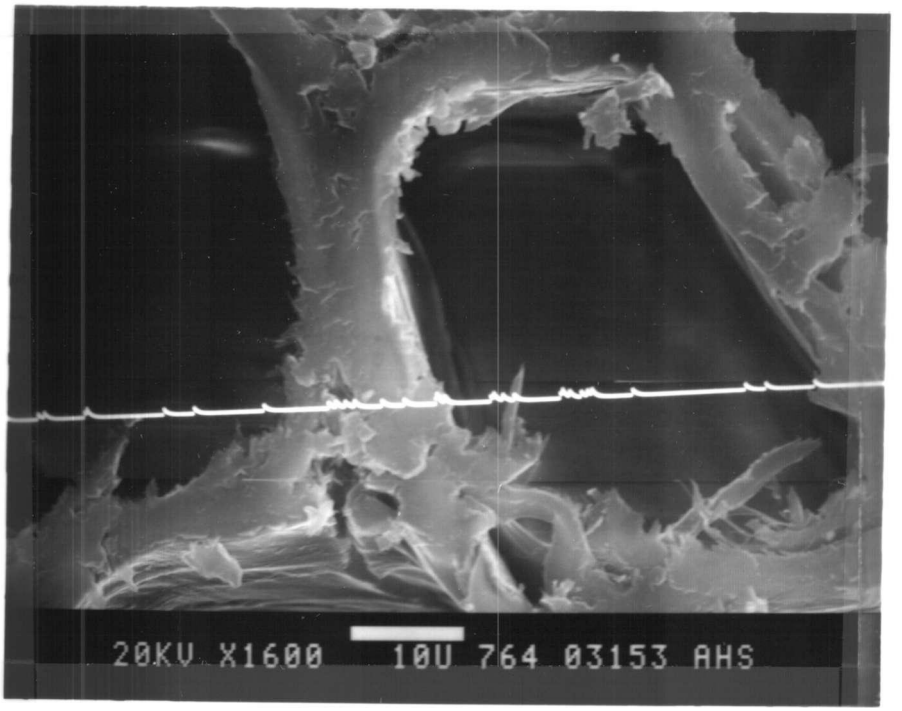

Figure 9. Energy dispersive X-ray analysis line scan across one cell wall of an untreated, control wafer. No detectable amounts of chlorine are present.

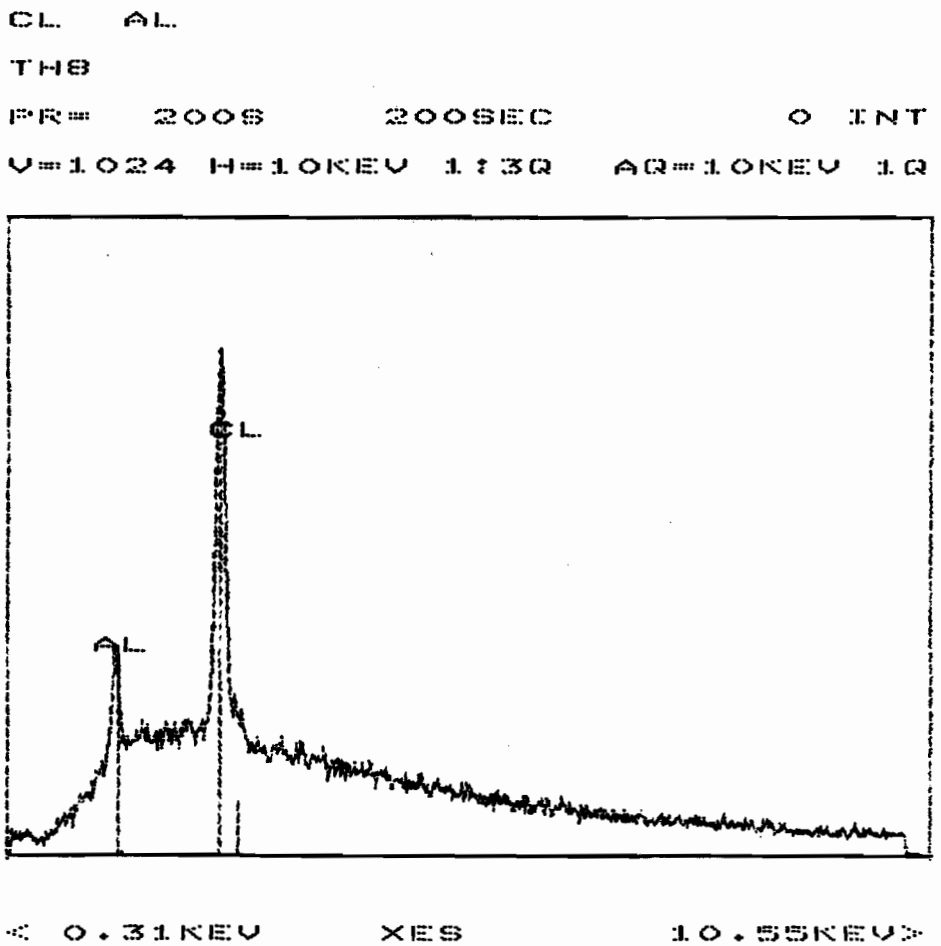

Figure 10. Representative energy dispersive X-ray spectral scan across extracted or heated, chloropicrin-treated wafers.

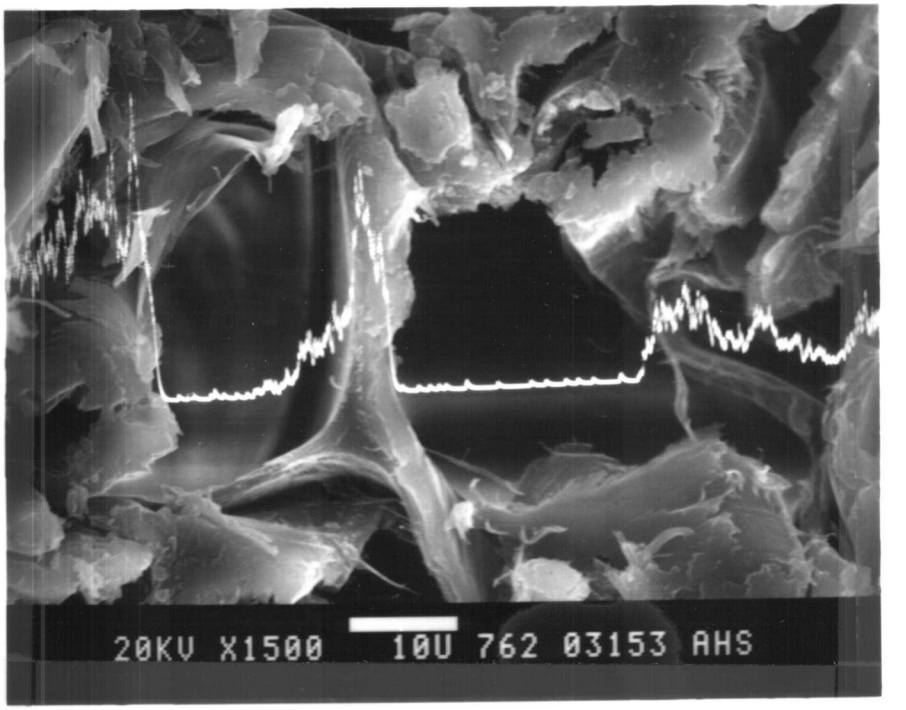

Figure 11. Energy dispersive X-ray analysis line scan across a cell wall of a heated, chloropicrin-treated sample that was sectioned dry.

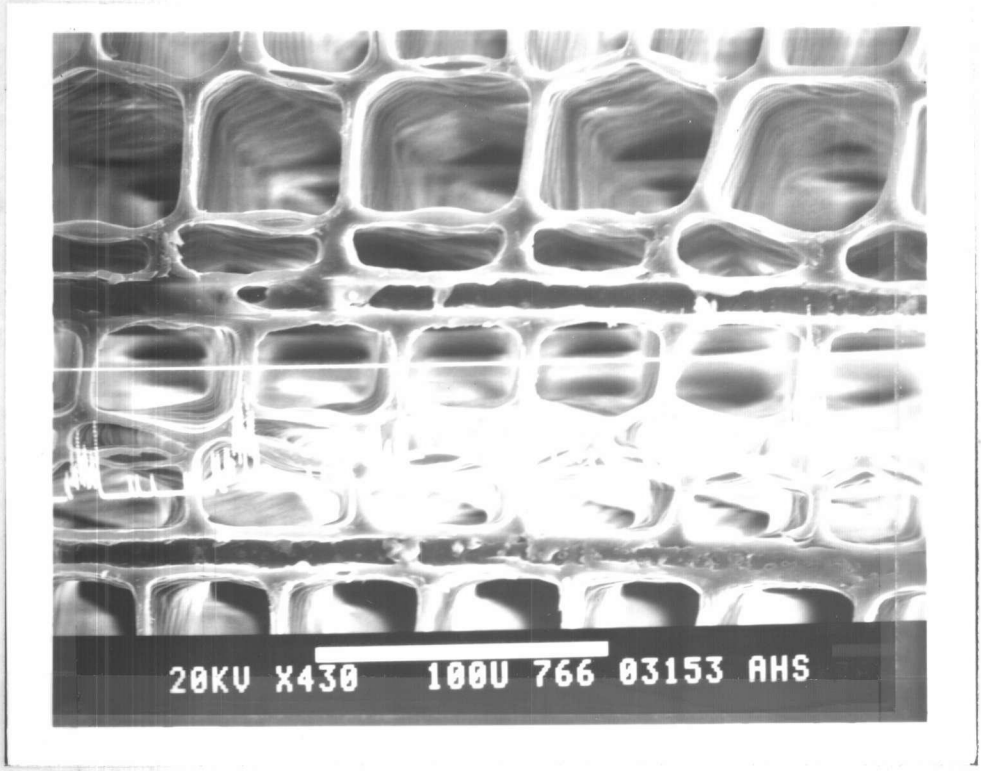

Figure 12. Energy dispersive X-ray analysis line scan across a cell wall of a heated, chloropicrin-treated sample that was sectioned wet.

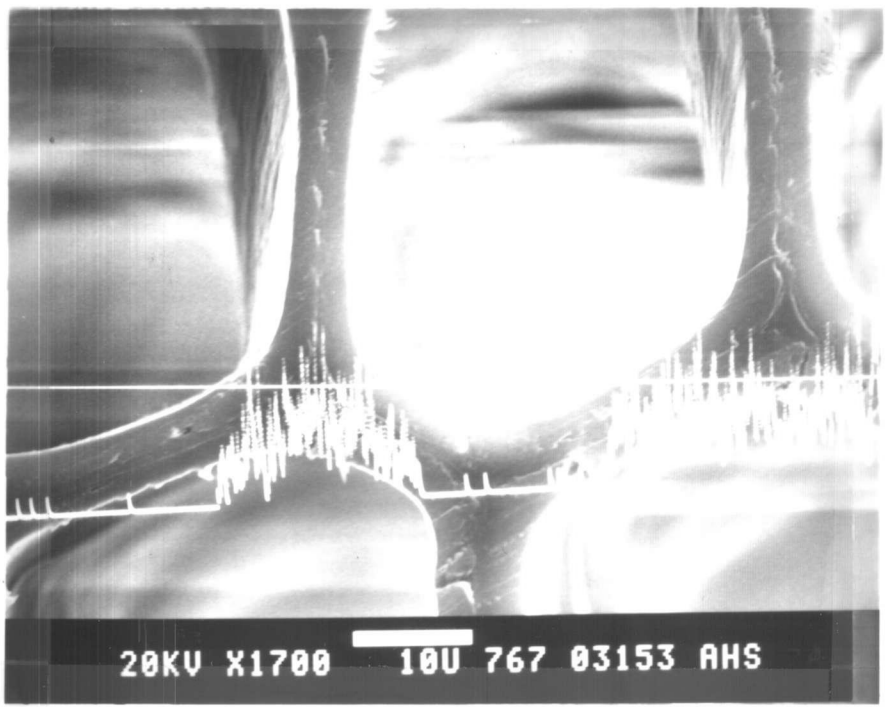

Figure 13. Representative energy dispersive X-ray line scan across acetone extracted or heated, chloropicrin-treated wood.

