Inhibition of Wood-Rotting Fungi by Ellagitannins in the Heartwood of Quercus alba

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ABSTRACT

Successive extraction of the heartwood of white oak (*Quercus alba*) with a series of solvents of increasing polarity revealed that fungistatic components were largely contained in the aqueous acetone extract. Both *Polyporus versicolor* and *Poria monticola* were inhibited in vitro by 1,000 ppm of the aqueous acetone extract. When this extract was impregnated into the normally decay-susceptible sapwood of cottonwood (*Populus deltoides*) at approximately the same concentration as that found in white oak heartwood, subsequent decay by *P. monticola* was reduced by 44%, but decay by *P. versicolor* was unaffected. Fractionation of the aqueous acetone extract followed by in vitro bioassays showed that the inhibitory compounds were water-soluble tannins. Chemical characterization by R_F values, chromogenic sprays, and hydrolysis demonstrated the toxic materials to be solely ellagitannins, the fungistatic effects of which were negated by the addition of polyvinyl pyrrolidone (PVP) or Tween 80 (polyoxyethylene sorbitan monooleate) to the growth medium. These data indicate that the heartwood of white oak is not decayed by *P. monticola* because certain ellagitannins are present. These compounds are thought to inhibit certain fungal proteins owing to their protein-binding capacity.

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The inhibitory effect of tannins on enzymes (8, 15) and on the growth of many fungi in culture (6) is well documented. Although a number of workers have suggested that the decay resistance of heartwood may be due to the presence of tannins [i.e., polyphenols having molecular weight in the range of 500 to 3,000 with 1-2 hydroxyl groups/100 mol wt (24, 26)], only Zabel (27), Anderson (2), and Rudman (18, 19) have reported experimental evidence supporting these claims. Polyphenols isolated from the heartwood of certain *Eucalyptus* species conferred decay resistance to wood that is normally susceptible to decay (19). Decay resistance was due to both the amount and toxicity of the polyphenolic materials (tannins) present.

The decay resistance of the heartwood of redwood (*Sequoia sempervirens*) to *Poria monticola* was greatly reduced when the wood was extracted with hot water (2). Cold-water extracts of the heartwood reduced the amount of decay by *P. monticola* and purified redwood tannin inhibited the growth of *Fomes annosus*. However, the hot-water extract after concentration was not fungistatic. While the chemical structure of the tannin was not determined, it was shown to be a condensed tannin built of catechin units.

The heartwood of white oak has been classed as decay resistant (21). Materials extracted from the heartwood with hot water were highly toxic to *Lenzites trabea* in malt extract agar (27). The amount and toxicity of the hot-water extractives were shown to vary with the radial position in the heartwood. Treatment of the hot-water extractives with hide powder almost entirely eliminated their toxicity. Zabel (27) did not demonstrate that the fungitoxic materials isolated would reduce the amount of decay when decay-susceptible wood was impregnated with them and subsequently exposed to wood-rotting fungi. The results did suggest that the tannins exert toxic effects on fungi which have been found in white oak heartwood.

The objectives of this study were to isolate the fungitoxic materials present in the heartwood of white oak, to characterize these materials chemically, to study their ability to reduce the growth of wood-decay fungi both in wood and in vitro, and to determine the mechanisms by which the growth of such fungi are inhibited.

MATERIALS AND METHODS.—*Chemical characterization.*—The main white oak (*Quercus alba* L.) sample used in this study was taken from a tree collected in Michigan which was ca. 25 years old, fast-growing, and with a heartwood somewhat paler in color than is characteristic for this species. Heartwood from the basal 1.5 m of the bole was reduced to sawdust, air-dried, and put through a Wiley mill with a 1-mm mesh screen. The ground heartwood was placed in desiccators containing P_2O_5 and dried under vacuum for 5 days. The dried wood (2,918 g) was placed in a column and extracted at room temperature successively with *n*-hexane, chloroform, acetone, acetone:water (9:1, v/v), and hot water. The extracts were evaporated to dryness in a rotary evaporator at 40-50 C, and the solid extracts stored at −10 to −18 C to reduce decomposition. To test for the presence of tannin, 2-3 mg of each of the extracts were added to a 0.5% solution of gelatin where the formation of a cloudy precipitate indicated a positive test. The acetone and acetone:water extracts were further subdivided by liquid-liquid extraction between water and ethyl acetate. The ethyl acetate fraction was recovered by vacuum evaporation; and the water fraction, by freeze-drying.

