

**The Inhibitory Effects of Some
Cinnamylphenols on the Marine Fungi
Dendryphiella Salina and *Corollospora Maritima***

C. A. BAILEY, K. K. PARRISH, AND J. D. BULTMAN

*Marine Biology and Biochemistry Branch
Ocean Sciences Division*

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<p>Nineteen cinnamylphenols were evaluated for fungicidal worth against the marine fungi <i>Dendryphiella salina</i> and <i>Corollospora maritima</i>. 2-Cinnamyl-4-nonylphenol and 2-phenyl-4-cinnamylphenol were completely ineffective against <i>Dendryphiella</i>; the remaining cinnamylphenols inhibited both fungi to varying degrees. 4-Cinnamylphenol, 4-(3-phenylpropyl)phenol, 2-cinnamyl-4,5-methelene-dioxyphenol, and 2-cinnamyl-4-methylphenol effectively inhibited both fungi, the criterion for effective inhibition being defined as 50% at 32 ppm; 2-cinnamyl-</p>		

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20. Abstract (Continued)

4-methylphenol and 2-cinnamyl-4-nonylphenol met this criterion against *Corollospora*. *Corollospora* was more sensitive to the cinnamylphenols than *Dendryphiella* at equivalent concentrations. There was some relationship between chemical structure and the biological activity of these compounds.

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**THE INHIBITORY EFFECTS OF SOME CINNAMYLPHENOLS
ON THE MARINE FUNGI *DENDRYPHIELLA SALINA*
AND *COROLLOSPORA MARITIMA***

INTRODUCTION

The Naval Research Laboratory (NRL) is engaged in developing a new, more effective method of protecting wood in marine service from destruction by borers. One approach involves evaluation of the natural resistance of Central American hardwoods toward these organisms and a study of the nature of this resistance. Only a handful of the 112 woods evaluated [1] withstood borer attack, and for some of these woods refractoriness toward the borers may be the consequence of a primary resistance to lignicolous marine fungi. These organisms are distributed ubiquitously; at least 121 species occur in the waters of 44 countries and the Panama Canal Zone [2]. Their role in the decomposition of wood and other cellulose in the marine environment is well documented [3-6]. Some investigators feel that lignicolous marine fungi, in addition to their direct involvement in the decomposition of wood, may contribute to the successful infestation of wood by teredinids and other marine wood-destroyers. Theoretically they precondition the wood surface, making it more hospitable to settling borer larvae. This hypothesized relationship between marine borers and marine fungi has evoked diverse opinions [7-10]; if such a relationship does exist, it is probably restricted to the teredinids. However, the concept is worth considering, because it could lead to a new approach toward protecting wood in marine service. Consequently, in the present NRL study of woods that are naturally marine-borer resistant, extractives obtained from these woods, and related synthetic derivatives, are evaluated for marine fungicidal worth. This report describes the evaluation of 19 cinnamylphenols as potential fungicides toward two lignicolous marine fungi.

EXPERIMENTAL PROCEDURE

The fungi used in this study were the ascomycete *Corollospora maritima* and the imperfect fungus *Dendryphiella salina*. Both fungi were obtained as agar slants: *Corollospora* from the American Type Culture Collection, serial 16932, and *Dendryphiella* from Portsmouth Polytechnic, Portsmouth, England. The fungi were subcultured on a modified glucose-yeast extract (GYE) medium (Table 1) in which seawater was substituted for distilled water; because of the natural occurrence of $MgSO_4$, $FeSO_4$, and KCl in seawater, they were omitted from the recipe. This nutrient medium was also used to prepare the experimental and control plates.

Table 1—Modified glucose-yeast extract medium used to prepare the assay plates

Component	Amount
Seawater	1 liter
Glucose	5.0 g/liter
Yeast extract	1.0 g/liter
$NaNO_3$	6.0 g/liter
Agar	15.0 g/liter

Stock solutions of the cinnamylphenols were prepared by dissolving 328 mg of each compound in 32 ml of absolute ethyl alcohol. From these stock solutions, volumes appropriate to provide final solute concentrations of 2, 8, 32, 128, and 512 ppm were transferred to 150-ml volumes of the nutrient medium; additional ethyl alcohol was added to each to adjust the final volume to 160 ml and, simultaneously, to adjust the final alcohol volume to 10 ml. Nutrient medium containing only alcohol comprised the solvent control.