Figure 14. Energy dispersive X-ray line scan across ray cell walls, tracheid cell walls, and a resin canal epithelial cell with included deposits, in extracted, chloropicrin-treated wood.

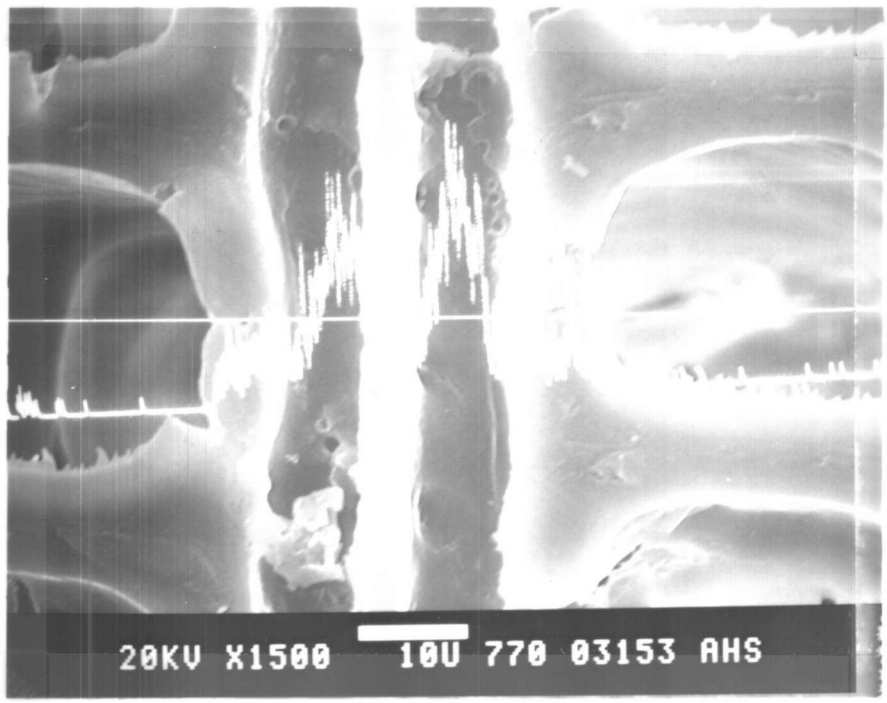

Figure 15. Energy dispersive X-ray line scan across cell wall material and extractive materials deposited in the lumens of rays in extracted, chloropicrin-treated wood.

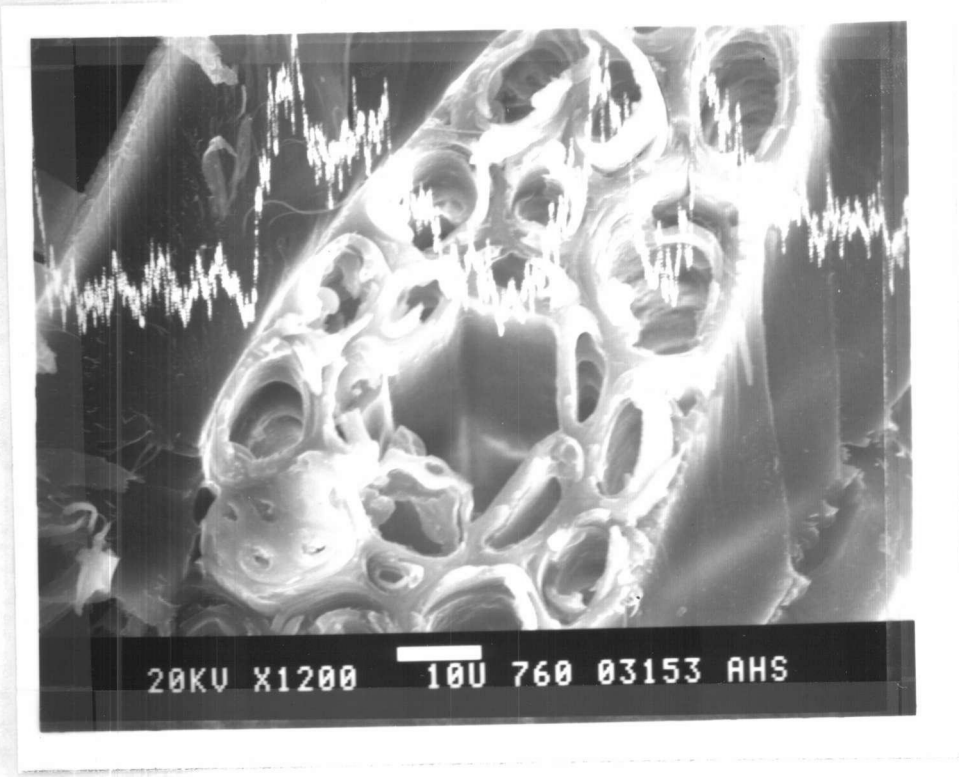

Figure 16. Energy dispersive X-ray line scan across fusiform ray in heated, chloropicrin-treated wood.

ray parenchyma cell walls (Figure 14) indicates that large amounts of chlorinated materials are present in these walls and also apparently in the extractive layer which coats the interior of these walls (Figure 15). Chlorinated materials within the cell walls of tracheids and deposits in the epithelial cells of resin ducts were not generally observed to be as highly chlorinated as ray parenchyma cell walls and extractive deposits (Figure 16).

These data indicate that chloropicrin or chlorinated breakdown products may selectively bind to lignin and to extractive material. Since lignin is a complex phenolic material and many extractives are known to be phenolic in nature, chloropicrin residues may be binding to phenolic compounds.

The fact that no detectable chlorine residues were found in liquid chloropicrin-treated wood is puzzling. There are two possible explanations for the difference in residues retained in the liquid and vapor treated samples. (i) Vapor phase oxidation of chloropicrin may be required to form reactive degradation products which can then bind to the wood; liquid phase treatment would exclude most oxygen to limit this reaction. (ii) Since the vapor phase treatment continued for a much longer period of time, binding of chloropicrin to wood may be a time dependent phenomenon.

IV. Infrared and Mass Spectrographic Analysis

A. Analysis of Chloropicrin and Derivitized Wood Components

Infrared analysis.--Representative infrared scans of chloropicrin (Figure 29, Appendix III) and the derived or synthesized compounds

from wood (Figures 30-35, Appendix III) were compared to scans of these same derivatives saturated with chloropicrin and allowed to aerate for 5 minutes (Figures 36-41, Appendix III). The major peaks for chloropicrin appeared to be superimposed over some of the treated derivative spectra and no new peaks were apparent. After 90 minutes no trace of chloropicrin could be detected in any of the spectra.

Absorbtion band shifts in the spectra in the range of 3200-3600 cm^{-1} could indicate that hydrogen bonding between chloropicrin and the hydroxyl groups on the derivative compounds was occurring, but no differences were noted in any sample when comparing untreated and treated spectra. Since a small percentage of each compound could be reacting with chloropicrin and the spectra of the reaction compound masked by that of the parent materials, this analysis indicates only that most of the derivative compounds tested remain unreacted with chloropicrin.

Mass spectroscopy.--Two small peaks in addition to those of chloropicrin and vanillin were present in the GC analysis of the reaction mixture of these two materials (Figure 17). These peaks were approximately 0.1% of the area of the chloropicrin peak. When unreacted chloropicrin and vanillin were injected simultaneously into the GC/MS only the peaks for the parent compounds were present, indicating that the reaction products were not artifacts produced in the injection port of the GC.

Mass spectral analysis of the first unidentified peak in Figure 17 (retention time = 11.07 m) yielded masses which corresponded to the isotopic fragments of the vanillin ion (average m/e = 152), and also of a parent ion with a major isotopic peak mass of 197. Subtracting these two ion masses yields a mass of 152.

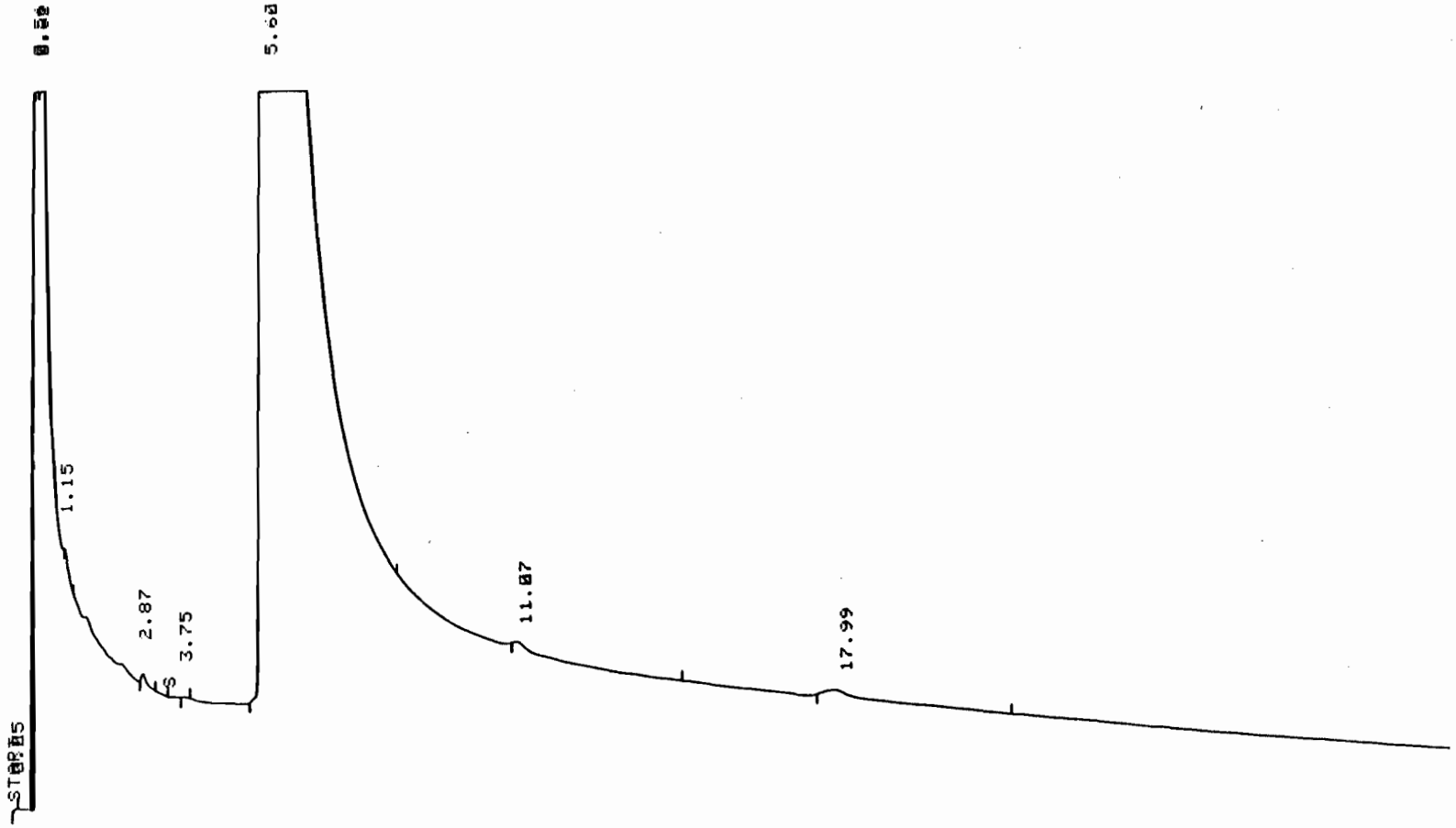

Figure 17. Gas chromatograph of a reacted mixture of chloropicrin (retention time = 0.52 m) and vanillin (retention time = 5.60 m). Reaction products present at retention times of 11.07m and 17.99 m.

