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Algicidal Activity of Stilbene Analogues

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Continuing our search for natural product and natural product-based compounds for the control of off-flavor in catfish, 29 stilbene analogues were synthesized and evaluated for algicidal activity against the 2-methylisoborneol (MIB)-producing cyanobacterium *Oscillatoria perornata*. The cis and trans isomers of 4-(3,5-dimethoxystyryl)aniline showed moderate and selective algicidal activity toward *O. perornata* with the lowest observed inhibitory concentration and lowest complete inhibition concentrations of 10 μM. This is the first report on selective stilbene algicidal activity toward a MIB-producing cyanobacteria species.

KEYWORDS: Algicide; stilbenes; resveratrol; pterostilbene; cyanobacteria; off-flavor

INTRODUCTION

Aquaculture is an integral part of the economy in Mississippi, United States, and the state supplies more than half of the U.S. demand for pond-raised channel catfish (*Ictalurus punctatus* Rafinesque) every year (*1*). Over the past 20 years, the U.S. catfish industry has been rapidly growing to meet the crescent demand for the fish. Thus, to maximize production, ponds are maintained at high fish-stocking densities (10000 to >25000 fish/ha), and average daily feeding rates are extremely high (75−125 kg/ha/day) (*2*). As a result, high nutrient levels in the ponds and favorable temperatures during the summer and fall promote the development of cyanobacteria (blue-green algae) blooms. Bloom-forming cyanobacteria (division Cyanophyta) have several undesirable characteristics. The negative attributes of bloom-forming cyanobacteria include the following: (i) because of slow growth rates, their production of oxygen and release into the surrounding water is less on a per unit biomass basis as compared to most types of eukaryotic phytoplankton (e.g., green algae); (ii) their formation of blooms and surface scum communities can lead to wide fluctuations in dissolved oxygen and total ammonia−nitrogen levels, especially during massive die-offs; (iii) some species produce toxins that are lethal to aquatic animals; and (iv) some species produce odorous compounds that can cause off-flavor in cultured aquatic animals (*3*). The latter characteristic is of great significance to catfish farmers since depuration of odorous compounds from catfish flesh to levels of acceptable flavor quality can take weeks or months depending upon such factors as pond water temperature, fish size, and lipid content of the fish flesh (*4*).

Cyanobacteria bloom formation constitutes one of the major problems for catfish farmers, particularly since certain types of filamentous cyanobacteria produce "earthy" and "musty" metabolites that are responsible for most off-flavor problems and can result in annual losses as high as U.S. $60 million for catfish farmers (*5*). These common off-flavors are due to the rapid absorption of the cyanobacterial metabolites 2-methylisoborneol (MIB) and geosmin into the fish flesh, thereby resulting in musty and earthy off-flavors, respectively. Off-flavor catfish are unmarketable and must be held by producers until MIB and geosmin are depurated from the fish flesh and subsequently deemed to be "on-flavor" by sensory personnel at the processing plant. Delayed harvest due to off-flavor results in direct and indirect economic losses, including additional feeding costs, interruption of cash flow, and loss of held catfish to disease, water quality problems, and bird depredation (*5*).

In west Mississippi catfish ponds, geosmin is less prevalent than MIB. The production of MIB in these ponds has been attributed to the presence of the filamentous cyanobacterium *Oscillatoria perornata* [Skuja] (*6*). The most common management approach used by catfish producers to mitigate musty off-flavor problems in pond-raised channel catfish is the application of algicides to production ponds to help reduce the abundance of *O. perornata*. The only algicides currently approved by the U.S. Environmental Protection Agency for use in food production ponds are copper-based products (e.g., copper sulfate) and diuron [*N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea]. However, the applications of these algicides to catfish ponds have several negative attributes including the following: (i) their low selective toxicity toward undesirable species of cyanobacteria as compared to preferred types of phytoplankton (e.g., green algae) requires careful application to catfish ponds to avoid poisoning and killing the entire phytoplankton community, which can lead to water quality deterioration; (ii) broad-spectrum toxicity of copper sulfate toward nontarget organisms (e.g., catfish); and (iii) the general public's sensitivity toward utilization of synthetic herbicides in food production (*5*). These concerns and the negative attributes of these algicides mentioned previously constitute the need for more reliable, selective, and environmentally safe compounds for managing earthy/musty off-flavor problems in catfish pond aquaculture.

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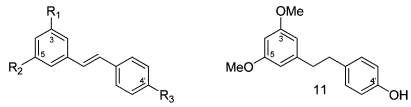


		R_1	R_2	R_3
1	(*E*)-1,3-dimethoxy-5-(4-nitrostyryl)benzene	OMe	OMe	NO_2
3	(*E*)-4-(3,5-dimethoxystyryl)aniline	OMe	OMe	NH_2
5	(*E*)-methyl 4-(3,5-dimethoxystyryl)benzoate	OMe	OMe	COOMe
7	(*E*)-4-(3,5-dimethoxystyryl)benzoic acid	OMe	OMe	COOH
8	(*E*)-1,3-dimethoxy-5-(4-methoxystyryl)benzene	OMe	OMe	OMe
10	(*E*)-3-(4-hydroxystyryl)-5-methoxyphenol	OH	OMe	OH
11	4-(3,5-dimethoxyphenethyl)phenol			
12	(*E*)-5-(4-methoxystyryl)benzene-1,3-diol	OH	OH	OMe
14	(*E*)-4-(3,5-dimethoxystyryl)phenyl dihydrogen phosphate	OMe	OMe	OPO_3H
15	(*E*)-4-(3,5-dimethoxystyryl)phenol	OMe	OMe	OH
16	(*E*)-5-(4-hydroxystyryl)benzene-1,3-diol	OH	OH	OH
19	(*E*)-2-(4-(3,5-dimethoxystyryl) phenoxy)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol	OMe	OMe	$OC_6H_{11}O_5$
25a	(*E*)-1-(4-chlorostyryl)-3,5-dimethoxybenzene	OMe	OMe	Cl
26a	(*E*)-1-(4-fluorostyryl)-3,5-dimethoxybenzene	OMe	OMe	F
27a	(*E*)-1-(4-bromostyryl)-3,5-dimethoxybenzene	OMe	OMe	Br
28a	(*E*)-1,3-dimethoxy-5-(4-(trifluoromethyl)styryl)benzene	OMe	OMe	CF3
31	(*E*)-4-(3,5-dimethoxystyryl)benzenethiol	OMe	OMe	SH
35a	(*E*)-5-styrylbenzene-1,3-diol	OH	OH	H