Supplemental experiments were with an acetone:water (19:1, v/v) extract of the heartwood of a white oak which grew in Wisconsin. This extract was also partitioned between ethyl acetate and water. Preliminary results of the chemical constituents of this extract have recently been published (5).

Chromatograms of the chloroform, acetone, acetone:water, and water extracts were made using Schleicher and Schnell (No. 2045-B) or Whatman No. 1 paper. The principal developing solvents for two-dimensional chromatograms were 1-butanol:27% acetic acid (1:1, v/v) (BAW) in the first direction, and 6% acetic acid (6 HA) in the second direction. The same solvents were used routinely for one-dimensional chromatograms and cochromatography. Other solvents used for cochromatographic identification were 3% NaCl in 0.1 N HCl and isopropanol-2N ammonia (2:1, v/v).

Chromatograms were viewed under short- (254 nm) and long-wave (365 nm) ultraviolet light. Chromogenic sprays were $FeCl_3$ (1%)-$K_3Fe(CN)_6$ (0.3%) (1:3, v/v); dSA (0.38% sulfanilic acid:30% H_2SO_4:0.34% $NaNO_2$) (10:1:2.2, v/v) with or without overspray of 20% Na_2CO_3; Gibbs' reagent (0.1% methanolic 2,6-dichloroquinone-4-chloroimide oversprayed with lead subacetate solution diluted with methanol (1:5, v/v); 2-methylindole (a dilute ethanolic solution of 2-methylindole to which a few drops of concentrated HCl had been added); dNPH (5 g dinitrophenylhydrazine in dilute HCl); ED-HCl (2.7% ethylenediamine dihydrochloride in water); pNA (0.05% *p*-nitrobenzendiazonium tetrafluoborate in water); vanillin (either a 1:1 mixture of ethanol saturated with vanillin and concentrated HCl or a 1:1 solution of 3% vanillin in ethanol and 2% *p*-toluenesulfonic acid in ethanol); and NSSC (15 g Na_2SO_3:3.5 g Na_2CO_3:350 ml water).

The water-soluble fractions of the acetone and acetone:water extracts appeared to contain identical ellagitannins, and samples were completely hydrolyzed by refluxing 10 mg of these fractions with 5 ml N H_2SO_4 for 2 hr. The solution was cooled to 1 C and filtered to remove the precipitate which was dissolved in methanol and chromatographed first in BAW and then in 6HA. The filtrate was passed through an ion exchange resin (Amberlite IR-4B[OH]), evaporated to dryness, redissolved in water, and cochromatographed with authentic glucose in ethyl acetate:pyridine:water (12:5:4, v/v). The chromatogram was developed successively in a saturated acetone solution of $AgNO_3$, 0.5 M NaOH in ethanol, and 25% sodium thiosulfate or with aniline phthalate reagent for the detection of sugars.

Complete alkaline hydrolysis was achieved by dissolving 3 mg of the fraction in 0.5 ml N NaOH in methanol, then adding 1 ml distilled water. After 90 min at 23 C, the solution was passed through an ion exchange resin (Amberlite IR-120 [H]), evaporated to near dryness, and chromatographed in BAW and then in 6HA or ethyl acetate:pyridine:water. Products were detected with $FeCl_3$-$K_3Fe(CN)_6$ and the $AgNO_3$ sugar reagent, respectively.