Taking into account the different isotopic masses present in the spectrum for both vanillin ion and the parent ion, and also the composition of the parent chemicals injected into the GC/MS, the probable identity for a fragment with a mass of 45 would be an NO_2 ion. Analysis of the second unidentified peak in Figure 17 (retention time = 17.99 m) yielded fragment masses corresponding to the masses of CCl_3^+ (average m/e = 119), and to the vanillin molecule which has probably lost a proton reducing its major isotopic peak mass to 151 (Figure 19). A parent ion mass (m/e = 270) which corresponds to the sum of the masses of vanillin (minus a proton) and CCl_3^+ is also present in the spectrum shown in Figure 19. In addition the isotopic fragment masses present about the 119 m/e peak and the 270 m/e peak in this spectrum are characteristic for the isotopes present in chlorinated materials. Breakdown of chloropicrin into NO_2 and CCl_3^+ transition states has been proposed to explain the mechanism for chloropicrin breakdown to phosgene and nitrosyl chloride (27), but experimental evidence of the existence of these products is lacking. My data indicate that a small portion of chloropicrin was broken down in the reaction mixture, and the breakdown products, NO_2 and CCl_3^+ were covalently linked to the vanillin molecule at some point which was not determined in this study. Further, this analysis suggests a possible mechanism for the covalent attachment of chloropicrin breakdown residues to wood.

As the IR analysis did not detect the reaction products of vanillin and chloropicrin, it is possible that some reaction with the other wood derivatives and chloropicrin may have occurred but the IR sensitivity was not great enough to detect the reaction products.

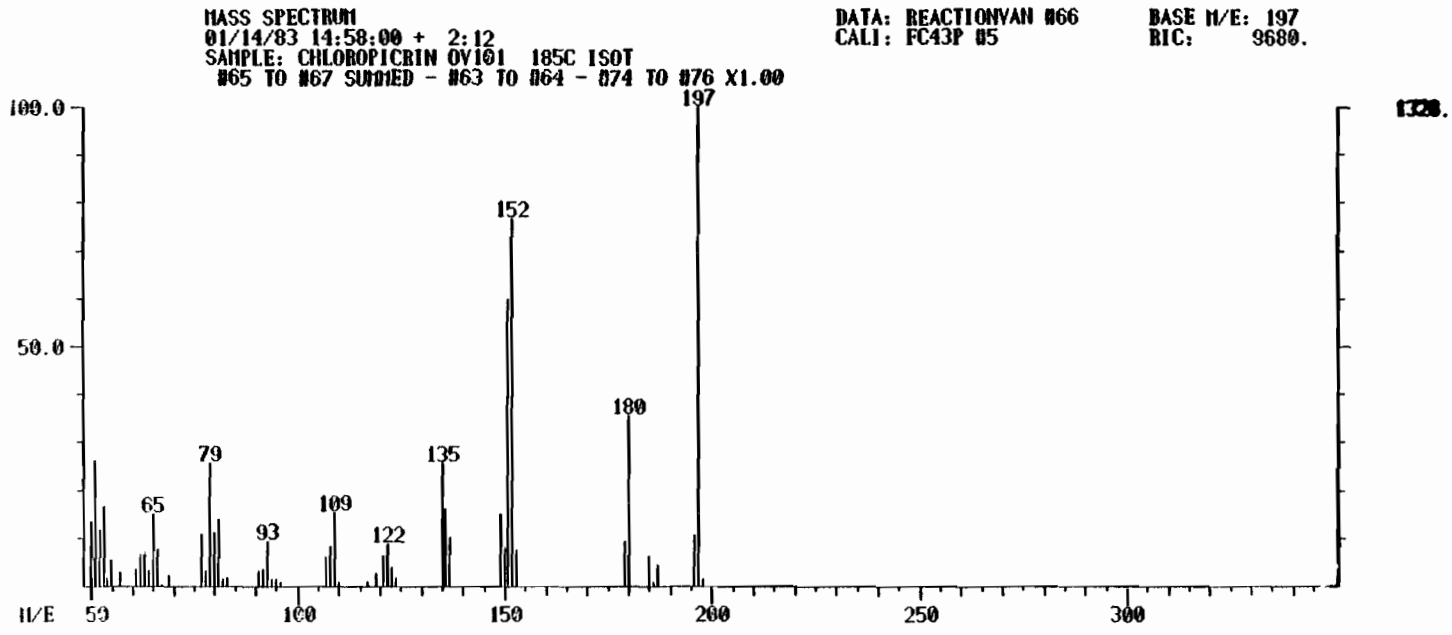

Figure 18. Mass spectrograph of chloropicrin/vanillin reaction product at 5.60 m (Figure 17). Parent ion mass = 197.

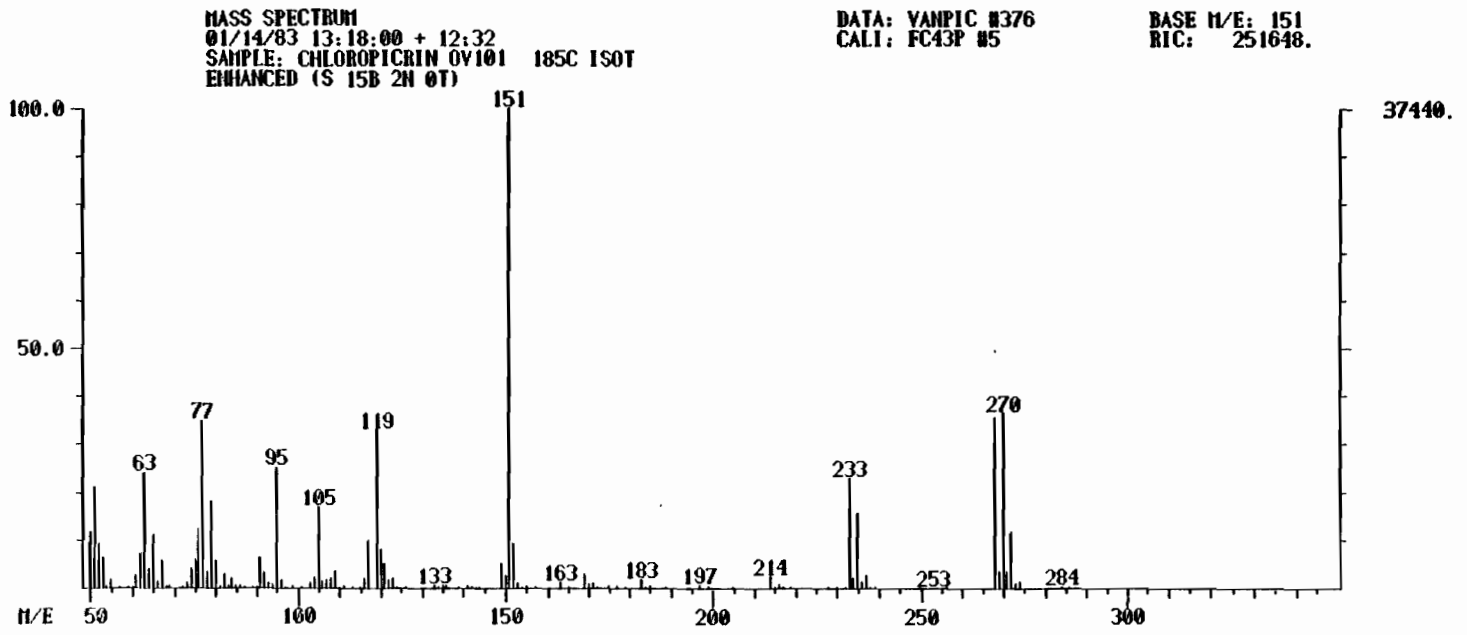

Figure 19. Mass spectrograph of chloropicrin/vanillin reaction product at 11.07 m (Figure 17). Parent ion mass = 270.

B. Analysis of Desorbed Gases from Chloropicrin Treated Wood

Only one peak was present in the vapor samples analyzed in the GC/MS. MS analysis indicated that this component was CCl_3^+ (Figure 20). This ion does not exist in the natural state, chloroform CCl_3H being its most probable stable chemical relative. Chloroform has a different retention time from chloropicrin on the GC suggesting that chloropicrin was being broken down in the MS to produce the two fragments CCl_3^+ and NO_2. Having no charge, NO_2 would not be detected in this spectrograph and CCl_3^+ would be the only ion apparent. When pure chloropicrin was injected directly into the MS without chromatographic separation, only the CCl_3^+ ion was apparent, offering further confirmation that chloropicrin (and not a breakdown product) was the chemical present in the vapor samples.

The volatile components which would be produced in the oxidative degradation of chloropicrin [phosgene and nitrosyl chloride (27, 3)], if formed in wood, should rapidly react with hydroxyl sites or phenolic materials to produce alkyl carbonates or nitrosation products, respectively (28), releasing free chlorine. Chlorine would also react with wood components or cations in solution. If these reactions do occur, no significant amount of volatile material other than chloropicrin would be detected in treated wood.

To determine if these breakdown products could attack wood derivitives, a preliminary experiment was performed where chloropicrin in vapor-phase was decomposed by heat, using procedures similar to that of Steacie et al. (39), and reacted with vanillin. The products isolated could not be positively identified from GC/MS data; however, no

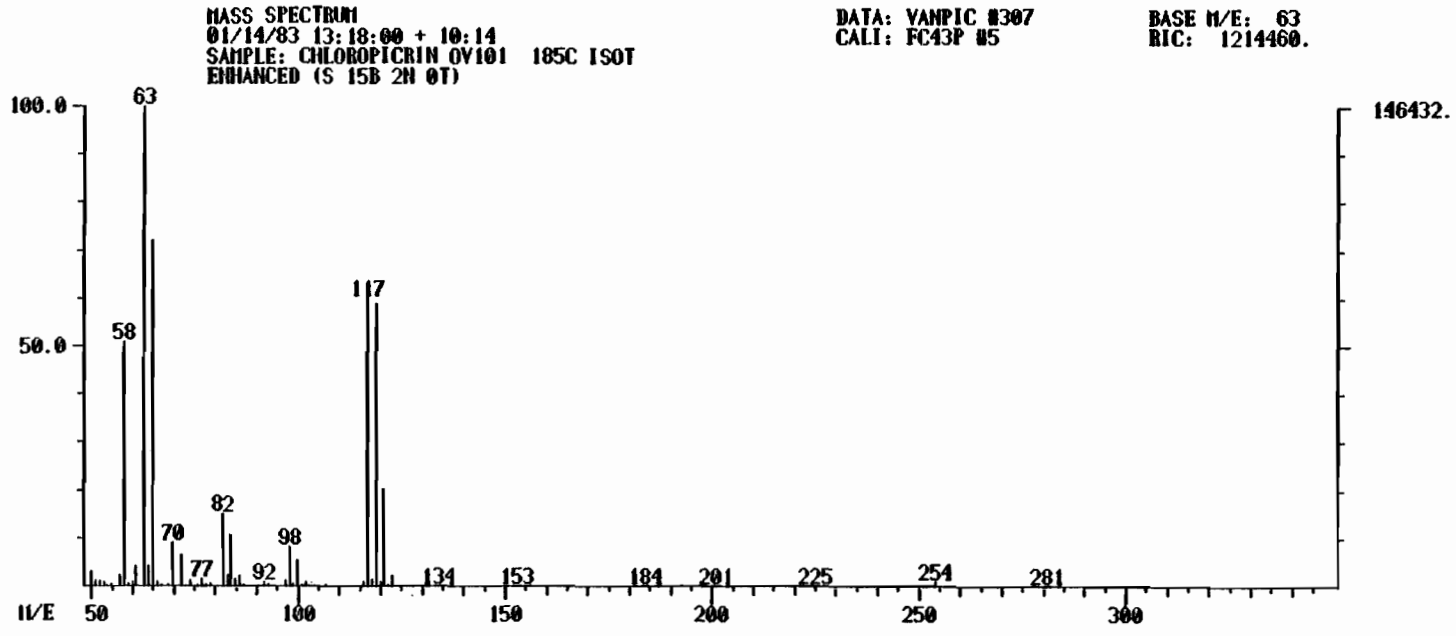

Figure 20. Mass spectrograph of chloropicrin vapor from treated wood showing only the mass fragment for CCl_3^+ (ion mass = 117, 118 and 119), and smaller mass fragments.

product was chlorinated and the simple alkyl carbonates or ring nitrosation products that would be expected from the reaction of phosgene or nitrosyl chloride with vanillin were not present. The large number of non-chlorinated compounds isolated suggests that heating of vanillin may have produced many of these products, and does not explain how chlorinated residues from chloropicrin may bind to wood. Since many of the breakdown residues were probably not volatile enough to be analyzed by GC/MS, additional research should be done on this problem to determine if significant amounts of the oxidative degradation products of chloropicrin are produced in wood and if these products react with wood derivitives.