Figure 1. Chemical structures of stibenes with *E*-configuration and compound **11**.

During the past decade, we have evaluated numerous natural and natural-based compounds to discover alternatives to the currently available algicides. A wide variety of chemical compounds and plant and marine extracts have undergone evaluation using a microplate bioassay in the laboratory as the first stage in the discovery process. Quinones are one group of compounds that were evaluated early in the research program, and they have provided several promising leads for subsequent efficacy testing in catfish aquaculture ponds (*1*).

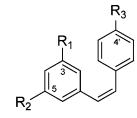
The stilbenes are known for their wide range of biological activities including antioxidant (*7*), anti-inflammatory (*8*), antileukemic (*9*), antibacterial (*10*), antifungal (*11*), antiplatelet aggregation (*12*), vasodilator (*13*), and antitumor (*14*) activities. Stilbenes are produced in plants in response to fungal attacks and are involved in the defense mechanism of plants.

Although previous research (*15, 16*) has found that certain types of stilbenes are toxic toward the coccoid cyanobacterium *Anacystis aeruginosa* (Zanardini) Drouet & Daily and the filamentous cyanobacterium *Gloeotrichia echinulata* (Smith) P.G. Richter at test concentrations of 1−200 mg/L, stilbenes have not been previously evaluated for selective toxicity toward the planktonic MIB producer *O. perornata*. In this report, the algicidal activities of stilbene analogues toward the MIB-producing *O. perornata* and a species of green algae were evaluated.

MATERIALS AND METHODS

Preparation and Synthesis of Stilbene Analogues. The synthesis of compounds **1**−**14** and **17** (**Figures 1** and **2**) has been described previously (*17*). Resveratrol (**16**) was purchased from Sigma-Aldrich (St. Louis, MO).

*(E)-2-(Acetoxymethyl)-6-[4-(3,5-dimethoxystyryl)phenoxy]tetrahydro-2H-pyran-3,4,5-triyl Triacetate (***18***).* Glucosylation of **15** (**Scheme 1**) was performed according to procedures reported by Orsini et al. (*18*). To a solution of **15** (256 mg, 1 mmol) in 1.25 M sodium hydroxide (NaOH) (5 mL) was added a solution of glucosyl bromide (411 mg, 1 mmol) and benzyltrietylammonium bromide (108 mg, 0.39 mmol) in chloroform. The reaction was vigorously stirred at 60 °C for 5 h. An additional amount of glucosyl bromide (200 mg) was added, and the reaction was heated for an extra 5 h. Ethyl acetate was added, and the reaction was washed with water. The organic phase was dried using $MgSO_4$, and the solvent was evaporated under reduced pressure. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.02−2.07 (m, 12H), 3.8 (s, 6H), 4.15 (d, 1H, J = 12 Hz), 4.28 (dd, 1 H, $J_{1,2}$ = 4 Hz, $J_{1,3}$ = 4 Hz), 5.07 (d, 1H, J = 8 Hz), 5.15 (t, 1H, J = 8 Hz), 4.26 (m, 2H), 6.37 (s, 1H), 6.63 (s, 2H), 6.96 (m, 5H), 7.42 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.6 (4C), 55.3 (2C), 61.9, 68.2, 71.1, 72, 72.6, 98.9, 104.4 (2C), 117.1 (2C), 127.7 (2C), 128.1, 132.5, 139.2, 156.4 (2C), 160.9 (4C), 169.3, 169.4, 170.6.



		R_1	R_2	R_3
2	(*Z*)-1,3-dimethoxy-5-(4-nitrostyryl)benzene	OMe	OMe	NO_2
4	(*Z*)-4-(3,5-dimethoxystyryl)aniline	OMe	OMe	NH_2
6	(*Z*)-methyl 4-(3,5-dimethoxystyryl)benzoate	OMe	OMe	COOMe
9	(*Z*)-1,3-dimethoxy-5-(4-ethoxystyryl)benzene	OMe	OMe	OMe
13	(*Z*)-5-(4-methoxystyryl)benzene-1,3-diol	OH	OH	OMe
17	(*Z*)-4-(3,5-dimethoxystyryl)benzoic acid	OMe	OMe	COOH
25b	(*Z*)-1-(4-chlorostyryl)-3,5-dimethoxybenzene	OMe	OMe	Cl
26b	(*Z*)-1-(4-fluorostyryl)-3,5-dimethoxybenzene	OMe	OMe	F
27b	(*Z*)-1-(4-bromostyryl)-3,5-dimethoxybenzene	OMe	OMe	Br
28b	(*Z*)-1,3-dimethoxy-5-(4-(trifluoromethyl)styryl)benzene	OMe	OMe	CF_3
35a	(*Z*)-5-styrylbenzene-1,3-diol	OH	OH	H

Figure 2. Chemical structures of stibenes with *Z*-configuration.