The nutrient medium, with and without added cinnamylphenols, was autoclaved at 140 kPA (20 psi) for 20 minutes and transferred to 15-by-100-mm petri plates. When solidified, medium containing the higher concentrations of the cinnamylphenols remained homogeneously opaque. The experimental plates, the solvent-control plates containing the alcohol, and the control plates containing only the nutrient medium were prepared in quadruplicate, the fourth plate being reserved for contingencies and to measure initial pH. To determine the stability of these chemicals to autoclaving, an additional set of plates were prepared from autoclaved medium to which the compounds were added aseptically at 8 ppm just before the plates were poured.

The center of each experimental plate was inoculated with a 6-mm circular plug of sporulating mycelium taken from the periphery of actively growing 3-to-4-week-old stock cultures of the fungi. The inoculated plates were incubated at room temperature in a humid environment (within large, closed glass jars) to prevent desiccation of the agar. After an induction period of 2 to 3 days to allow the new colonies to become established, the diameter of maximum growth for each colony was selected, and daily growth was measured along this diameter. Since the two fungi grew at different rates on the control plates, the experiments varied temporally. The pH of the medium in each set of plates was determined at the start and the termination of the experiment. When the fungi did not continue to grow on the inoculum plug, or did not grow from the plug onto the experimental medium, the fungus was subcultured to determine if lack of growth was caused by a fungistatic or fungicidal action by the particular cinnamylphenol in the medium.

Finally, in comparing the relative merits of potential fungicides, it is useful to establish a level of fungicide performance as effective inhibition of the organisms. This is a subjective judgment. In the present study a 50% inhibition in fungal growth at a solute concentration of 32 ppm, as compared to the growth on the control plates, was arbitrarily selected as the criterion for effective inhibition.

RESULTS AND DISCUSSION

General Comments

The chemical names and formulas of the cinnamylphenols are presented in Table 2, and their abilities to inhibit the growth of *Dendryphiella* and *Corollospora* are presented in Table 3. The data show anomalies regarding the inhibitory effects imposed upon the fungi by some of the cinnamylphenols. Undoubtedly some of the anomalies can be attributed to experimental error, such as temperature fluctuations, pH changes during growth, accumulation of detrimental metabolic waste products, or nonhomogeneous dispersion of the solute

in the medium; others may be related to the inherent variability associated with experimentation upon living systems. However, adequately dispersing insoluble material in an aqueous medium is always a problem, so that nonhomogeneity of the solute is most probably the major cause of these observed anomalies. In subsequent work other dispersion techniques will be tried when preparing the experimental medium.

Comparison between the fungal growth on media containing the unautoclaved cinnamylphenols and on media which were autoclaved with the compounds present at the same concentration (8 ppm) indicated that autoclaving had no deleterious effect upon these compounds. Also, fungal growth on the solvent controls was not significantly different from that on the basic controls.

The initial pH of the medium in the plates varied from 6.5 to 7.0; the final pH at the termination of the experiments varied from 6.5 to 8.4. The rise in pH was due primarily to an accumulation of nitrogenous waste products, and the more vigorously growing cultures produced a larger pH change. When no growth occurred, the pH did not change.

When fungi which remained viable on the inoculating plugs but did not grow onto the test agar were subcultured on a suitable medium, normal growth resumed. Confinement of growth to the plugs suggests that the cinnamylphenols in these cases were functioning fungistatically. When the fungi with no apparent growth on the inoculating plugs were subcultured, neither the hyphae nor the spores germinated.

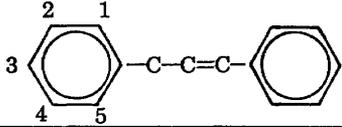
Only six of the cinnamylphenols were able to meet or exceed the established criterion for effective fungal inhibition (50% inhibition at a solute concentration of 32 ppm). Four of these, 4-cinnamylphenol (compound I, obtusastylene), 4-(3-phenylpropyl)phenol (II, dihydro-obtusastylene), 2-cinnamyl-4,5-methylenedioxyphenol (X), and 2-methyl-4-cinnamylphenol (VI) were effective against both organisms; 2-cinnamyl-4-methylphenol (XII) and 2-cinnamyl-4-nonylphenol (XVI) were effective against only the more sensitive *Corollospora*. Several of the other cinnamylphenols provided better than 50% inhibition of fungal growth at 128 ppm or 512 ppm, but at these solute concentrations these compounds are not considered to be fungicidal. Also, those compounds which inhibited *Corollospora* by 50% or more at solute concentrations of 2 ppm and 8 ppm but whose inhibition was not sustained at 32 ppm are not considered as effective inhibitors.