V. Microscopy

Periodic light microscopic observations of chloropicrin treated and control wafers showed that fungal attack did not occur until after 3 months incubation. This may have coincided with a reduction in moisture content of the wafers to a range favoring fungal attack.

In wafers attacked by G. applanatum, typical white rot bore holes were produced in the earlywood of both control and treated wafers at locations from 10 mm above, to depths up to 25 mm below the agar surface. Perforation type bore holes (Figure 21), similar to those previously reported (25, 29), were also present in this same region of the earlywood and in the latewood of both control and treated wafers. Some erosion of the interior cell walls was also apparent, especially in the latewood (Figure 22).

Both perforation type and typical bore holes were observed in the treated and control wafers attacked by Poria carbonica (Figure 23).

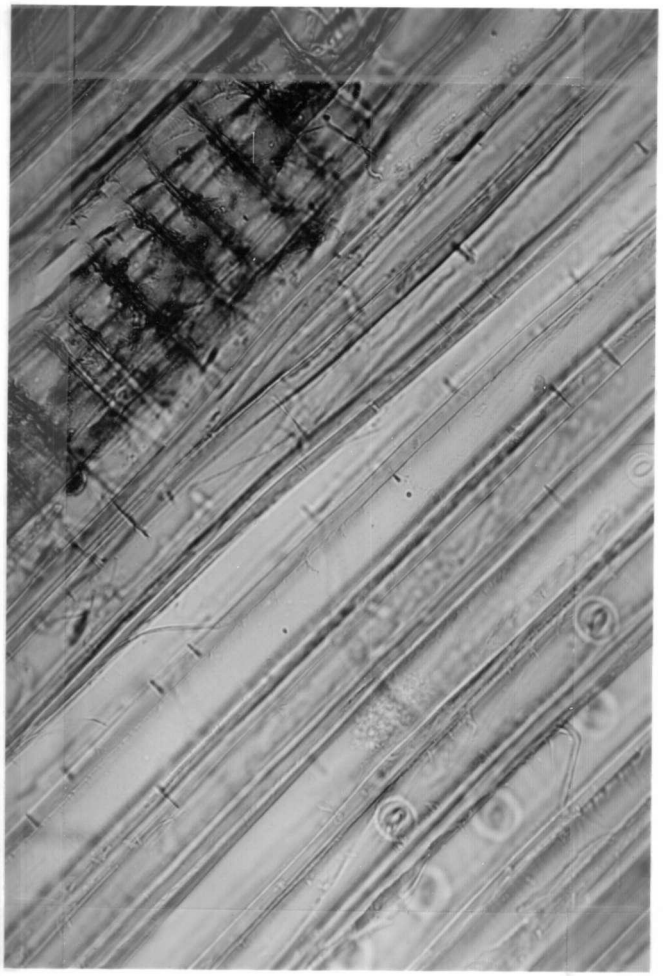

Figure 21. Perforation type bore holes produced by the fungus Ganoderma applanatum in the cell walls of Douglas-fir.

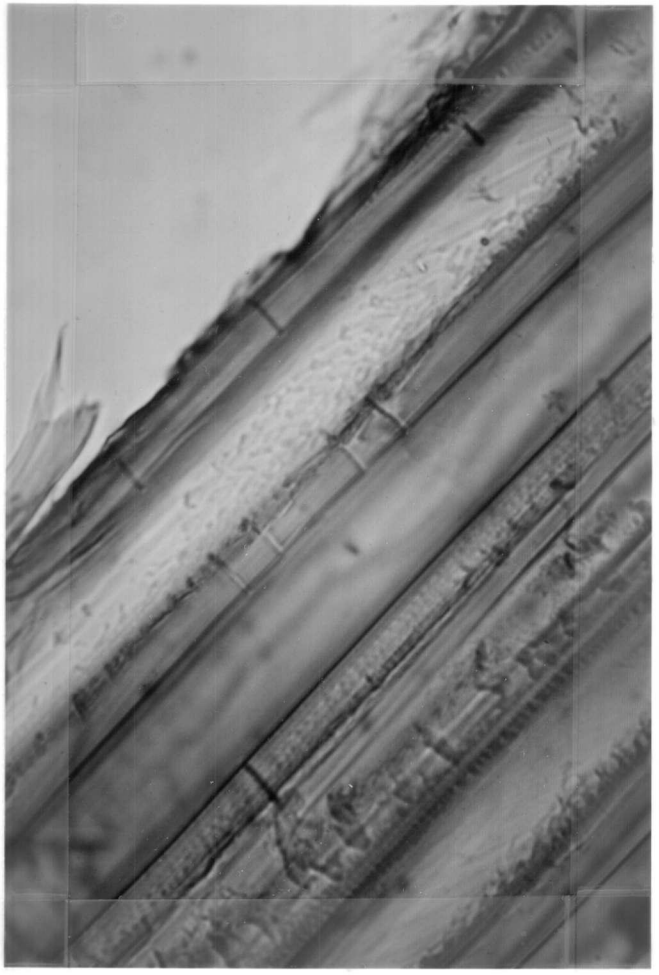

Figure 22. Erosion produced by the action of Ganoderma applanatum on the lumen surface of Douglas-fir cell walls.

Figure 23. Perforation type and typical bore holes produced by the fungus Poria carbonica in the cell walls of Douglas-fir.

Bore holes were present in some samples from 5 mm above, to 20 mm below the agar surface. The predominant attack, as evidenced by numbers of bore holes, occurred in the range of 1-5 mm below the agar surface.

For both fungi no differences in the amount or severity of attack--as evidenced by bore hole formation and erosion--were observed between control and treated wafers.

Observations of the cytoplasm in the cells of G. applanatum showed that vacuolation or lysis of the fungal cells, similar to that reported by Bravery et al. (6) in preservative-treated wood, was much more prominent in general in the treated samples (Figures 24 and 25) than in control samples (Figure 26). Extensive vacuolation of P. carbonica hyphae in treated wood was not observed although some lysis of cell walls was apparent (Figure 27) and contrasted with the unaffected hyphae present in control samples (Figure 28).

The attack of chloropicrin treated wood by the two decay fungi indicates that the treatment cannot be depended on to protect wood under extreme decay hazard conditions. The apparent disruption of the fungal protoplasm in the treated wood of at least the white rot species suggests that treatment residues in wood do have an inhibitory effect on the fungi and may possibly limit fungal attack of the wood under less severe decay exposure conditions. The differences observed in the disruption of the cellular material of the two fungi suggests that P. carbonica may be more resistant to the treatment than G. applanatum, possibly reflecting differences in the wood components utilized by the fungi and those to which chloropicrin or its breakdown residues may be bonding, or it may simply indicate the variability of residue retention in the samples.

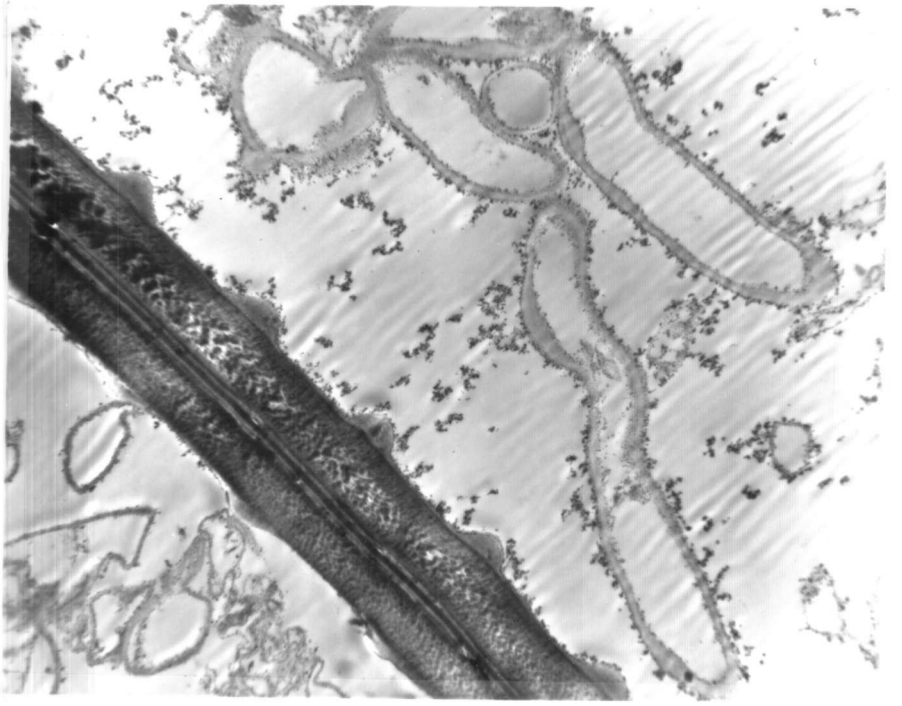

Figure 24. Vacuolated and lysed hyphae of Ganoderma applanatum in chloropicrin-treated Douglas-fir wood. 4000X.

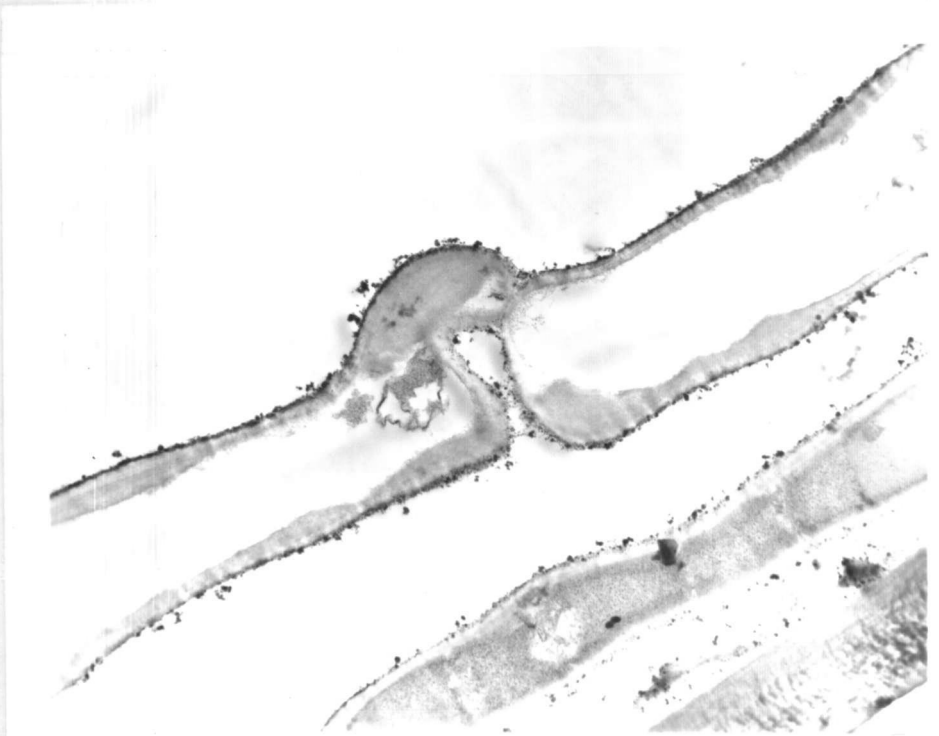

Figure 25. Vacuolated hyphae of Ganoderma applanatum with clamp connection in chloropicrin treated Douglas-fir wood. 7500X.