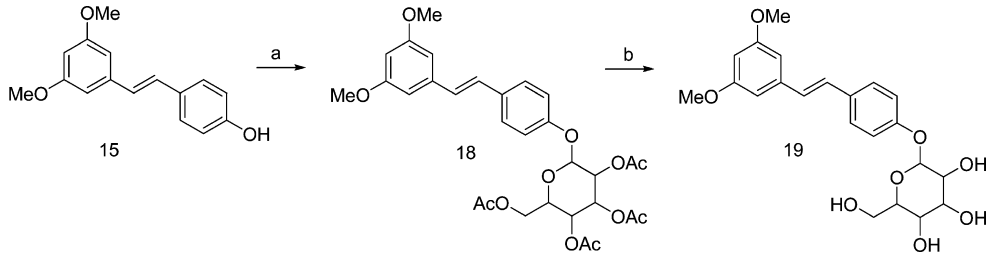
*(E)-2-[4-(3,5-Dimethoxystyryl)phenoxy]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (***19***).* To a solution of **18** (100 mg, 0.17 mmol) in methanol (MeOH) (20 mL) was added 10 mL of 0.2 M of methanolic sodium methoxide (NaOMe) (*18*) (**Scheme 1**). The reaction was stirred for 2 h at room temperature, and Dowex 50W-X8 (H^+) was added until neutral pH was reached. The resin was filtered, and the solvent was removed. The crude mixture was purified by column chromatography eluting with chloroform/methanol (98:2) and gave 46 mg of **19** (27% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.14 (m, 1H), 3.23 (m, 2H), 3.32 (s, 1H), 3.44 (m, 1H), 3.66 (m, 1H), 3.74 (s, 6H), 4.57 (s, 1H), 4.87 (d, 1H, J = 8 Hz), 5.02 (s, 1H), 5.09 (s, 1H), 5.31 (s, 1H), 6.37 (s, 1H), 6.72 (s, 2H), 7.02 (m, 3H), 7.20 (d, 1H, J = 16 Hz), 7.50 (d, 2H, J = 8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 60.6 (2C), 66.1, 75.1, 78.7, 82, 82.5, 105, 105.7, 109.7 (2C), 121.8 (2C), 132.1, 133.1 (2C), 133.1, 136.1, 144.7, 162.5, 166 (2C). Positive ion ESI-HRMS: calcd for $C_{22}H_{26}NaO_8$ $[M + Na]^+$, 441.15254; found, 441.15252.

General Procedure for the Synthesis of Compounds **25a**,**b** *and* **28a**,**b.** Aryl aldehyde, potassium hydroxide (KOH) (102 mg, 1.81 mmol), and 18 crown-6 were added to a solution of phosphonium salt **20** in dichloromethane (DCM) (*19*) (**Scheme 2**). The reaction was stirred at room temperature for 12 h. The reaction was poured into water and extracted with ethyl acetate (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified through flash chromatography eluting with hexanes/ethyl acetate (98:2).

*1-(4-Chlorostyryl)-3,5-dimethoxybenzene (***25a**,**b***).* The reaction of (3,5-dimethoxybenzyl)triphenylphosphonium **20** (300 mg, 0.6 mmol) and 4-chlorobenzaldehyde **21** (85 mg, 0.6 mmol) afforded **25a**,**b**. Compound **25a**, 53 mg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.83 (s, 6H), 6.41 (s, 1H), 6.66 (s, 2H), 7.01 (s, 2H), 7.32 (d, 2H, J = 8 Hz), 7.42 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.6 (2C), 100.3, 104.8 (2C), 127.9 (2C), 128, 129 (2C), 129.5, 133.5, 135.8, 139.2, 161.2 (2C). Positive ion ESI-HRMS: calcd for $C_{16}H_{16}O_2Cl$ $[M + H]^+$, 275.0839; found, 275.0846. Compound **25b**, 50 mg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.68 (6H), 6.36 (s, 1H), 6.40 (s, 2H), 6.55 (dd, 2H, $J_{1,2}$ = 8 Hz, $J_{1,3}$ = 4 Hz), 7.21 (s, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.4 (2C), 100.0, 106.8 (2C), 128.5 (2C), 129.5, 130.5 (2C), 131.1, 133.0, 135.8, 138.9, 160.8 (2C). Positive ion ESI-HRMS: calcd for $C_{16}H_{16}O_2Cl$ $[M + H]^+$, 275.0839; found, 275.0834.

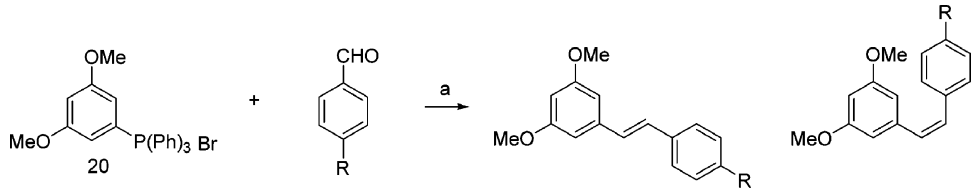
*1,3-Dimethoxy-5-[4-(trifluoromethyl)styryl]benzene (***28a**,**b***).* Reaction of (3,5-dimethoxybenzyl)triphenylphosphonium **20** (300 mg, 0.6 mmol) and 4-(trifluoromethyl)benzaldehyde **24** (84 μL, 0.6 mmol) afforded **28a**,**b**. Compound **28a**, 4.7 mg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.84 (s, 6H), 6.43 (t, 1H, J = 2 Hz), 6.68 (d, 2H, J = 2.4 Hz), 7.10 (d, 2H, J = 2.8 Hz), 7.59 (s, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.6 (2C), 100.7, 105 (2C), 125.8 (3C), 126.8 (2C), 127.8. 131.3, 138.8 (2C), 140.8, 161.2 (2C). Positive ion ESI-HRMS: calcd for $C_{17}H_{16}O_2F_3$ $[M + H]^+$, 309.1102; found, 309.1089. Compound **28b**, 22 mg. ^{1}H NMR

Scheme 1[a]



[a] Conditions: (a) glucosyl bromide, benzyltrietylammonium bromide, NaOH; (b) NaOMe, Dowex 50W-X8, MeOH.

