Wood Decay Inhibition by Tropical Hardwood Extractives and Related Compounds

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ABSTRACT

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Pine blocks were impregnated with the extractives obtusaquinone, obtusastyrene, or lapachol, or with the synthetic compound 2-benzyl-4,6-di-*t*-butylphenol. The blocks were sterilized by steam or by exposure to ethylene oxide and then subjected to decay by *Gloeophyllum trabeum*, *Poria placenta*, or *Coriolus versicolor*. Obtusastyrene and obtusaquinone were most effective against the brown-rot fungi, controlling them at concentrations of 3 and 3.5%, respectively. Weight losses by *C. versicolor* were reduced from 23% (steamed blocks) to 4% by a 4% concentration of obtusastyrene and to 6% by a similar concentration of obtusaquinone. Lapachol was effective at its highest concentration (4%) only against *P. placenta*. Benzylphenol, at a similar concentration, reduced all decay-associated weight losses to 8% or below. Ethylene oxide sterilization resulted in decreased decay by *G. trabeum* and *P. placenta* on treated wood. In control (nontreated) wood however, no significant differences in decay susceptibility were detected between blocks sterilized by different methods.

The heartwood of some trees contains toxic substances that render it resistant to attack by decay fungi. The isolation of these extractives, determination of their toxicity to fungi, insects, and marine borers, and identification of their chemical structures may lead to development of new, environmentally safe wood preservatives. The need for such investigations is reflected by the concern of environmental groups and governmental agencies over the potential health and ecological hazards associated with some of the wood preservatives in current use.

Recent biological and chemical studies of the natural resistance of wood to biodeterioration has dealt largely with wood from tropical forests. For example, a workshop held in 1974 (4) brought together 35 international collaborators studying the chemistry of the natural bioresistance of tropical woods—predominantly woods native to Panama—and the effect of their protective constituents (extractives) on biological systems. Many of the studies reported at that workshop dealt with investigations of the toxicity of the extractives obtusaquinone isolated from *Dalbergia retusa* Hemsl. (cocobolo), obtusastyrene isolated from *D. obtusa* Lecomte, and lapachol isolated from *Tabebuia guayacan* (Seem.) Hemsl. (guayacan) to marine animals (4,16), marine fungi (5,9), insects (7), and terrestrial mold fungi (10). However, with the exception of lapachol (12), these compounds have not been tested for toxicity to terrestrial wood decay fungi.

The present laboratory tests were conducted to determine the protection afforded wood against typical wood decay fungi (Basidiomycetes) through its impregnation with the preceding extractives. In addition a phenolic compound, 2-benzyl-4,6-di-*t*-butylphenol, synthesized by Jurd (*unpublished*) was included in the testing.

MATERIALS AND METHODS

Preparation of test blocks. Sapwood of ponderosa pine (*Pinus ponderosa* Laws.) was milled into 1.9-cm (0.75-in.) cubes. These were stored in an atmosphere of 70% relative humidity (RH) until they reached equilibrium moisture content and then were individually weighed. The blocks were then packed, 15 at a time, in a pressure/vacuum chamber with nylon mesh spacers to keep them separated. After charging, the chamber was evacuated to about 4 mm and kept at this vacuum for 1 hr to remove the air from the blocks. The impregnating solution, consisting of the test chemical in acetone solvent, was then introduced into the evacuated chamber and the chamber was pressurized with nitrogen gas at 689.5 kPa (100 lb/in.^{2}) for an additional hour. The treated blocks were removed one at a time, rapidly wiped of excess solution, and immediately reweighed to obtain the amount of solution pickup. This weight was converted to volume by dividing by the density of the acetone (0.79 g/cm^3). The absolute quantity (hence percent pickup) of the solute in each cube could then be determined from its solution concentrations. The quantity of acetone evaporated during this weighing process never exceeded 0.01 g and it was insignificant relative to establishing the concentration of the solute in wood. Control blocks were prepared by impregnation with solvent only. Prior to placement into decay test, the blocks were aired for 10 days, then reconditioned at 27 C and 70% RH and reweighed to establish a decay weight loss baseline.