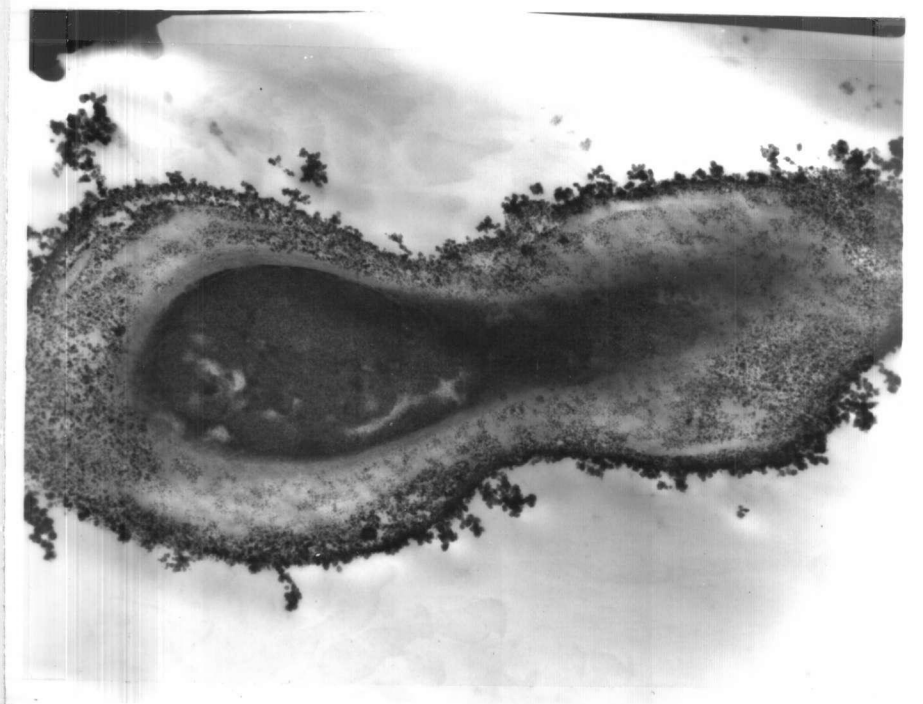

Figure 26. Normal appearing hyphae of Ganoderma applanatum in untreated Douglas-fir. 10,600X.

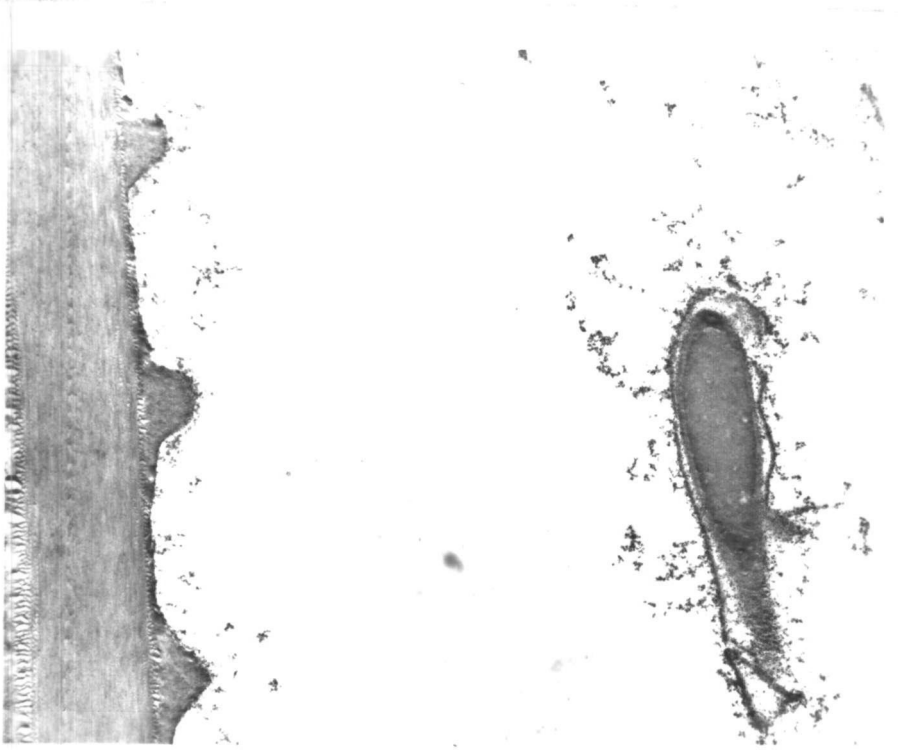

Figure 27. Lysed hyphae of Poria carbonica in chloropicrin treated Douglas-fir. 7200X.

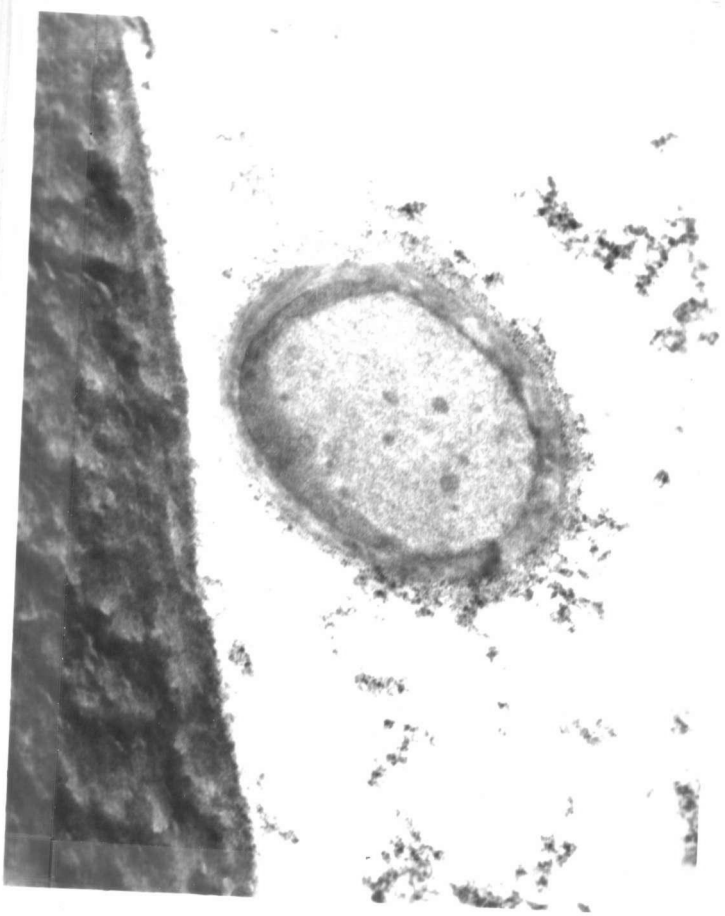

Figure 28. Normal appearing hyphae of Poria carbonica in untreated Douglas-fir. 9400X.

CONCLUSIONS

(1) Wood does not swell when treated with chloropicrin; however, the lower retention of chlorinated residues in wood with greater amounts of decay suggests that chloropicrin may hydrogen bond to wood. Hydrogen bonding between chloropicrin and wood (or water molecules sorbed to wood) may occur only at exterior microsites in the wood structure where the added bulk of the chloropicrin molecule would not cause the wood lignocellulosic matrix to separate. Hydrogen bonds and van der Waals forces may play a significant role in the retention and slow release of chloropicrin as shown by the mass spectrographic analysis of volatiles from wood treated 4 years prior.

(2) A 1-1.5% residue of chlorinated material is present in aerated treated wood. The residue cannot be removed by heating, repeated acetone extraction, or high vacuum treatment suggesting that covalent bonds between chloropicrin breakdown products and wood may be formed. Thin layer chromatography of acetone-extracted, treated wood also indicates that a chlorinated residue is covalently bound to wood extractives.

(3) Mass spectrograph data confirm that chloropicrin degradation products can covalently bond to phenolic wood derivitives suggesting that they could bond to phenolic wood extractives and to lignin. Energy dispersive X-ray analysis shows that greater amounts of chlorinated materials are found in phenolic rich sites of treated wood, offering further support that chloropicrin residues chemically bond to lignin and phenolic extractives in wood.

(4) Chloropicrin desorption studies and energy dispersive X-ray analysis show that the amount of chlorinated residues retained by treated samples is affected by the amount of chemical applied or the length of contact time with the wood, or both. A time dependent effect could be explained by the slow breakdown of chloropicrin to more reactive species which would react with wood; however, preliminary studies to test the reactivity of chloropicrin degradation products with wood derivitives were inconclusive.

(5) Light microscopic analysis shows that chloropicrin treatment cannot be expected to protect wood from fungal attack under severe decay hazard conditions. Preliminary micromorphological analysis of the fungi invading treated wood shows that the treatment does have an inhibitory effect, especially on Ganoderma applanatum, as evidenced by lysis and vacuolation of the fungal hyphae. It was not determined whether the fungi played an active role in releasing the fungitoxic moiety from the wood, or if this material was passively released into the fungal environment.

SUGGESTIONS FOR FUTURE RESEARCH

This thesis uncovered many new problems which need to be examined in greater detail. Research in the following areas would help further our understanding of the microdistribution and retention of chloropicrin in wood.

- Chemical and biological assays should be performed to determine: (1) the time/concentration effects of chloropicrin retention in wood, (2) the extent that extractive content of wood affects chloropicrin retention, and (3) the fungitoxicity of chloropicrin treated wood and its potential for use as a long-term wood preservative after simulated leaching and weathering treatments.
- Transmission electron microscopy studies should be done using an electron dense stain to locate chlorine residues in the wood ultrastructure. Fungal invasion of treated wood should be studied to determine if enzymatic attack is necessary to release chlorinated moieties from the wood.
- Specific mechanisms for the degradation of chloropicrin in wood should be determined. Chlorinated residues in treated wood, and the wood components these residues bond to should be identified. Reactions of chloropicrin and wood derivitives in the dark should be compared to light catalyzed reactions.

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APPENDICES

APPENDIX 1

Preparation of Samples for Light and Electron Microscopy

Light microscopy.--Wafers were soaked in 25% ethanol until soft enough for sectioning on the sliding microtome. Ten to 20 μm thick, 50 mm long sections were made of the region to both sides of the point of contact with the agar surface. Sections were stained with safrarin O and picroanaline blue according to procedures outlined by Wilcox (44), and mounted in diluted Karo syrup.

Scanning electron microscopy.--Small sections above and below the agar were split, mounted, and air dryed for later coating under vacuum with gold/palladium.

Transmission electron microscopy.--Small 1 mm square samples were removed above and below the agar, and were fixed and stained with 2.5% potassium permangenate for 30 minutes. Samples were imbedded in Spurr's standard medium (38) and sectioned on the ultra microtome. The sections were collected on copper wire grids for microscopic observation.

APPENDIX II

Modified Soil-Block Test

1. Fill incubation bottle about half full of screened soil (screening to eliminate lumps). Use a measuring cup.
2. Place a circle or square (depending on shape of bottle) of filter paper on top of the soil.
3. Pour onto the filter paper, evenly, sufficient water to give the soil a moisture content of 60%, based on oven-dry weight of the soil.[9]
4. Place the weighed test block on the filter paper (the block ordinarily is dry), close the bottle with the lid loosely turned down, and autoclave about 15 minutes at 15 or 20 pounds of steam.
5. Allow the bottles to thoroughly cool, then inoculate the filter paper.
6. Allow up to 3 weeks for the fungus to grow on the filter paper. Shorter period is OK if the paper is covered earlier.
7. Incubate at 80°F for 12 weeks (or for a shorter period with small blocks). The 12-week period is standard for 3/4 inch cubes.