Scheme 2[a]



[a] Conditions: (a) NaOH or KOH, 18 crown-6, DCM; or BuLi, THF.

($CDCl_3$, 400 MHz): δ 3.63 (s, 6H), 6.35 (s, 3H), 6.63 (dd, 2H, $J_{1,2}$ = 12 Hz, $J_{1,3}$ = 12 Hz), 7.37 (d, 2H, J = 8 Hz), 7.50 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.3 (2C), 100.3, 106.9 (2C), 125.2, 125.3, 129.3 (2C), 129.4 (2C), 132.5 (2C), 138.6, 141.1, 160.9 (2C). Positive ion ESI-HRMS: calcd for $C_{17}H_{16}O_2F_3$ $[M + H]^+$, 309.1102; found, 309.1098.

*1-(4-Fluorostyryl)-3,5-dimethoxybenzene (***26a**,**b***).* To a solution of phosphonium salt **20** (300 mg, 0.6 mmol), 4-fluorobenzaldehyde **22** (133 mg, 1.07 mmol), and catalytic amounts of 18 crown-6 was added 1.5 mL of a 50% sodium hydroxide (NaOH) in aqueous solution (*20*). The reaction was stirred at room temperature for 12 h. The reaction was poured into water and extracted with ethyl acetate (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified through flash chromatography eluting with hexanes/ethyl acetate (98:2) and afforded **26a**,**b**. Compound **26a**, 71 mg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.83 (s, 6H), 6.43 (s, 1H), 6.67 (s, 2H), 6.97 (s, 1H), 7.05 (m, 3H), 7.46 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.5 (2C), 100.1, 104.7 (2C), 115.7, 115.9, 128.2, 128.3 (2C), 128.6, 133.6, 139.4, 161.2 (2C), 163.8. GCMS: *m*/*z* 257 [M − H]. Compound **26b**, 51 mg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.67 (6H), 6.33 (s, 1H), 6.39 (s, 2H), 6.54 (s, 2H), 6.93 (m, 2H), 7.26 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.4 (2C), 100.0, 106.9 (2C), 115.2, 115.4, 129.6, 130.4, 130.8, 130.9, 133.3, 139.1, 160.8 (2C), 163.3. GCMS: *m*/*z* 257 [M − H].

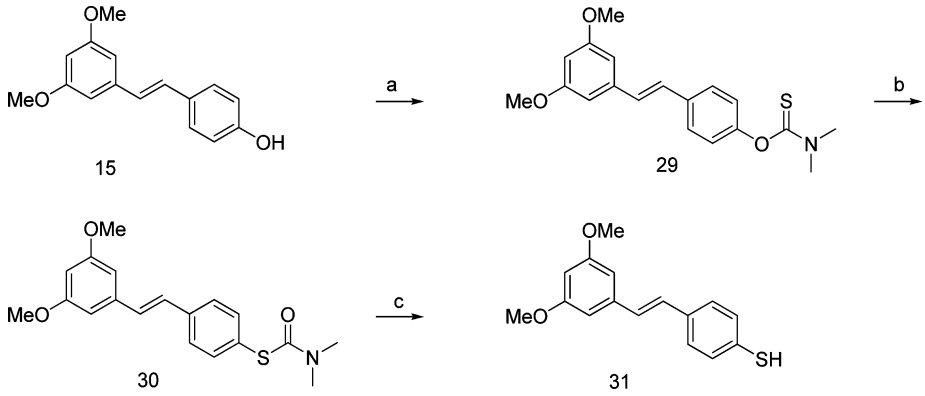
*1-(4-Bromostyryl)-3,5-dimethoxybenzene (***27a**,**b***).* To a cold solution (−78 °C) of phosphonium salt **20** (300 mg, 0.6 mmol) in THF was added *n*-butyllithium (BuLi) (245 μL, 0.6 mmol) (*21*), and the resulting solution was stirred under inert atmosphere for 2 h (**Scheme 2**). A solution of 4-bromobenzaldehyde **23** (112.5 mg, 0.6 mmol) in THF was added dropwise, and the mixture was stirred for 12 h at room temperature. The resulting suspension was poured into water and extracted with DCM (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified through flash chromatography eluting with hexanes/ethyl acetate (40:1) and gave **27a**,**b**. Compound **27a**, 45 mg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.83 (s, 6H), 6.42 (s, 1H), 6.66 (s, 2H), 7.00 (s, 2H), 7.35 (d, 2H, J = 8 Hz), 7.47 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 55.6 (2C), 100.4, 104.8 (2C), 121.6, 128.1, 128.3 (2C), 129.6, 132 (2C), 136.3, 139.1, 161.2 (2C). Positive ion ESI-HRMS: calcd for $C_{16}H_{16}O_2Br$, 319.0334; found, 319.0336. Compound **27b**, 31 mg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.67 (s, 6H), 6.33 (m, 1H), 6.37 (m, 2H), 6.53 (dd, 2H, $J_{1,2}$ = 12 Hz, $J_{1,3}$ = 16 Hz), 7.13 (d, 2H, J = 8 Hz), 7.35 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.4 (2C), 100.0, 106.8 (2C), 121.6, 129.5, 130.8 (2C), 131.2, 131.5 (2C), 136.2, 138.9, 160.8 (2C). Positive ion ESI-HRMS: calcd for $C_{16}H_{16}O_2Br$, 319.0334; found, 319.0330.