Chemical impregnation. Tested chemicals were obtusaquinone, obtusastyrene, lapachol, and 2-benzyl-4,6-di-*t*-butylphenol. In preliminary tests, test blocks were impregnated at two concentrations: ~1.0% and either 2.0, 2.5, or 3.0% of the wood weight. Final tests included concentrations of about 3.5 and 4.0% plus, and in the case of obtusastyrene and the benzylphenol compound, 3.0%. Structural formulas for each of the test compounds are given in Fig. 1.

Decay testing. The effect of chemical impregnation of the wood upon its decay resistance was determined by use of the ASTM standard method of testing wood preservatives (1). The test fungi were *Gloeophyllum trabeum* (Pers.) Murr. (isolate Mad-617); *Poria placenta* (Fr.) Cke. (isolate Mad-698), and *Coriolus versicolor* L. ex Fr. (isolate Mad-697).

In preliminary tests, the blocks were steamed for 5 min before being placed in soil bottles for exposure to decay fungi. A short sterilization procedure was deemed advisable to ensure little or no breakdown of the treating chemicals. However, due to contamination problems encountered, subsequent testing included sterilization of blocks either by steaming for 20 min, as recommended in the ASTM test method (1), or by exposure to

ethylene oxide. The latter was carried out by placing control and treated blocks in rubber-stoppered glass cylinders (Fig. 2). Tubing was inserted into the rubber stoppers. Cylinders were placed into desiccators and maintained at 100% RH for 48 hr. Four milliliters of ethylene oxide were then introduced into each cylinder. Excess gas was bled off and the cylinders were sealed for 48 hr. Following this, the cylinders were flushed for 72 hr with filtered air.

Following 12 wk of exposure to test fungi, the blocks were again conditioned and weighed and their weight losses were calculated.

RESULTS AND DISCUSSION

The decay resistance imparted to the wood blocks by impregnation with the test compounds was measured by the percent of weight loss in blocks after 12 wk of exposure to wood decay fungi (Tables 1 and 2).

Preliminary decay test. The results of initial tests in which pine blocks were impregnated with low concentrations of test compounds are provided in Table 1. Inhibition of fungal activity varied from almost nil with obtusaquinone (at a concentration of 1.0%) and lapachol (0.9%) to highs between 71 and 80% —dependent upon the test fungus—obtained with the benzylphenol compound at a concentration of 3.2%. Since all concentrations of each test compound were deemed insufficient to effect control of decay, further testing at higher concentration of solutes in the test blocks was initiated.

Mold contamination was severe in many of the blocks—particularly those infected with *C. versicolor*, resulting in the omittance in Table 1 of test data associated with this fungus. Consequently more effective sterilization techniques were necessary.

Final decay test. *Statistical details.* Average percent weight losses sustained in the final decay test are included together with their standard deviations in Table 2. As can be noted, the values obtained for the various treatments had greatly differing variances. Such heterogeneity of variance can affect the validity of statistical tests. To help solve this problem a transformation of the data was performed. Steel and Torrie (15) recommend a square root transformation for data consisting of percentages primarily between zero and 20%. This transformation was used on the data and the treatments on this scale proved to have equal variances.

Effect of sterilization method. As expected, both methods of sterilization effectively controlled contamination. When weight losses were compared, no significant differences in magnitude were found between steamed and gassed blocks when *C. versicolor* was used as the test fungus (Table 2). However, with *G. trabeum* and *P. placenta* significantly lower ($P < 0.01$) weight losses occurred in the treated blocks that were gas-sterilized as compared to those that were steamed. Measurements of control (nontreated) blocks showed no significant difference between sterilization methods. The use of propylene oxide as a sterilant on creosote-treated wood has had a similar effect on *Lentinus lepideus* (8,13). However, when ethylene oxide was used on nontreated ponderosa pine and red alder, the decay efficacy of four wood-decay fungi was not affected (14). Gas sterilization apparently affects toxicity of wood—generally preservative-treated wood—on a very selective basis and then only a few specific fungi.