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[9]The water at step 3 should contain in solution enough malt extract to provide 5% extract by weight of soil (extract and soil proportions on dry-weight basis).

The oven-dry weight of soil in a bottle (on which the weight of water and malt extract to added is calculated) is the average determination for 3 or 4 bottles which are extra for the purpose. (If the soil is measured into the bottles it can be assumed that the amounts in the test bottles are essentially the same as those in these extra bottles).

APPENDIX III

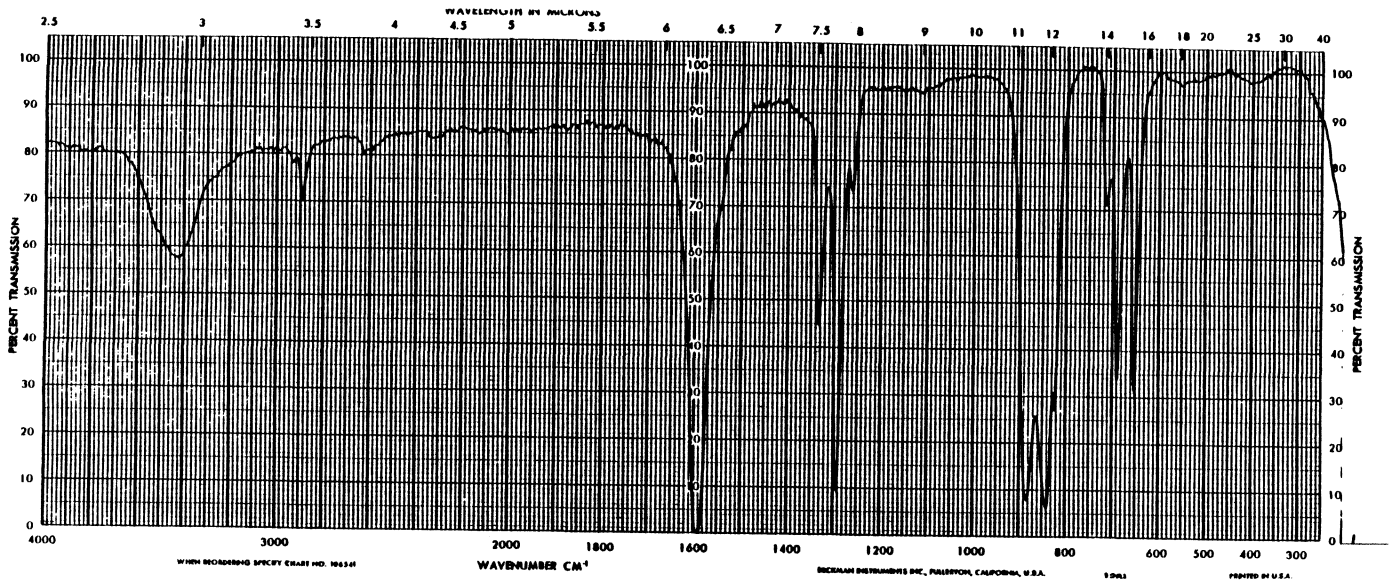

Figure 29. Infrared spectral scan of chloropicrin in potassium bromide.

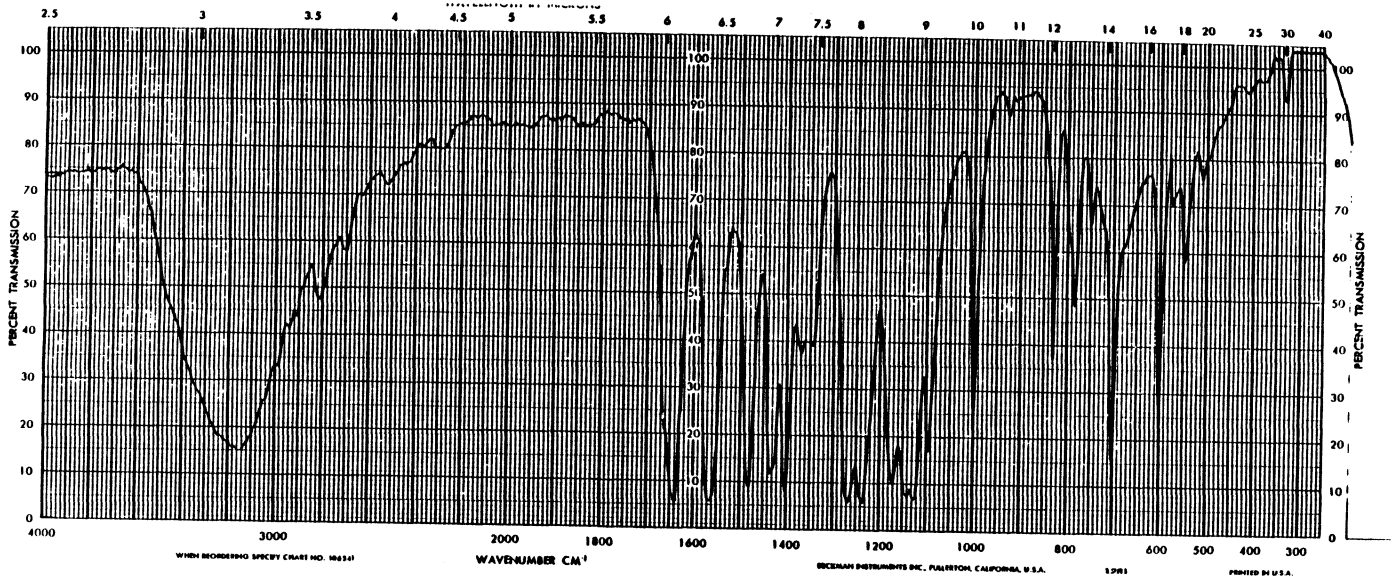

Figure 30. Infrared spectral scan of vanillin (in potassium bromide).

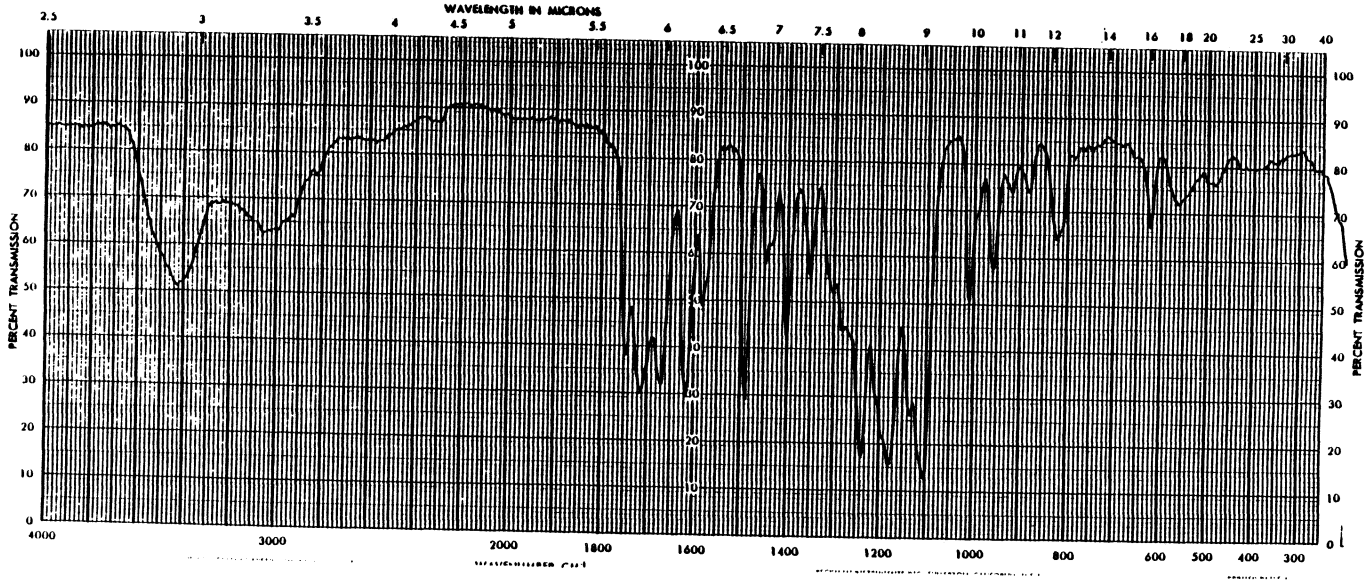

Figure 31. Infrared spectral scan of acetyl ferullic acid (in potassium bromide).

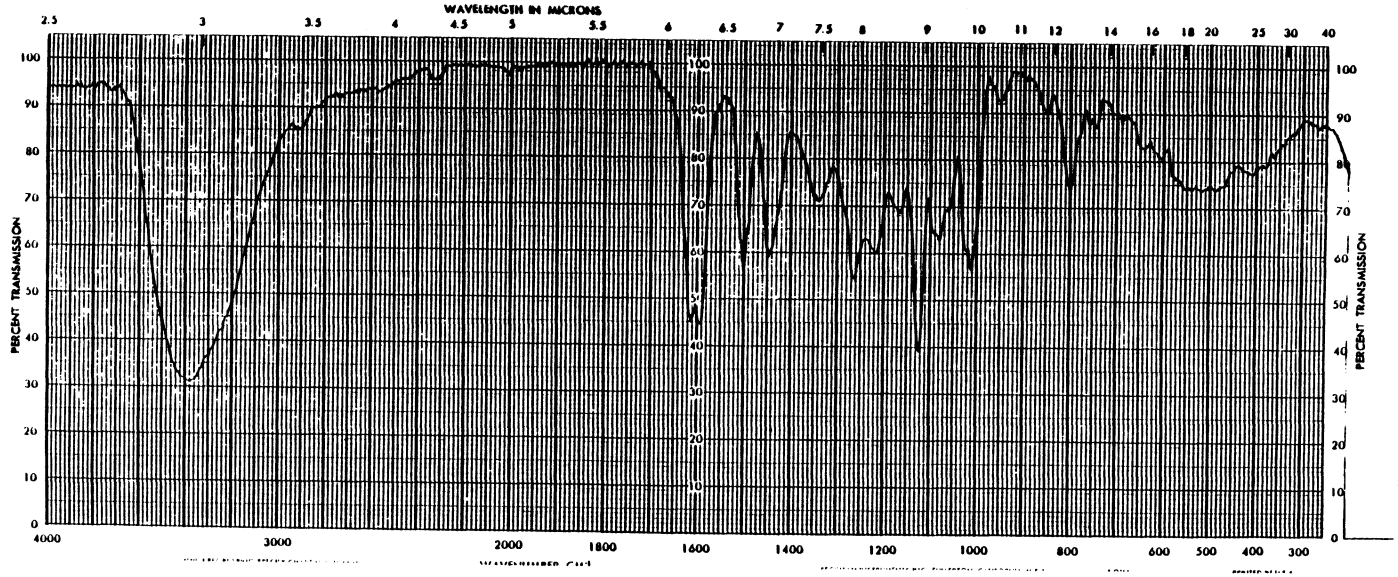

Figure 32. Infrared spectral scan of d-catechin (in potassium bromide).