*(E)-O-4-(3,5-Dimethoxystyryl)pheny Dimethylcarbamothioate (***29***).* Dimethylaminothio-carbonyl chloride (58 mg, 0.46 mmol), 4-dimethylamino pyridine (DMAP) (4.7 mg, 0.039 mmol), and triethylamine (TEA) (109 μL, 0.78 mmol) were added to a solution of **15** in dioxane (*22*) (**Scheme 3**). The reaction was stirred at reflux for 30 h and poured into water. The aqueous phase was extracted with ethyl acetate (3 × 10 mL), and the organic solvent was combined and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the crude extract was purified using flash chromatography eluting with hexanes/ethyl acetate (7:3) to give 68 mg of **29**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.33 (s, 3H), 3.45 (s, 3H), 3.83 (s, 6H), 6.41 (s, 1H), 6.66 (s, 2H), 7.00 (s, 1H), 7.07 (m, 3H), 7.51 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 38.8, 43.4, 55.6 (2C), 100.3, 104.9 (2C), 123.2 (2C), 127.4 (2C), 128.5, 129.1, 135.2, 139.5, 153.7, 161.2 (2C), 188.2. Positive ion ESI-HRMS: calcd for $C_{19}H_{22}NO_3S$ $[M + H]^+$, 344.13204; found, 344.13657.

*(E)-4-(3,5-Dimethoxystyryl)benzenethiol (***31***).* Compound **29** (68 mg, 0.19 mmol) was diluted in tetradecane (*22*) (10 mL) and heated to 240 °C for 12 h. After it was cooled, a filtration column was performed to eliminate tetradecane. The column was washed several times with hexanes and then ethyl acetate and afforded 40 mg of **30** (**Scheme 3**). Lithium aluminum hydride ($LiAlH_4$) (5.8 mg, 0.14 mmol) was added to a solution of **30** (50 mg, 0.14 mmol) in ether (Et_2O) (*23*) at 0 °C. The reaction was stirred for 3 days at room temperature. One molar HCl was added, and the reaction was extracted with ethyl acetate. The organic phase was combined and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and purified using flash column chromatography eluting with chloroform/methanol (85:15) to give 10 mg of **31**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.83 (s, 6H), 6.40 (s, 1H), 6.66 (s, 2H), 7.00 (s, 2H), 7.25 (d, 2H, J = 8 Hz), 7.37 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.6 (2C), 100.2, 104.7 (2C), 127.4 (2C), 128.5, 128.6, 129.8 (2C), 130.4, 134.9, 139.4, 161.2 (2C). Negative ion ESI-HRMS: calcd for $C_{16}H_{16}O_2S$ $[M - H]^-$, 271.07927; found, 271.07827.

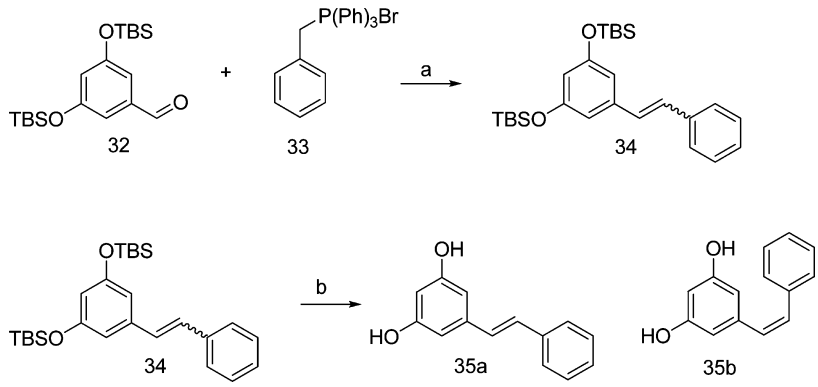
*5-Styrylbenzene-1,3-diol (***35a**,**b***).* To a cold solution (−78 °C) of phosphonium salt **33** (236 mg, 0.54 mmol) in THF was added *n*-butyllithium (BuLi) (338 μL, 0.54 mmol) (*21*), and the resulting solution was stirred under inert atmosphere for 2 h. A solution of aldehyde **32** (200 mg, 0.54 mmol) in THF was added dropwise, and the mixture was stirred for 12 h at room temperature (**Scheme 4**). The

Scheme 3[a]



[a] Conditions: (a) Dimethylaminothiocarbomoyl chloride, DMAP, TEA, dioxane; (b) tetradecane; (c) $LiAlH_4$, Et_2O.

Scheme 4[a]



[a] Conditions: (a) *n*-BuLi, THF, 12 h; (b) TBAF, THF.

resulting suspension was poured into water and extracted with DCM (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified through flash chromatography eluting with hexanes/ethyl acetate (40:1) and gave 92 mg of **34**. To a solution of **34** (92 mg, 0.21 mmol) in THF was added tetrabutylammnonium fluoride (TBAF) (*18*) (546 μL, 0. 546 mmol). The solution was stirred for 20 min, poured into water, and extracted with ethyl acetate.