Bioassays.—The brown-rot fungus *Poria monticola* Murr. (Madison 698) and the white-rot fungus *Polyporus versicolor* L. ex Fr. (Madison 697), synonymous with *Coriolus versicolor* (L.) Quel., *Polystictus versicolor* (L.) Sacc., and *Trametes versicolor* (L. ex Fr.) Lloyd, were grown in Erlenmeyer flasks containing 50 ml of malt extract medium (30 g/liter). Flasks were inoculated with a 4-mm^2 malt agar block taken from an actively growing culture. The acetone, acetone:water, or hot-water extracts (1 mg/ml) and their water fractions (0.5 or 1 mg/ml medium) were added to each flask which previously had been autoclaved. Some extracts proved to be sterile, and others required sterilization by Millipore filter (0.5-μ pores). Puratized Agricultural Spray (PAS) (7.5% phenyl mercuri triethanol ammonium lactate) at the rate of 1 mg/ml was used as an example of a commercial fungicide. For each fungus, four to five flasks/treatment were used in each experiment. Each experiment was repeated one or more times. Initial pH of media was 4.5 to 5.0. Flasks were placed on a reciprocal shaker for 2 weeks at 27-29 C. The final pH values of controls for *P. monticola* and *P. versicolor* were 3.0-3.2 and 4.6, respectively. The contents were filtered through tared No. 3 Whatman filter paper, dried, and weighed to determine the dry weight of fungal material in each flask.

A similar procedure was used to bioassay the water fractions of the acetone:water extracts from both trees. The fractions (1 mg/ml) were added to malt extract to which Tween 80 (polyoxyethylene sorbitan monooleate) (1 mg/ml medium) or polyvinyl pyrrolidone (= PVP, ca. mol wt 24,500; 2 mg/ml medium) or both had been added. Both compounds protect enzymes against the inhibitory effects of tannins (7, 8, 12). The Tween 80 was added to the medium which was then autoclaved, and the PVP, which was sterilized by passage through a Millipore filter, was added after the medium had been autoclaved.

Blocks (ca. 1.4 g each) of cottonwood sapwood (*Populus deltoides* Bartr.) were impregnated (1) with one of the extracts from the Michigan tree, steam-sterilized at 99 C for 15 min, and placed on glass triangles situated on the surface of the fungal culture (either *P. versicolor* or *P. monticola*) on malt agar (17). After 4 weeks at 27 C, the blocks were removed and the mycelium was carefully brushed off. All blocks were dried for 48 hr at 95 C, and the dry weights determined. The dry weight loss during the exposure period was used as a relative measure of decay susceptibility. The experiment was repeated twice.

The decay resistance of the intact sapwood and heartwood of the white oak (Michigan tree) was determined by the agar-block method (17). Blocks (1.5 g) were exposed to either *P. versicolor* or *P. monticola* for 6 weeks, and the loss in dry weight was calculated. Sapwood and heartwood were represented by 10 and 15 blocks, respectively, exposed to each fungus; 10 blocks of the heartwood were from the basal portion, and 5 from the upper section of the log used in this study.

RESULTS.—*Chemical characterization.*—The total amount of extractives dissolved by all solvents combined was 7.1% oven dry wt of the heartwood with the bulk of the material dissolving in either acetone:water or hot water (Table 1). The hexane extract did not contain phenolic compounds detectable by chromatography, and this extract was not characterized. With pNA and dSA, three phenolic spots were detected in the chloroform extract. The most prominent of these (R_F 0.85 BAW, 0.45 6HA) had an intense blue fluorescence when exposed to ultraviolet light. Separation using preparative chromatography and examination by cochromatography in four solvents and ultraviolet spectra (including spectral shifts by the addition of N ethanolic sodium ethylate followed by N HCl) revealed that this compound was identical with scopoletin, previously identified in the heartwood of plum and red oak (11, 23).