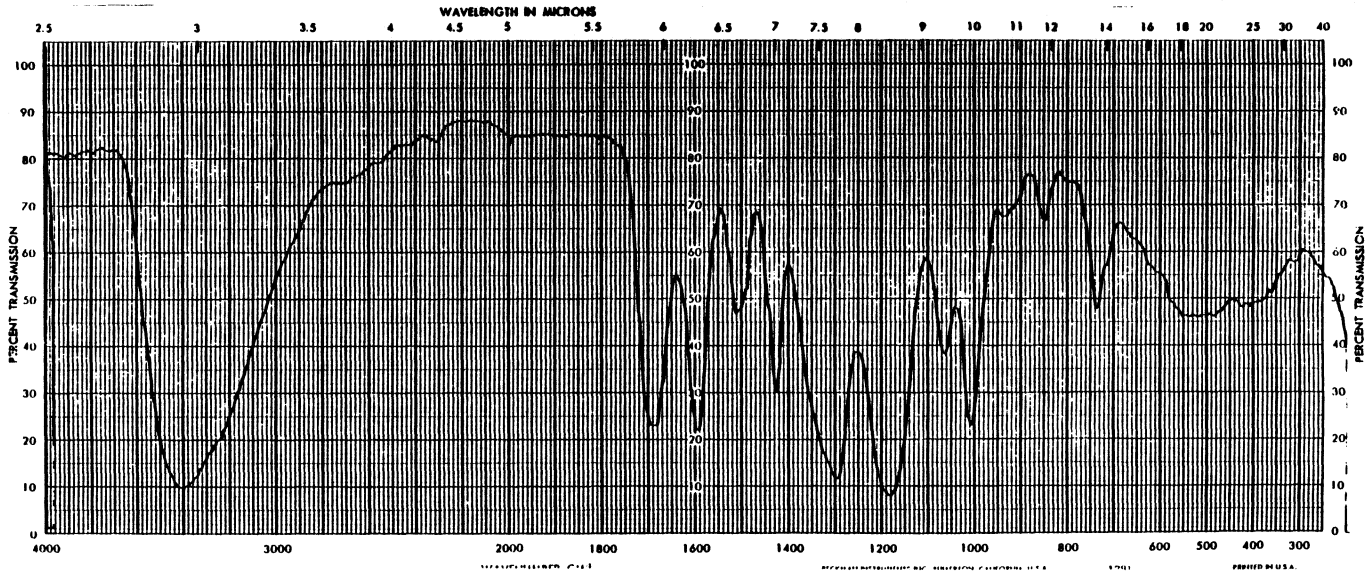

Figure 33. Infrared spectral scan of tannic acid (in potassium bromide).

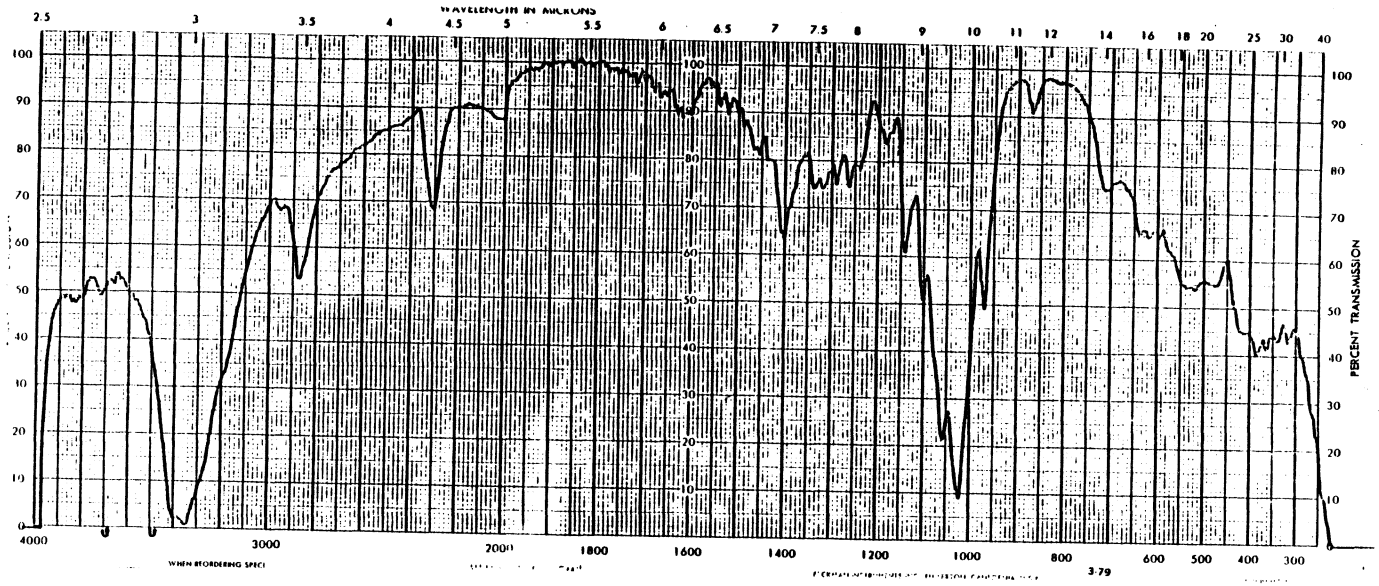

Figure 34. Infrared spectral scan of cellobiose (in potassium bromide).

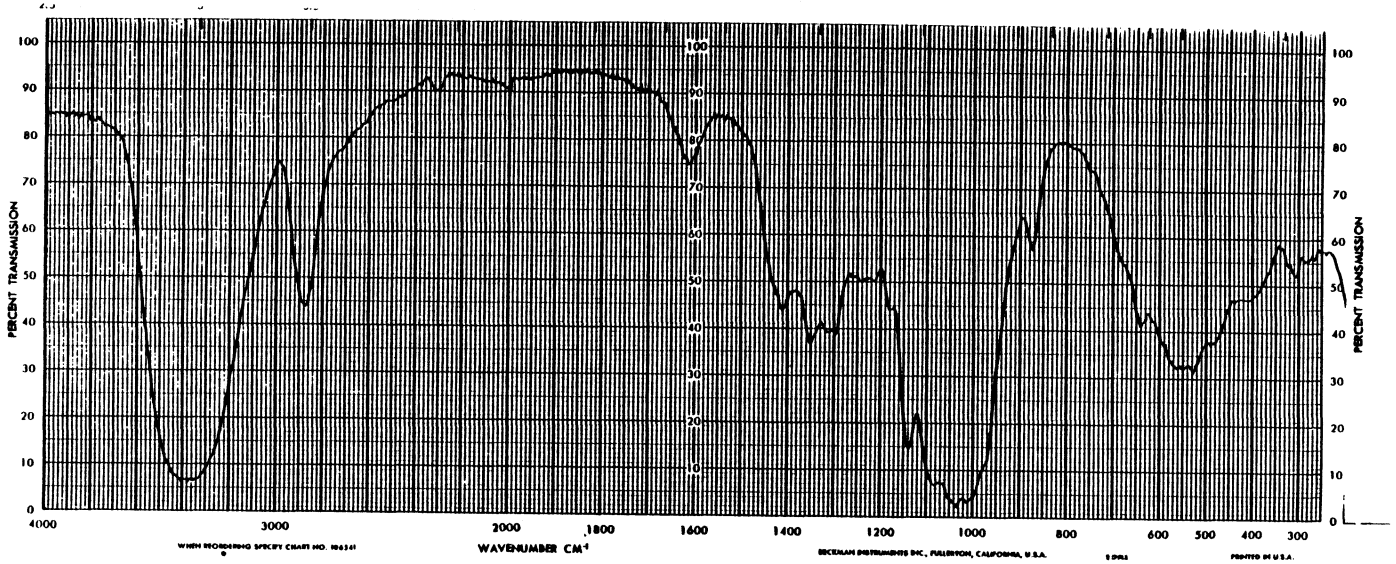

Figure 35. Infrared spectral scan of solka flok (in potassium bromide).

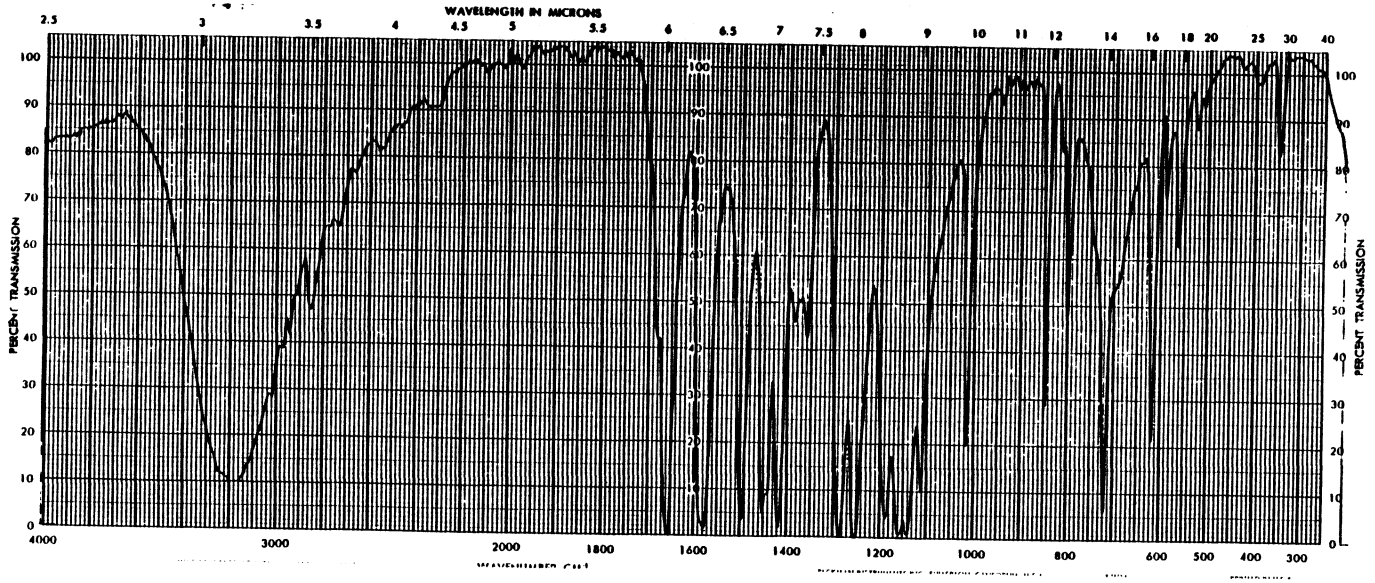

Figure 36. Infrared spectral scan of vanillin reacted with chloropicrin and aerated for five minutes (in potassium bromide).

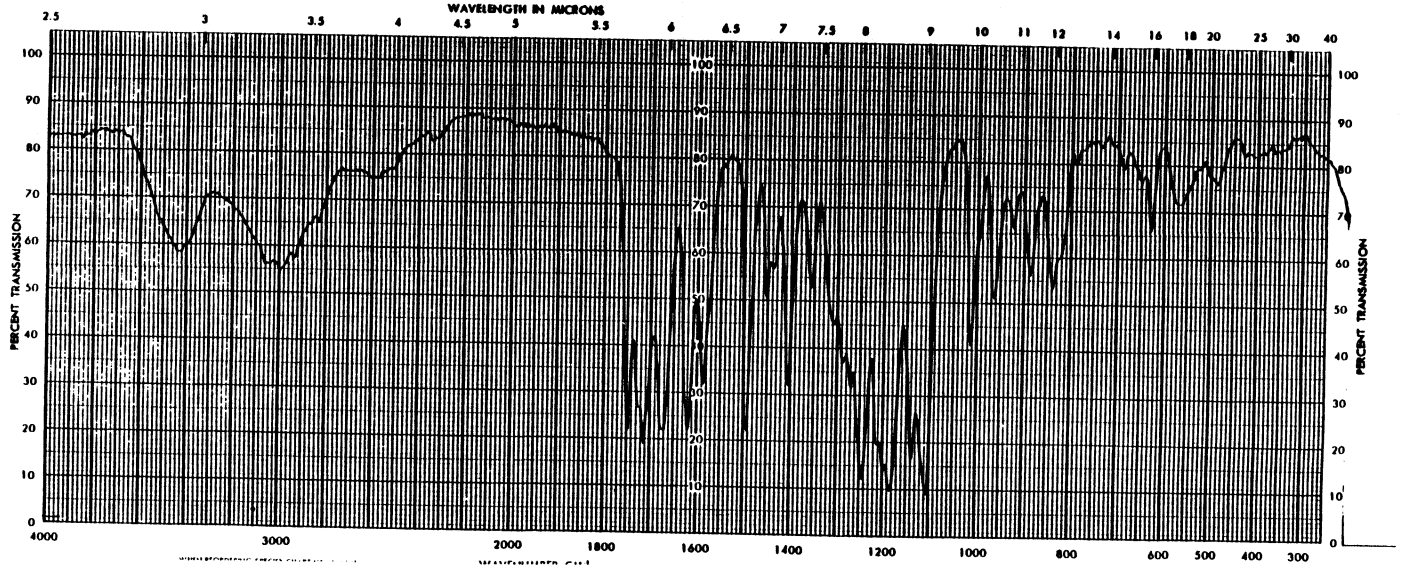

Figure 37. Infrared spectral scan of acetyl ferullic acid reacted with chloropicrin and aerated for five minutes (in potassium bromide).