The solvent was removed under reduced pressure, and the crude extract was purified using flash chromatography eluting with hexanes/ether (65:35) to afford **35a**,**b**. Compound **35a**, 9 mg. ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 6.30 (s, 1H), 6.58 (s, 2H), 7.09 (s, 2H), 7.25 (t, 1H, J = 8 Hz), 7.35 (t, 2H, J = 8 Hz), 7.56 (d, 2H, J = 8 Hz). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 102.5, 105.3 (2C), 126.6 (2C), 127.7, 128.5, 128.8 (2C), 129, 137.6, 139.7, 158.9 (2C). HRMS: calcd for $C_{14}H_{11}O_2$ [M − H], 211.07590; found, 211.07558. Compound **35b**, 15 mg. ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 6.24 (d, 1H, J = 4 Hz), 6.26 (s, 2H), 6.51 (dd, 2H, $J_{1,2}$ = 12 Hz, $J_{1,3}$ = 8 Hz), 7.17−7.29 (m, 5H). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 101.9, 107.3 (2 C), 127.2, 128.3 (2C), 129 (2C), 130, 130.6, 137.5, 139.4, 158.7 (2C). Negative ion ESI-HRMS: calcd for $C_{14}H_{11}O_2$ [M − H]$^-$, 211.07590; found, 211.07594.

Algicide Bioassay. The cyanobacterium *O. perornata* (Skuja) [synonym of *Planktothrix perornata* (Skuja) Anagnostidis & Komárek] was isolated from a water sample obtained from a catfish pond located in west Mississippi, United States. A culture of the green alga *Selenastrum capricornutum* Printz [synonym of *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák] was obtained from Dr. J. C. Greene, U.S. Environmental Protection Agency (Corvallis, OR). These cultures were maintained separately in continuous, steady state culture systems to provide a source of cells growing at a constant rate. The culture conditions used were the same as those outlined in Schrader et al. (1997), except that the cell density as measured spectrophotometrically (750 nm) was maintained at 0.12−0.21 A_{750nm} for *O. perornata* and at 0.10−0.15 A_{750nm} for *S. capricornutum*. To help evaluate toxic selectivity of each test compound, the green alga *S. capricornutum* was used as a representative species for green algae (division Chlorophyta) since *Selenastrum* spp. are common in freshwater ponds in the southeastern United States (*24*).

The bioassay was performed in a similar manner as outlined by Schrader et al. (1997). Various analogues of stilbenes were dissolved separately in methanol to provide stock solution concentrations of 0.2, 2.0, 20.0, 200.0, and 2000.0 μM for each analogue. Initially, each stock solution was aseptically pippetted into the bottom of separate wells (10 μL per well) of a 96-well microplate (Costar; Corning, Inc., Corning, NY), and the methanol was allowed to evaporate completely before the addition of 200 μL of either cyanobacterial or unialgal culture from the continuous culture systems. Final test concentrations of each analogue were 0.01, 0.1, 1.0, 10.0, and 100.0 μM. Wells of controls contained only cyanobacterial or unialgal culture. Three replications of each analogue concentration and controls were used. Microplates were placed in a Percival Scientific model I-36LL growth chamber (Percival Scientific, Inc., Boone, IA) maintained at 28−30 °C and illuminated by overhead fluorescent lamps (20 W) at a light intensity of 18−24 μE/m^2/s. At 24 h intervals over a 4 day period, the optical density of each well was measured at 650 nm using a Packard model SpectraCount microplate photometer (Packard Instrument Co., Meriden, CT). Mean values of the optical density measurements for each concentration and controls were graphed, and graphs were used to determine, for each stilbene analogue, the lowest observed effect concentration (LOEC; lowest concentration to adversely affect growth) and the lowest complete inhibition concentration (LCIC; lowest concentration to completely inhibit growth).

RESULTS AND DISCUSSION

The results of the screening of stilbenes as algicides are shown in **Table 1**. For the compounds with methoxy groups at the 3- and 5-positions, the analogue with amino group at the 4′-position

Table 1. LOEC and LCIC of Stilbenes Screened for Agicide Activity

	O. perornata		*S. capricornutum*	
stilbene	LOEC (μM)	LCIC (μM)	LOEC (μM)	LCIC (μM)
1	10	>100	>100	>100
2	10	>100	10	>100
3	10	10	10	100
4	10	10	10	100
5	>100	>100	>100	>100
6	100	100	>100	>100
7	>100	>100	>100	>100
8	100	100	>100	>100
9	100	100	>100	>100
10	100	100	>100	>100
11	100	100	100	100
12	>100	>100	>100	>100
13	100	100	>100	>100
14	100	100	10	100
15	100	100	10	100
16	>100	>100	>100	>100
17	>100	>100	>100	>100
19	100	100	100	>100
25a	100	>100	>100	>100
25b	100	>100	>100	>100
26a	100	>100	100	>100
26b	100	100	100	>100
27a	>100	>100	100	>100
27b	100	>100	>100	>100
28a	>100	>100	>100	>100
28b	100	>100	>100	>100
31	10	>100	>100	>100
35a	>100	>100	>100	>100
35b	100	>100	1	>100

seems to be preferred for algicide activity. Among the analogs differing in functional group substitution at the 4′ position (including methoxy, carboxy, thiol, hydroxyl, phosphate, and nitro groups) only the amino-bearing stilbenes were active. On the basis of LCIC results, **3** and **4** showed moderate activities and selectivity against cyanobacteria as compared to the green alga at the concentrations tested. These compounds are cis and trans isomers, which indicate that the configuration of the molecule is not imperative to impart algicidal activity.

In a previous study by Schrader et al. (*25*), the pyridine-based herbicides diquat (6,7-dihydrodipyrido[1,2-α:2′,1′-*c*]pyrazinediium) and paraquat (1,1′-dimethyl-4,4′-bipyridinium) had the lowest LCIC (0.1 μM) and were selectively toxic for *Oscillatoria* cf. *chalybea* (later redesignated as *O. perornata*). Results from this present study and the one performed by Schrader et al. showed that nitrogen-containing molecules show the best activities against *O. perornata*. In addition to diquat and paraquat, halogenated herbicides showed moderate inhibitory activity against *O. perornata* (*25*). Diclofop [(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid] and bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) had LOEC values of 10 μM but were highly selective against *O. perornata*. Because of the selective algicidal activity of these two aromatic halogenated herbicides, compounds **25a**−**28b** were synthesized for evaluation; however, none of these analogues were active.