The Cinnamylphenols and *Dendryphiella salina*

Colony Development

In most cases the developing mycelium covered the inoculating plug and spread onto the test medium, extending radially toward the periphery of the plate. The initial lag of 2 to 3 days was followed by measurable hyphal growth, and within 3 to 4 days sporulation began close to the plug, moving outward as growth progressed. The dark spores gave the mycelial mat a gray-green color; a 2-mm hyaline band surrounded the periphery of each mat. Fungal growth on the control media took 1 to 2 weeks to cover the plates. As the colonies aged and expanded, copious growth of the fungi produced white aerial hyphae which gave the mats a floccose appearance. When severe fungal inhibition occurred, apical growth was restricted,

Table 2—Chemical names and formulas of the cinnamylphenols evaluated

Compound						
No.	Name	1	2	3	4	5
I	4-cinnamylphenol (obtusastylene)	H	H	OH	H	H
II	4-(3-phenylpropyl)phenol	Compound I with a saturated propene bridge				
III	2,3-dihydroxy-4-cinnamylphenol	OH	OH	OH	H	H
IV	3-hydroxy-4-cinnamylphenol	OH	H	OH	H	H
V	2-isopropyl-4-cinnamyl-5-methylphenol	CH ₃	H	OH	CH(CH ₃) ₂	H
VI	2-methyl-4-cinnamylphenol	H	CH ₃	OH	H	H
VII	2,6-dimethyl-4-cinnamylphenol	H	CH ₃	OH	CH ₃	H
VIII	2-phenyl-4-cinnamylphenol	H	C ₆ H ₅	OH	H	H
IX	2,6-di-t-butyl-4-cinnamylphenol	H	C(CH ₃) ₃	OH	C(CH ₃) ₃	H
X	2-cinnamyl-4,5-methylenedioxyphenol	OH	H	O-CH ₂ -O		H
XI	2-cinnamyl-4-hydroxy-5-methoxyphenol	OH	H	CH ₃ O	OH	H
XII	2-cinnamyl-4-methylphenol	OH	H	H	CH ₃	H
XIII	2-cinnamyl-4-ethylphenol	OH	H	H	C ₂ H ₅	H
XIV	2-cinnamyl-4-methoxyphenol	OH	H	H	CH ₃ O	H
XV	2-cinnamyl-4-butylphenol	OH	H	H	C(CH ₃) ₃	H
XVI	2-cinnamyl-4-nonylphenol	OH	H	H	C ₉ H ₁₉ *	H
XVII	2-cinnamyl-4,6-di-t-butylphenol	OH	C(CH ₃) ₃	H	C(CH ₃) ₃	H
XVIII	2-cinnamyl-4-methyl-6-t-butylphenol	OH	C(CH ₃) ₃	H	CH ₃	H
XIX	2-methoxy-4-(2-propenyl)-5-cinnamylphenol	H	OH	CH ₃ O	H	CH ₂ CH=CH ₂

*nonyl.

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Table 3—Percent inhibition of growth of *Dendryphiella salina* and *Corollospora maritima* as a function of the concentration of the cinnamylphenols evaluated. Shading indicates inhibition of at least 50%.

Com- pound No.	Inhibition of Growth (%) as a Function of Solute Concentration									
	<i>Dendryphiella salina</i>					<i>Corollospora maritima</i>				
	2 ppm	8 ppm	32 ppm	128 ppm	512 ppm	2 ppm	8 ppm	128 ppm	32 ppm	512 ppm
I	0	3	67	100	100	0	86	86	100	100
II	6	1	71	100	100	0	100	100	100	100
III	19	33	28	50	61	26	25	37	33	18
IV	4	10	15	21	16	0	8	0	14	0
V	30	27	33	41	70	8	12	4	50	85
VI	0	27	62	70	80	59	34	63	42	53
VII	17	10	4	3	32	54	54	49	62	100
VIII	0	0	0	0	0	22	36	38	33	69
IX	14	21	22	33	38	42	26	45	38	39
X	29	25	75	79	68	73	83	92	100	100
XI	0	11	10	11	14	0	14	5	12	7
XII	1	12	48	58	81	42	51	92	93	100
XIII	12	13	41	47	51	27	48	36	45	51
XIV	13	11	14	40	50	65	51	49	59	100
XV	13	40	45	51	68	18	5	9	41	42
XVI	0	0	0	0	0	31	18	59	60	81
XVII	15	17	18	31	41	26	18	34	35	47
XVIII	18	19	20	24	31	41	22	42	55	41
XIX	1	3	14	26	51	0	0	8	46	39