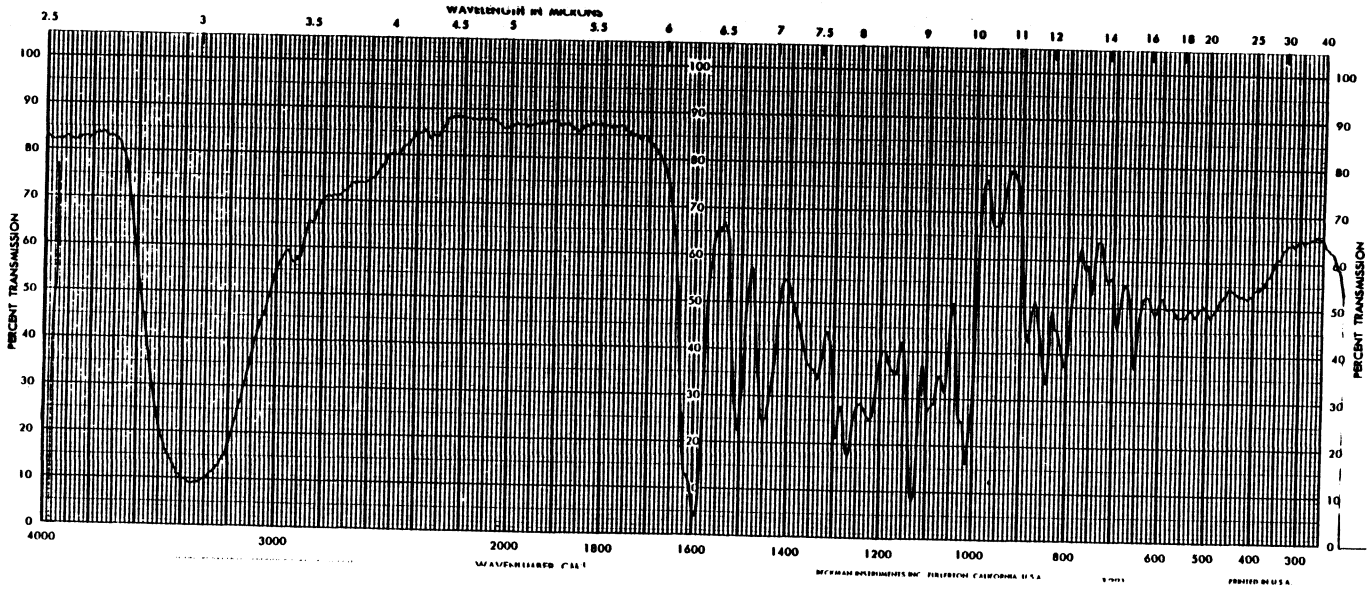

Figure 38. Infrared spectral scan of d-catechin reacted with chloropicrin and aerated for five minutes (in potassium bromide).

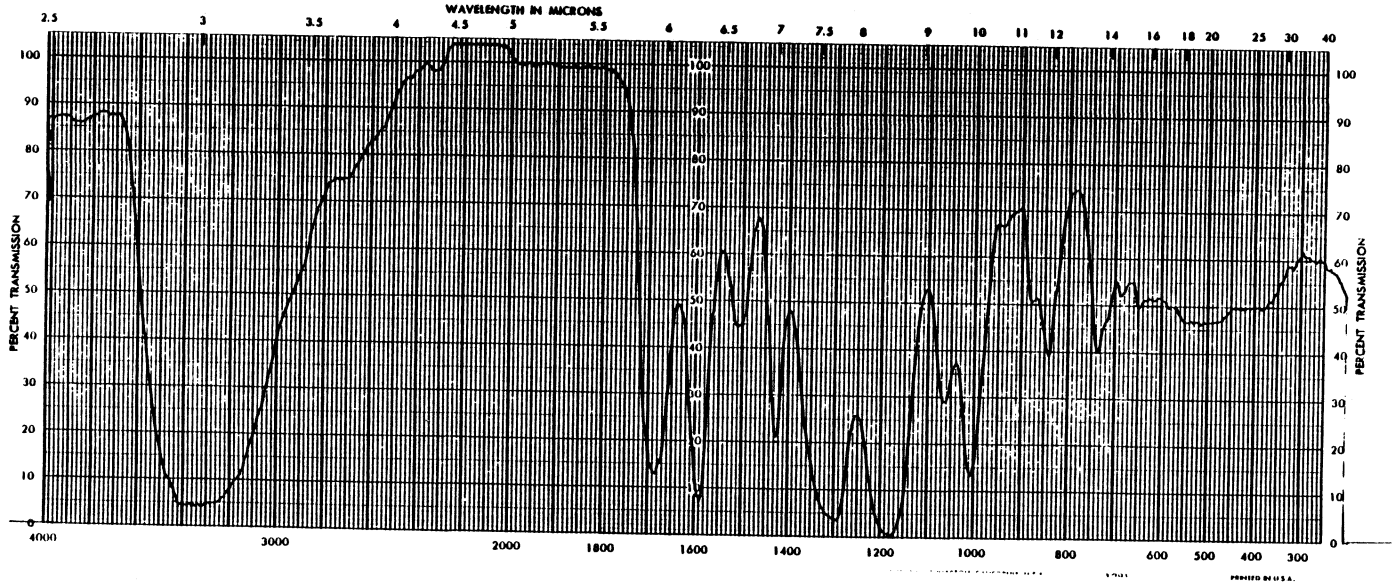

Figure 39. Infrared spectral scan of tannic acid reacted with chloropicrin and aerated for five minutes (in potassium bromide).

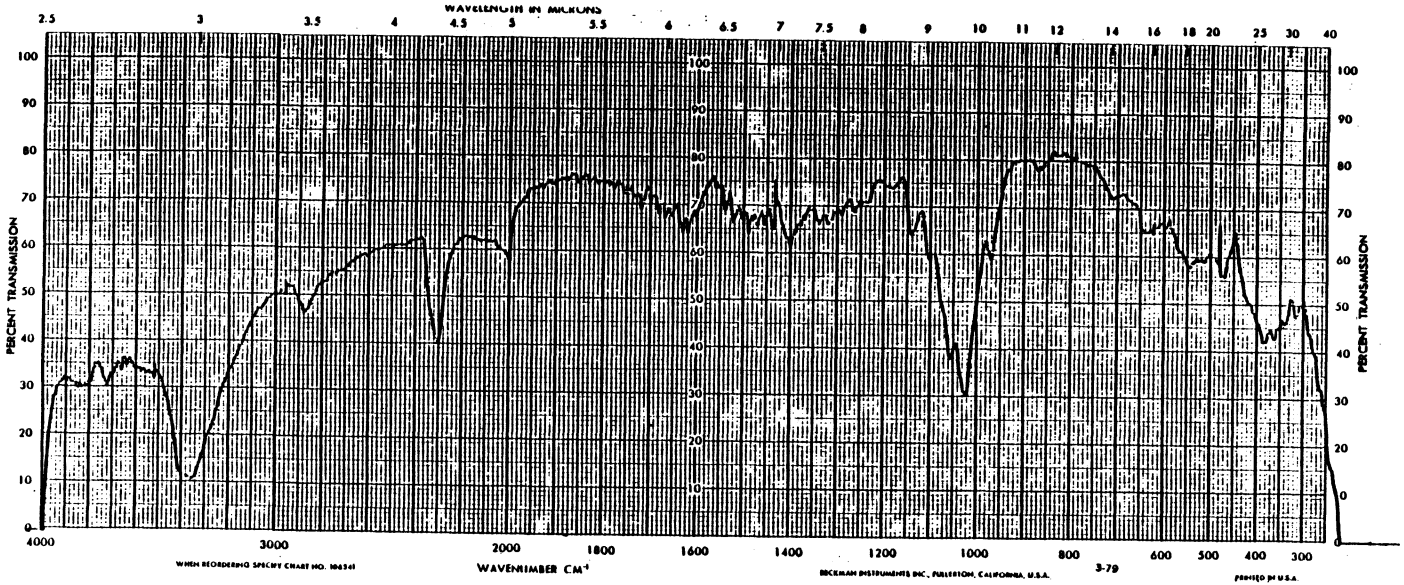

Figure 40. Infrared spectral scan of cellobiose reacted with chloropicrin and aerated for five minutes (in potassium bromide).

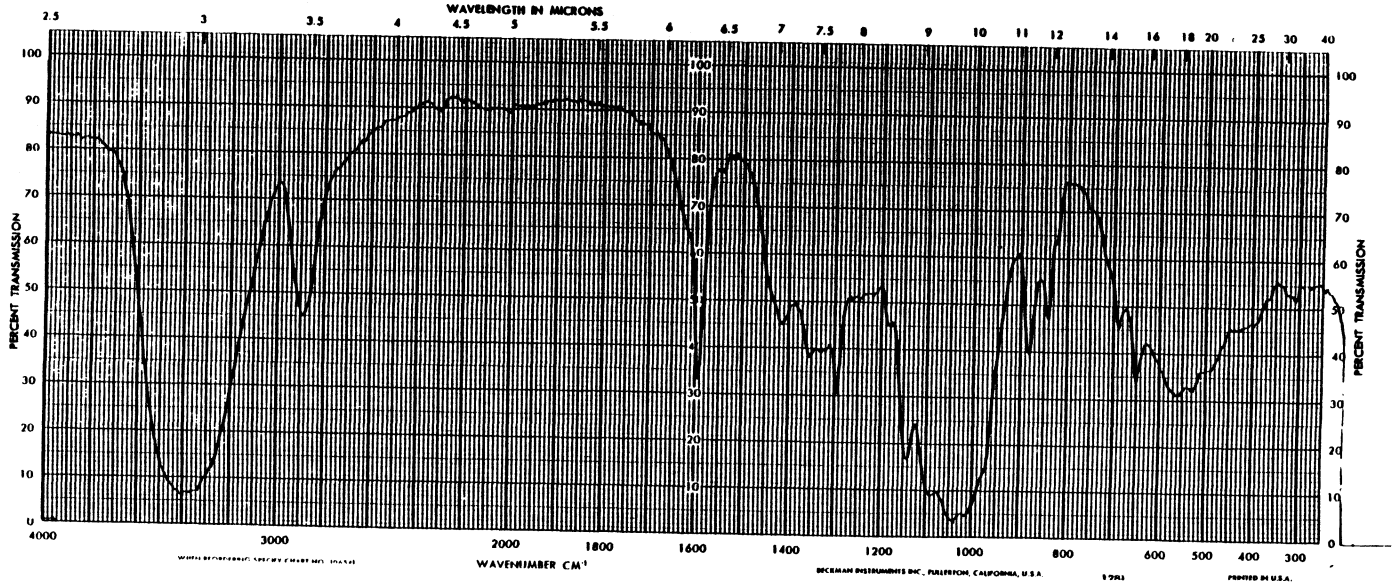

Figure 41. Infrared spectral scan of solka flok reacted with chloropicrin and aerated for five minutes (in potassium bromide).