Resveratrol (**16**) and pterostilbene (**15**), while attributed with health-promoting properties, are also phytoalexins. Both compounds have shown inhibitory activity against some plant pathogenic fungi. Pterostilbene was reported to be more fungitoxic than resveratrol against *Botrytis cinerea*, *Cladosporium cucumerinum*, *Pellicularia sasakii*, *Pyricularia oryzae*, and *Plasmopora viticola* (*26*). Pterostilbene, but not resveratrol, inhibited mycelial growth of fungi involved in esca of grapevine (*27*). Resveratrol has shown activity against some human pathogenic bacteria (*20, 28*−*30*) and fungi (*31*), as well as the protozoan parasite *Leishmania major* (*32*). While **15** and **16** reportedly show activity against various types of microorganisms, these compounds were not active against *O. perornata*.

Synthetic **14** and the naturally occurring stilbene **15** had LOEC values of 10 μM toward *S. capricornutum* but were less active against *O. perornata*, thereby indicating selectivity toward the green alga. On the basis of the bioassay results, methoxy groups at 3′- and 5′-positions are preferred for algicide activity since the hydroxylated compounds represented by **10**, **12**, **13**, and **16** were inactive toward *O. perornata* at the concentrations tested.

Compound **35a** was previously reported to possess activity against the green algae *Ankistrodesmus arcuatus* Korshikov and *Chlamydomonas gigantea* Dill and the cyanobacteria *A. aeruginosa* and *G. echinulata* at concentrations of 1−200 mg/L (*6*). In our study, we evaluated **35a** as well as its cis isomer, **35b**. Our results indicated that **35b** was more toxic than **35a** against *O. perornata* and *S. capricornutum* based upon LOEC results of 100 μM (21.2 mg/L) and 1 μM (0.2 mg/L), respectively. Conversely, **35a** did not inhibit the growth of either *O. perornata* or *S. capricornutum* at the highest test concentration of 21.2 mg/L. Although **35b** is more toxic than **35a** toward *O. perornata*, it is selectively toxic toward *S. capricornutum* based upon LOEC results; therefore, this isomer is unsuitable for selective control of *O. perornata*.

The toxicity of promising cyanobactericidal stilbenes toward nontarget organisms in the pond (e.g., catfish) would need to be investigated. Among the stilbenes tested, toxicity data have been reported thus far only for resveratrol; that is, renal toxicity observed at 3000 mg per kilogram body weight per day in rats (*33*). The stilbenes generally have poor water solubility. This problem can be overcome by the synthesis of water-soluble analogues, which is a subject of future work.

In summary, the algicidal activities of 29 natural and synthetic stilbene analogues were investigated. Among these analogues, **3** and **4** showed moderate algicidal activity toward *O. perornata*. This is the first report of the selective algicidal activity of certain stilbenes toward a MIB-producing species of cyanobacteria. This research has provided a new class of compounds for further investigation toward the discovery of environment-friendly algicides.

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LITERATURE CITED

(1) Schrader, K. K.; Nanayakkara, N. P. D.; Tucker, C. S.; Rimando, A. M.; Ganzera, M.; Schaneberg, B. T. Novel derivatives of 9,10-anthraquinone are selective algicides against the musty-odor cyanobacterium *Oscillatoria perornata*. *Appl. Environ. Microbiol.* **2003**, *69*, 5319–5327.

(2) Zimba, P. V.; Grimm, C. C. A synoptic survey of musty/muddy odor metabolites and microcystin toxin occurrence and concentration in southeastern USA channel catfish (*Ictalurus punctatus* Ralfinesque) production ponds. *Aquaculture* **2003**, *218*, 81–87.

(3) Paerl, H. W.; Tucker, C. S. Ecology of blue-green algae in aquaculture ponds. *J. World Aquacult. Soc.* **1995**, *26*, 109–131.

(4) Howgate, P. Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: A review of sensory aspects and of uptake/depuration. *Aquaculture* **2004**, *234*, 155–181.

(5) Tucker, C. S. Off-flavor problems in aquaculture. *Rev. Fish. Sci.* **2000**, *8*, 45–88.