Effect of treatment upon wood decay. Obtusastyrene controlled both brown-rot fungi, *G. trabeum* and *P. placenta* completely at a concentration of 3.5%. White rot, which is caused by *C. versicolor*, was restricted to a weight loss of 4% (8.0% in gassed blocks) with an

TABLE 1. Weight losses in pine wood blocks treated with low concentrations of antifungal compounds and then exposed for 12 wk to two wood decay fungi

Compound retention (%)	Percent weight loss[a]	
	G. trabeum	*P. placenta*
Control (acetone solvent)	46.5	58.7
Obtusaquinone		
1.0	42.7	53.9
2.1	42.3	37.1
Obtusastyrene		
1.0	13.5	52.4
2.5	8.9	37.1
Lapachol		
0.9	41.7	52.9
2.9	41.8	49.4
Benzylphenol[b]		
1.0	37.2	52.9
3.2	13.3	12.3

[a] All weight loss figures are averages of five replications.
[b] 2-benzyl-4,6-di-*t*-butylphenol.

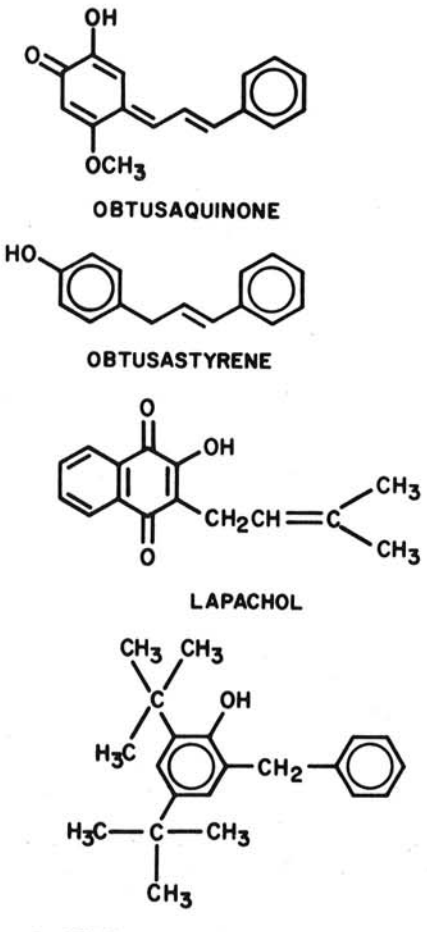

Fig. 1. Structural formulas of compounds tested for effectiveness against wood-decaying fungi.

Fig. 2. Apparatus used to gas-sterilize wood decay test blocks. The glass cylinder is sealed at each end and the blocks are undergoing gas treatment.

TABLE 2. Weight losses in pine wood blocks treated with higher concentrations of antifungal compounds, steam- or gas-sterilized, then exposed for 12 wk to three wood decay fungi

Compound retention (%)	Percent weight loss[a]					
	G. trabeum		*P. placenta*		*C. versicolor*	
	Steamed	Gassed	Steamed	Gassed	Steamed	Gassed
Control (acetone solvent)	32.7 (12.1)	23.5 (10.5)	38.2 (6.6)	39.0 (4.3)	23.3 (5.5)	25.1 (6.6)
Obtusaquinone						
3.5	4.0 (4.0)	1.5 (1.6)	0.2 (0.3)	0.1 (0.2)	9.0 (5.4)	7.5 (3.0)
4.0	5.6 (6.0)	1.4 (3.2)	0.1 (0.1)	0.0 (0.0)	6.0 (6.4)	3.0 (3.1)
Obtusastyrene						
3.0	0.9 (1.2)	0.0 (0.0)	1.7 (1.4)	0.0 (0.1)	9.3 (5.5)	11.0 (4.4)
3.5	0.2 (0.2)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	4.5 (2.7)	9.4 (2.5)
4.1	0.3 (0.2)	0.3 (0.6)	0.4 (0.7)	0.0 (0.0)	4.1 (3.3)	8.0 (4.2)
Lapachol						
3.5	22.0 (6.5)	11.6 (6.3)	1.5 (1.0)	0.8 (0.8)	7.2 (4.9)	10.2 (2.6)
4.0	27.9 (7.7)	25.6 (10.2)	3.8 (3.4)	1.6 (1.6)	10.3 (6.4)	9.7 (4.8)
Benzylphenol[b]						
3.0	25.5 (11.4)	8.1 (4.3)	4.2 (2.5)	0.8 (1.2)	8.0 (1.6)	9.0 (1.6)
3.4	14.4 (6.1)	8.2 (3.3)	5.5 (2.1)	1.1 (0.9)	8.3 (3.7)	10.0 (2.4)
3.9	6.6 (3.6)	2.0 (1.0)	3.7 (2.6)	0.6 (0.6)	4.9 (3.2)	8.2 (5.7)