Over 40 individual compounds were detected on BAW × 6HA chromatograms of the acetone extract. On the basis of R_F values and reaction to chromogenic sprays, ellagic acid, a trace of gallic acid, the three ellagitannins, H-1, H-2, and H-3, and a number of guaiacyl and syringyl derivatives of smaller molecular weight were identified. The remainder of the compounds had R_F values in BAW in excess of

TABLE 1. Some characteristics of extracts from white oak heartwood obtained with solvents of increasing polarity

Solvent used	% Total dry wt	Appearance	Reaction with 0.5% gelatin
Hexane (skelly-B)	0.2	Yellow amber gum	Not tested
Chloroform	0.6	Brown amorphous solid	None
Acetone	0.9	Orange-brown amorphous solid	Small amount of precipitate
Acetone:water	3.4	Amber amorphous solid	Large amount of precipitate
Hot water	2.0	Black glass	None
Total	7.1		

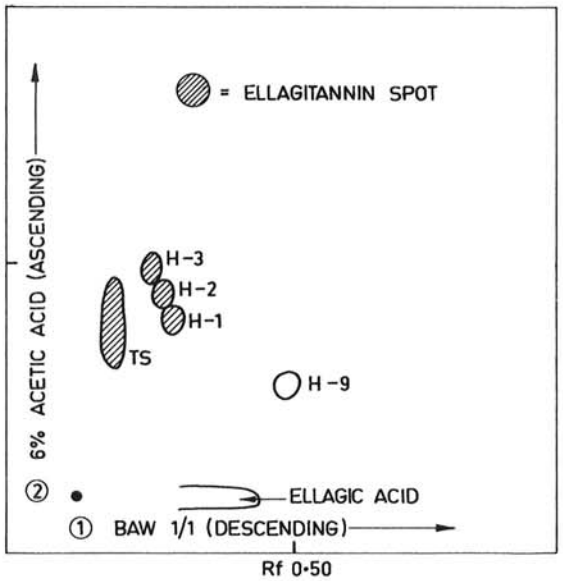

Fig. 1. Diagrammatic representation of the two-dimensional paper chromatographic separation of ellagitannins from the heartwood of white oak.

0.45. Aldehydes and ketones could not be detected, and their absence was unexpected, as previous work (4, 5) has shown similar extracts from *Q. alba* to contain a number of aromatic aldehydes. Catechins and leucoanthocyanins were not detected, although Hathway (9) found substantial amounts in oak bark.

Only five strong spots were located on the BAW X 6HA chromatograms of the acetone:water extract obtained from the Michigan tree. These were the ellagitannins H-1, H-2, and H-3, ellagic acid, and H-9 (Fig. 1). The extract from the Wisconsin tree was similar, but lacked H-9 and contained significant amounts of three additional compounds with R_F values > 0.75 in BAW. Chromogenic sprays indicated no aldehydes, ketones, and catechins in these extracts. Components of the hot-water extract were poorly resolved by BAW X 6HA; no further work was done with this extract because bioassay showed no fungitoxicity.

Water fractions of the acetone:water extract were similar for both trees and were primarily ellagitannins (Fig. 1, Table 2), as shown by paper chromatography. The water fraction of the acetone extract contained ellagitannins in lower concentration than the water fractions of the acetone:water extracts as well as a number of other compounds. Partial degradation in hot aqueous solution or hydrolysis in NaOH or H_2SO_4 gave large amounts of ellagic acid and moderate amounts of glucose, but no detectable gallic acid. Further characterization of the ellagitannins is given in Table 3. Cochromatography with the ellagitannins D-6 and D-13 from *Eucalyptus* sp. (10, 22) followed by development with NSSC showed H-1 and H-3 to be similar (if not identical) with D-6 and D-13, respectively. Very distinctive colors are formed between NSSC and the various ellagitannins (10). H-1, H-2, and H-3 form the same colors with NSSC at the same R_F values in BAW and 6HA as the *Eucalyptus* ellagitannins D-6, D-12, and D-13, respectively. Hence it appears that the ellagitannins in white oak are comprised of hexahydroxydiphenoyl (HHDP)-glucoses.