(6) Martin, J. F.; Izaguirre, G.; Waterstrat, P. A planktonic *Oscillatoria* species from Mississippi catfish ponds that produces the off-flavor compound 2-methylisoborneol. *Water Res.* **1991**, *25*, 1447–1451.
(7) Stivala, L. A.; Savio, M.; Carafoli, F.; Perucca, P.; Bianchi, L.; Maga, G.; Forti, L.; Pagnoni, U. M.; Albini, A.; Prosperi, E.; Vannini, V. Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol. *J. Biol. Chem.* **2001**, *276*, 22586–22594.
(8) Kimura, Y.; Okuda, H.; Arichi, S. Effects of stilbenes on arachidonate metabolism in leukocytes. *Biochim. Biophys. Acta* **1985**, *834*, 275–278.
(9) Mannila, E.; Talvitie, A.; Kolehmainen, E. Antileukemic compounds derived from stilbenes in *Picea abies* bark. *Phytochemistry* **1993**, *33*, 813–816.
(10) Mahady, G. B.; Pendland, S. L.; Chadwick, L. R. Resveratrol and red wine extracts inhibit the growth of CagA+ strains of *Helicobacter pylori* in vitro. *Am. J. Gastroenterol.* **2003**, *98*, 1440–1441.
(11) Creasy, L. L.; Coffee, M. Phytoalexin production potential of grape berries. *J. Am. Soc. Hortic. Sci.* **1988**, *113*, 230–234.
(12) Chung, M. I.; Teng, C. M.; Cheng, K. L.; Ko, F. N.; Lin, C. N. An antiplatelet principle of *Veratrum formosanum*. *Planta Med.* **1992**, *58*, 274–276.
(13) Inamori, Y.; Kubo, M.; Tsujibo, H.; Ogawa, M.; Saito, Y.; Miki, Y.; Takemura, S. The ichthyotoxicity and coronary vasodilator action of 3,3′-dihydroxy-alpha,beta-diethylstilbene. *Chem. Pharm. Bull. (Tokyo)* **1987**, *35*, 887–890.
(14) Schneider, Y.; Duranton, B.; Gosse, F.; Schleiffer, R.; Seiler, N.; Raul, F. Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. *Nutr. Cancer* **2001**, *39*, 102–107.
(15) Arlt, H. G., Jr.; Conroy, N. E. Algicidal 2,2′-methylenebis(*trans*-3,5-dihydroxystilbene). U.S. Patent 3,531,533, 1970.
(16) Sheers, E. H. *trans*-3,5-Dihydroxystilbene as algicide. U.S. Patent 3,577,230, 1971.
(17) Mizuno, C. S.; Ma, G.; Khan, S.; Patny, A.; Avery, M. A.; Rimando, A. M. Design, synthesis, biological evaluation and docking studies of pterostilbene analogs inside PPAR alpha. *Bioorg. Med. Chem.* **2008**, *16*, 3800–3808.
(18) Orsini, F.; Pelizzoni, F.; Bellini, B.; Miglierini, G. Synthesis of biologically active polyphenolic glycosides (combretastatin and resveratrol series). *Carbohydr. Res.* **1997**, *301*, 95–109.
(19) De Medina, P.; Casper, R.; Savouret, J.-F.; Poirot, M. Synthesis and biological properties of new stilbene derivatives of resveratrol as new selective aryl hydrocarbon modulators. *J. Med. Chem.* **2005**, *48*, 287–291.
(20) Zhang, J.-T.; Dai, W.; Harvey, R. G. Synthesis of higher oxidized metabolites of dibenz[a,j]anthracene implicated in the mechanism of carcinogenesis. *J. Org. Chem.* **1998**, *63*, 8125–8132.
(21) Pettit, G. R.; Grealish, M. P.; Jung, M. K.; Hamel, E.; Pettit, R. K.; Chapuis, J. C.; Schmidt, J. M. Antineoplastic agents. 465. Structural modification of resveratrol: Sodium resverastatin phosphate. *J. Med. Chem.* **2002**, *45*, 2534–2542.
(22) Conner, S. E.; Mantlo, N. B.; Zhu, G.; Herr, R. J. Preparation of bicyclic derivatives as PPAR modulators. WO 2005/066136 A1.
(23) Hasegawa, J.-Y.; Hamada, M.; Miyamoto, T.; Nishide, K.; Kajimoto, T.; Uenishi, J.-I.; Node, M. The application of phenylmethanethiol and benzenethiol derivatives as odorless organo-sulfur reagents in the synthesis of thio-sugars and thioglycosides. *Carbohydr. Res.* **2005**, *340*, 2360–2368.
(24) Boyd, C. E.; Tucker, C. S. *Pond Aquaculture Water Quality Management*; Boyd, C. E., Tucker, C. S., Eds.; Kluwer Academic Publishers: Massachusetts, 1998; 700 pp.
(25) Schrader, K. K.; de Regt, M. Q.; Tidwell, P. D.; Tucker, C. S.; Duke, S. O. Compounds with selective toxicity towards the off-flavor metabolite-producing cyanobacterium *Oscillatoria* cf. chalybea. *Aquaculture* **1998**, *163*, 85–99.
(26) Langcake, P. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, e-viniferin a-viniferin and pterostilbene. *Physiol. Plant Pathol.* **1981**, *18*, 213–226.
(27) Alessandro, M.; Di Marco, S.; Osti, F.; Cesari, A. Bioassays on the activity of resveratrol, pterostilbene and phosphorous acid towards fungi associated with esca of grapevine. *Phytopathol. Mediterr.* **2000**, *39*, 357–365.
(28) Jung, H. J.; Seu, Y. B.; Lee, D. G. Candicidal action of resveratrol isolated from grapes on human pathogenic yeast *C. albicans*. *J. Microbiol. Biotechnol.* **2007**, *17*, 1324–1329.
(29) Kukric, Z. Z.; Topalic-Trivunovic, L. N. Antibacterial activity of *cis*- and *trans*-resveratrol isolated from *Polygonum cuspidatum* rhizome. *Acta Period. Technol.* **2006**, *37*, 131–136.
(30) Lu, C.-C.; Lai, H.-C.; Hsieh, S.-C.; Chen, J.-K. Resveratrol ameliorates *Serratia marcescens*-induced acute pneumonia in rats. *J. Leukocyte Biol.* **2008**, *83*, 1028–1037.
(31) Jung, H. J.; Hwang, I. A.; Sung, W. S.; Kang, H.; Kang, B. S.; Seu, Y. B.; Lee, D. G. Fungicidal effect of resveratrol on human infectious fungi. *Arch. Pharmacal Res.* **2005**, *28*, 557–560.
(32) Kedzierski, L.; Curtis, J. M.; Kaminska, M.; Jodynis-Liebert, J.; Murias, M. In vitro antileishmanial activity of resveratrol and its hydroxylated analogues against *Leishmania major* promastigotes and amastigotes. *Parasitol. Res.* **2007**, *102*, 91–97.
(33) Crowell, J. A.; Korytko, P. J.; Morrissey, R. L.; Booth, T. D.; Levine, B. S. Resveratrol-associated renal toxicity. *Toxicol. Sci.* **2004**, *82*, 614–619.

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