[a] All weight loss figures are averages of five replications except for results of obtusaquinone tests at 3.5%, which are based on 10 replications. Figures in parentheses are standard deviations.
[b] 2-benzyl-4,6-di-*t*-butylphenol.

obtusastyrene concentration of about 4% (Table 2). At $P = 0.05$, obtusastyrene was significantly better than all the other treatments; however, at $P = 0.01$ it did not differ significantly from obtusaquinone. This effect may be due to the large values obtained for obtusaquinone-treated, steamed blocks exposed to *G. trabeum*, when compared to similar values for obtusastyrene. Both obtusastyrene and obtusaquinone were significantly better ($P < 0.01$) than lapachol and benzylphenol. In field trials against cooling tower fungi (soft-rot fungi), obtusastyrene has proved more effective than obtusaquinone and lapachol in protecting wood blocks impregnated with these compounds (9). In laboratory trials also, obtusastyrene more effectively inhibited growth and reproduction of *Chaetomium* and *Pestalotia* than obtusaquinone (5).

Obtusaquinone controlled *P. placenta* decay at a concentration of 3.5%. At a 4% loading, decay caused by *G. trabeum* and *C. versicolor* was reduced to the 5–6% level (Table 2). This compound is present in *Dalbergia retusa* in a concentration of about 3% of the weight of the wood and is considered to be the most effective chemical isolated from this species to date (4). In addition, *D. retusa* has been reported to be the most borer- and decay-resistant wood tested among 112 different tropical woods (4). Consequently one would expect obtusaquinone to perform more effectively than shown here (Table 2). This indicates that other extractives present in this wood, such as isoflavone and dalbergioquinol (4), may contribute more than expected to the decay resistance of *D. retusa*. Four phenolic compounds isolated from incense cedar heartwood were found by Anderson et al (2) to contribute greater resistance to wood in mixture than the sum of their separate effects. A similar synergistic effect may be operative relative to *D. retusa* heartwood extractives.

Lapachol, the major extractive associated with the decay-resistant heartwood of *T. quayacan* (6), was less effective than any of the other compounds tested. Wood impregnated with lapachol also was less resistant to cooling tower soft-rot fungi than that treated with either obtusaquinone or obtusastyrene (9). In the present study, at the highest retention (4%) lapachol essentially controlled only *P. placenta*. However, while it had little effect against *G. trabeum* at that concentration, it did reduce decay by *C. versicolor* to somewhat less than half of that occurring in the controls (Table 2). Rudman (12) used impregnated sawdust rather than wood blocks and found that 1% (w/w) of lapachol reduced decay caused by *G. trabeum* and *P. placenta* (*P. monticolor*) from 100% (controls) to 47 and 45%, respectively. However, in the present work, a 0.9% retention of lapachol reduced decay by only about 10% of that induced in the controls (Table 1). These differences are assumed to be due to the dissimilarity in methods employed in the two studies. However, Rudman (11), by using his sawdust technique to test decay inhibitive activity of the extractives λ-thujaplicin and carvacrol, found that his results agreed with those of Anderson et al (3), who used wood blocks to test these same extractives. In comparing the two results, however, we find that while the weight losses for wood blocks impregnated with λ-thujaplicin were similar for both methods used, the results obtained with the carvacrol were dissimilar. Sawdust impregnated with carvacrol underwent weight losses at 49 and 37% when attacked by *G. trabeum* (*L. trabea*) and *Lentinus lepideus*, respectively (12). In impregnated wood blocks these losses were 18 and 20%.

The benzylphenol compound was significantly more effective than lapachol. At a retention of 3.9%, all weight losses were reduced to 8% or below. As with obtusaquinone and lapachol, it proved most effective against *P. placenta* (Table 2).

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