the fungi forming small, compact, circular mats with heavy sporulation about the inoculating plugs. Hyphal or spore forms with physical abnormalities did not develop on any of the plates except those composed of medium containing 2-methyl-4-cinnamylphenol (VI) or 2,6-di-*t*-butyl-4-cinnamylphenol (IX); in these cases the spores developed a light yellow-green color which increased in intensity as the solute concentration increased.

Growth Inhibition

Dendryphiella was generally more resistant toward the cinnamylphenols than *Corollospora*. The cinnamylphenols most effective in inhibiting the growth of *Dendryphiella* were 4-cinnamylphenol (I) and 4-(3-phenylpropyl)phenol (II). Both compounds met the criterion for effective inhibition of fungal growth of *Dendryphiella* at 32 ppm and completely inhibited its growth at 128 ppm. Only two other phenols, 2-methyl-4-cinnamylphenol (VI) and 2-cinnamyl-4,5-methylenedioxyphenol (X), were able to inhibit *Dendryphiella* effectively at 32 ppm; nine others were inhibitory at 512 ppm, and only I and II were able to inhibit the growth of the fungus completely and then only at 128 ppm and 512 ppm. Finally, 2-phenyl-4-cinnamylphenol (VIII) and 2-cinnamyl-4-nonylphenol (XVI) were completely ineffective at preventing fungal growth at any concentration. Although not completely ineffective, 3-hydroxyl-4-cinnamylphenol (IV) and 2-cinnamyl-4-hydroxy-5-methoxyphenol (XI) possessed virtually no fungicidal activity toward either fungus.

The Cinnamylphenols and *Corollospora maritima*

Colony Development

Corollospora grew more slowly than *Dendryphiella*, and growth on the plates was allowed to continue 4 to 8 weeks before the experiments were terminated. While expanding, the fungal colonies formed concentric zones of white hyphae and black sporulating hyphae. Asexual sporulation began 7 to 14 days after initial growth onto the plates, and some colonies developed a slimy, water-logged mycelium. Normal spore and hyphal development occurred on all plates except those with 2-methyl-4-cinnamylphenol (VI) in the medium. The fungus on these plates developed a cream color; minimal or no sporulation was produced.

Growth Inhibition

The most effective compounds inhibiting the growth of *Corollospora* were also 4-cinnamylphenol (I) and 4-(3-phenylpropyl)phenol (II), although four others met the criterion for effective inhibition at 32 ppm. The 4-cinnamylphenol (I) inhibited the growth of *Corollospora* by 86% at 8 and 32 ppm and completely at 128 ppm, and 4-(3-phenylpropyl)phenol (II) inhibited this fungus completely at 8 ppm, which makes this compound the most potent of the phenols in this series. In addition, 2-cinnamyl-4,5-methylenedioxyphenol (X) and 2-cinnamyl-4-methylphenol (XII) both inhibited growth of *Corollospora* by 92% at 32 ppm, with the former compound (X) still being effective at a solute concentration of 2 ppm. Unlike some of the other cinnamylphenols that appeared to be very inhibitory at 2 ppm, the performance of the methylenedioxy derivative at the higher solute concentrations suggests that its performance at 2 ppm is not anomalous. Although not quite

meeting the criterion of effective inhibition at 32 ppm, 2,6-dimethyl-4-cinnamylphenol (VII) and 2-cinnamyl-4-methoxyphenol (XIV) completely inhibited this fungus at a solute concentration of 512 ppm.

Generally, *Corollospora* was more sensitive to the cinnamylphenols at equivalent solute concentrations than *Dendryphiella*. For example, the average level of inhibition of *Dendryphiella* was 31% at 32 ppm and 52% at 512 ppm; for *Corollospora* the corresponding averages were 44% and 62%. Why *Corollospora* is more sensitive is not known; however, it might be related to the slower growth of this fungus, which gives it a greater opportunity to assimilate the solute. To reiterate, the most striking difference in behavior between these fungi was their response to 2-phenyl-4-cinnamylphenol (VIII) and 2-cinnamyl-4-nonylphenol (XVI). These compounds were completely ineffective in inhibiting *Dendryphiella* at any solution concentration, but the 4-nonyl derivative (XVI) effectively inhibited *Corollospora* at 32 ppm, and the 2-phenyl derivative (VIII) exerted some inhibitor effect on the fungus, particularly at 512 ppm.