Bioassays.—In vitro tests with cultures of *P. versicolor* and *P. monticola* revealed that the acetone:water extract was the only toxic material (Table 4). The ellagitannin-containing fraction (water fraction) of this extract (as well as of the acetone extract) had even greater toxicity. When decay-susceptible wood was impregnated, only the acetone:water extract provided protection against decay by *P. monticola*, and none was effective in reducing decay by *P. versicolor* (Table 5). These results are in general agreement with the data obtained when the intact sapwood and heartwood were subjected to the same two decay fungi. Weight losses (% dry wt) were 42 and 23% for the sapwood

TABLE 2. Distribution of ellagitannins in extracts from white oak heartwood

Extract	Liquid-liquid fraction %[a]			Compounds in water fraction[b]	Hydrolysis products of water fraction
	Water-soluble	Ethyl acetate-soluble	Insoluble in either solvent		
Michigan tree					
Acetone	42	34	24	H-1, H-2, & H-3 plus many additional compounds	Not tested
Acetone:water	65	15	20	H-1, H-2, & H-3 TS, trace H-9	Ellagic acid, glucose
Wisconsin tree					
Acetone:water	68	14	18	H-1, H-2, H-3, TS	Ellagic acid, glucose

[a] The acetone and acetone:water extracts were suspended in water and continuously extracted with ethyl acetate in a liquid-liquid extractor.

[b] TS = tannin streak; H-1, H-2, and H-3 are code symbols for different ellagitannins; H-9 = code symbol for a compound of unknown chemical composition; see Fig. 1.

TABLE 3. Characterization of the ellagitannins from the heartwood of white oak

Component[a]	$R_F \times 100$ BAW	$R_F \times 100$ 6HA	Color[b] SUV	Color[b] UV
TS	4	30-50	Purple	nv[c]
H-1	13	42	Purple	nv
H-2	12	50	Purple	nv
H-3	9	55	Purple	nv
H-9	51	31	Intense blue	nv

Reaction with					
	NSSC		pNA		
$FeCl_3$-K_3 $Fe(CN)_6$	3 min	12 hr	Before overspray	After overspray	Gibbs' reagent
Blue	Pale pink, violet	Very pale tan			
Blue	Pink, orange changing quickly to orange brown	Gray brown	Brown	Tan	Tan
Blue	Purple	Yellow brown	Brown	Tan	Tan
Blue	Reddish purple	Gray brown	Brown	Tan	Tan
nr[d]	nr	nr	nr	nr	nr

[a] TS = tannin streak; H-1, H-2, and H-3 are code symbols for different ellagitannins; H-9 = code symbol for a compound of unknown chemical composition; see Fig. 1.
[b] In short- and long-wave ultraviolet light (SUV and UV, respectively).
[c] Not visible.
[d] No reaction.

TABLE 4. Growth of *Polyporus versicolor* and *Poria monticola* in cultures containing various extracts of the heartwood of white oak (1 mg/ml) and test compounds

Extract	Growth (% of control) of *P. versicolor*	Growth (% of control) of *P. monticola*
Acetone–Michigan tree	96	99
Water fraction of acetone extract	94	25**[a]
Acetone:water–Michigan tree	78*	66*
Water fraction of acetone:water extract	93	13**
Acetone:water–Wisconsin tree	51**	65**
Water fraction (1 mg/ml) of acetone:water extract (0.5 mg/ml)	95 nt[b]	8** 76**
Hot water	134*	106
Gallic acid	37**	113
Ellagic acid	68**	100
Ellagitannins (D-6 & D-13 + others) from the heartwood of *Eucalyptus regnans* F. Muell.	nt[b]	72*
PAS[c]	4**	0**

[a] ** or * indicates value differs significantly from control at 1% or 5% level, respectively.
[b] Not tested.
[c] Puratized agricultural spray.

and 31 and 13% for the heartwood when these tissues were exposed for 6 weeks to *P. versicolor* or *P. monticola*, respectively. The decay of heartwood by *P. monticola* was reduced to a greater degree (44 versus 26%) than was decay by *P. versicolor*.

TABLE 5. Weight loss of cottonwood blocks impregnated with various extractives from white oak heartwood exposed to *Polyporus versicolor* and *Poria monticola* for 4 weeks at 27 C

Treatment	Weight loss (% dry wt) *P. versicolor*	Weight loss (% dry wt) *P. monticola*
Acetone alone	29[a]	30
Acetone extract (0.7%)[b]	28	26
Acetone:water (9:1) alone	24	29
Acetone:water extract (2.94%)	27	16[c]
Hot water extract (2.24%)	23	31
PAS[d] (1 mg/ml)	4	0
Check (water)	26	28

[a] Each value is the average of five blocks.
[b] Percentage increase in initial block weight due to uptake of extractives.
[c] Statistically different from acetone:water alone, 1% level.
[d] Puratized agricultural spray.

The addition of PVP or Tween 80 to the growth medium totally overcame the fungistatic effects of the ellagitannins (Table 6). In fact, the combined presence of ellagitannins, PVP, and Tween 80 appears to have stimulated the growth of *P. monticola*.

DISCUSSION.–The inhibitory effect of the ellagitannins probably is due to a structurally critical interaction between the tannin molecule and the fungal proteins, with the pyrogallol grouping of the HHDP forming hydrogen bonds with the free amino and amido groups of the proteins. Tween 80 and PVP are capable of splitting tannin-protein complexes,

TABLE 6. Growth of *Poria monticola* in cultures containing ellagitannins[a] from white oak, PVP[b], and Tween 80 (polyoxyethylene sorbitan monooleate)

Treatment	Growth (mg dry wt) of *P. monticola* on Michigan tree extract	Wisconsin tree extract
Tween 80 (1,000 ppm)	77	71
Tween 80 + ellagitannins (1,000 ppm)	91	89
PVP (2,000 ppm)	63	76
PVP + ellagitannins	100	72
PVP + Tween 80	73	72
PVP, Tween 80 + ellagitannins	102	92
Ellagitannins	9	6
Check (growth in malt extract alone)	70	

[a] Water fraction of acetone:water extract; see Table 2.
[b] Polyvinyl pyrrolidone.

thus regenerating enzyme activity (8, 12). That the fungistatic effect of the ellagitannins could be completely overcome by the addition of PVP or Tween 80 (Table 6) indicates that the ellagitannin-protein complex was due to hydrogen bonds rather than to more stable complexes involving covalent bonds (8, 15). The tannin-protein may affect the membrane permeability of the fungus, or perhaps the tannin may be an uncoupling agent.

Rudman (19) has previously suggested that vicinal trihydroxy groups (of tannins) as well as a certain minimum molecular size are important factors in determining the toxicity of phenolics in the heartwood of certain species of *Eucalyptus*. Compounds of small molecular weight (i.e., gallic acid and ellagic acid) were not toxic to *P. monticola* in vitro (Table 4), nor did they increase the decay resistance of sawdust subsequently exposed to *P. monticola* (18, 20). In general, the minimum molecular weight for inhibitory activity of enzymes by tannins appears to be about 500 (26). The minimum molecular weight for HHDP-glucose would be 481, but the actual molecular weight of most of the previously studied ellagitannins is higher (22).

Zabel (27) showed that the amount and toxicity of the tannins in oak heartwood decreases radially from the outer heartwood towards the pith. This phenomenon has been explained in terms of polymerization of the phenolics involved (3). However, in the slightly acidic environment of oak heartwood, gradual detoxification may be achieved by hydrolysis of the ellagitannin into ellagic acid and glucose. Once decay begins, additional degradation of the tannins could occur via fungal hydrolases (16). Since the heartwood is dead, there would be no replacement of inhibitory materials to offset losses. Hence, the heartwood gradually would become more decay-susceptible as the fungistatic compounds were destroyed by enzymatic detoxification, polymerization, or hydrolysis.