Structural Relationships

Important information to be abstracted from the evaluation of the biological activity of an analogous series of compounds concerns the relationships between the activity and the kind and/or placement of substituent groups in the chemical structure of the parent compound, in this case 4-cinnamylphenol (I). Unfortunately this series of cinnamylphenols provided little information on the effects of substituent groups.

Generally the simpler compounds with the smaller substituents, such as ethyl or methyl groups, on the phenolic ring were the most effective against both fungi. An increase in hydroxylation of the phenolic ring decreased the inhibitory effects of 4-cinnamylphenol (I), particularly toward *Corollospora*. 3-Hydroxy-4-cinnamylphenol (IV) and 2,3-dihydroxy-4-cinnamylphenol (III) were also found to be 2 to 4 times less effective than 4-cinnamylphenol (I) toward a wide variety of other microorganisms (11).

The most dramatic change in activity resulting from molecular alteration occurred with the opening of the methylenedioxy ring of 2-cinnamyl-4,5-methylene-dioxyphenol (X) to form 2-cinnamyl-4-hydroxy-5-methoxyphenol (XI). For *Dendryphiella* at 32 ppm, inhibition of growth decreased from 75% for the former compound to 10% for the latter; for *Corollospora*, inhibition decreased from 92% to 5% respectively.

The outstanding structural feature of these compounds is the steric hindrance of the phenolic hydroxyl; however, there was no strong contribution by this structural feature toward biological activity. The highly hindered phenols were generally more inactive than the partially hindered phenols; however, within the latter groups activity ranged from poor to good.

SUMMARY

Nineteen cinnamylphenols were evaluated as fungicides against the obligate marine fungi *Dendryphiella salina* and *Corollospora maritima*. With the exception of 2-phenyl-4-cinnamylphenol (VIII) and 2-cinnamyl-4-nonylphenol (XVI), which were completely ineffective against *Dendryphiella* at all solute concentrations, all of these compounds inhibited the growth of both fungi to some degree. However, only four compounds, 4-cinnamylphenol (I, obtusastylene), 4-(3-phenylpropyl)phenol (II-dihydro-obtusastylene), 2-methyl-4-cinnamylphenol (VI), and 2-cinnamyl-4,5-methylenedioxyphenol (X) were considered as effective inhibitors of the growth of both fungi, the arbitrarily selected criterion for effective inhibition being 50% at 32 ppm; 2-cinnamyl-4-methylphenol (XII) and 2-cinnamyl-4-nonylphenol (XVI) met this criterion with *Corollospora*. The most active compounds toward both fungi were 4-cinnamylphenol (I), which completely inhibited their growth at 128 ppm, 4-(3-phenylpropyl)phenol (II), which completely inhibited growth of *Dendryphiella* at 128 ppm and growth of *Corollospora* at 8 ppm, and 2-cinnamyl-4,5-methylenedioxyphenol (X), which inhibited the growth of *Corollospora* by 73% at 2 ppm. The least effective compounds toward both fungi were 3-hydroxy-4-cinnamylphenol (IV) and 2-cinnamyl-4-hydroxy-5-methoxyphenol (XI). Generally *Corollospora* was more sensitive to the cinnamylphenols than *Dendryphiella* at equivalent solute concentrations.

There was some relationship between chemical structure and activity. The simpler compounds were frequently more effective, and an increase in the hydroxylation of the phenolic ring of 4-cinnamylphenol (I) decreased its activity. A marked decrease in activity toward both fungi occurred with the opening of the methylenedioxy ring of 2-cinnamyl-4,5-methylenedioxyphenol (X) to form 2-cinnamyl-4-hydroxy-5-methoxyphenol (XI). Also, the highly hindered phenols were more inactive than the partially hindered phenols; however, within the latter group, activity ranged from poor to good. Much of the anomalous experimental data probably resulted from nonhomogeneous dispersion of the insoluble phenols in an aqueous medium; in future work other dispersion techniques will be tried.

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