As previously suggested (19, 27), durability of heartwood is dependent both on the toxicity of its extractives and on their concentration. The nondurable heartwood of *Eucalyptus regnans* contains the same toxic components (tannins) present in the extractives from the durable heartwoods of *E. microcorys* and *E. triantha*, but the concentration in *E. regnans* is much lower than in the latter two species (19). If the ellagitannins from *E. regnans* are present in high enough concentration (Table 4), they are capable of inhibiting the growth of *P. monticola*.

Ellagitannins were not present in detectable quantities in an acetone:water (9:1) extract of the heartwood (decay susceptible) of northern red oak (*Quercus rubra*), but a previous study (23) indicated they may occur in trace amounts. In the sapwood of white oak, ellagitannins are present, but in reduced amounts compared with the heartwood (Hart & Hillis, *unpublished data*). A twofold dilution of the water fraction (Table 4) almost completely eliminated any measurable inhibition against *P. monticola*. Other decay-susceptible woods may also contain ellagitannins or other fungistatic materials, but in too low a concentration to prevent decay. However, in white oak heartwood there apparently is an adequate amount (ca. 2%) of ellagitannins present to reduce the rate of decay by *P. monticola* and perhaps by other fungi.

With *P. monticola* there was a close correlation between inhibition measured in in vitro tests, in intact wood, and in impregnated wood. The weight loss of intact white oak heartwood caused by *P. monticola* was 44% less than that of the sapwood. Similarly, decay-susceptible wood after impregnation with about the same amount of ellagitannins as in oak heartwood had 45% less weight loss than the control (Table 5). On the other hand, with *P. versicolor*, while the intact heartwood was more durable than the sapwood and the acetone:water extract did inhibit fungal growth as measured in vitro (Table 4), it was impossible to demonstrate the fungistatic nature of any of the extracts when activity was assessed in impregnated wood blocks (Table 5). The reasons for this phenomenon are not known. Amongst the causes may be subcellular differences in distribution of the inhibitory components between the impregnated cottonwood blocks and intact oak heartwood, or the inhibitory effect may have been reduced by the woody substrate as recently demonstrated for pinosylvin (14).

Gallic and ellagic acids may be of some importance in the durability of white oak heartwood to *P. versicolor* (Table 4), but it is doubtful if they play a major role. The amount of gallic acid present was small and was contained in the nontoxic acetone fraction. A substantial amount of ellagic acid was present in both the acetone and acetone:water extracts, yet only the latter was toxic to *P. versicolor*. When either compound was incorporated into sawdust, the susceptibility of the wood to decay by a number of wood-decay fungi was not influenced.

In any case, both the intact heartwood of the Michigan tree and the extractives from it were less inhibitory to *P. versicolor* than to *P. monticola*. Possible reasons for this phenomenon are numerous.

The extracellular enzymes of *P. monticola*, a brown-rot fungus, and *P. versicolor*, a white-rot fungus, have been shown to differ in their mode of action and molecular size (25). The different extracellular enzymes of the two fungi may possess different receptor sites; i.e., they are stereospecific. Certainly there is a variation in the protein-tannin binding capacity of different tannin-protein mixtures, depending both on their molecular size and chemical structure. Rudman (20) previously showed that wood extractives do not always control the growth of a broad spectrum of fungal species, but may be quite specific.

Another possibility is that the toxic materials differ in distribution on different layers of the cell wall. *P. versicolor* first attacks the S_3 layer, then the S_2, and finally the S_1, whereas *P. monticola* starts with the S_2 layer (13, 25). A third possibility is that *P. versicolor* possesses a more effective polyphenol oxidase system for detoxification of the ellagitannins than does *P. monticola* (16). The isolate of *P. versicolor* used in this study possessed very strong laccase activity (rapid oxidation of α-naphthol), whereas the isolate of *P. monticola* produced only weak laccase activity. Neither fungus was positive for tyrosinase; both failed to oxidize *p*-cresol or tyrosine. Additional work will be required to elucidate these differences, and to determine whether the durability of white oak heartwood to other fungi is due to the presence of ellagitannins or to other factors.

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