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Synthesis of Water-Soluble 9,10-Anthraquinone Analogues with Potent Cyanobactericidal Activity toward the Musty-Odor Cyanobacterium *Oscillatoria perornata*

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A series of water-soluble 9,10-anthraquinone analogues were prepared and evaluated for their selective toxicity toward *Oscillatoria perornata*, which grows in catfish production ponds and causes "musty" off-flavor in channel catfish (*Ictalurus punctatus*). Water-soluble mono- and dicationic salts were prepared by conjugating various small amines directly or through a methylene or ethylene bridge to the 9,10-anthraquinone nucleus. One of the dicationic salts, 2-[*N*-(1′-methyl-4′-*N*,*N*-diethylaminobutyl)aminometyl]anthraquinone diphosphate, exhibited very high water solubility and potent selective toxicity toward *O. perornata*. However, the tendency of this compound to potentially bind to suspended sediments may be the reason for its limitations in controlling *O. perornata* in catfish production ponds. The monocationic salt, 2-[*N*-(1′-methylethyl)]aminomethyl]anthraquinone monophosphate, showed good solubility and high selective toxicity toward *O. perornata*. Neutral water-soluble analogues prepared by conjugating terta- or pentaethylene glycol directly or by a methylene bridge to the 9,10-anthraquinone nucleus had less activity than the parent compound.

KEYWORDS: Algicide; anthraquinone; channel catfish; *Ictalurus punctatus*; 2-methylisoborneol; *Oscillatoria perornata*

INTRODUCTION

The cyanobacterium (blue-green alga) *Oscillatoria perornata* [Skuja] (previously designated *Oscillatoria* cf. *chalybea*) grows in channel catfish (*Ictalurus punctatus*) production ponds in the southeastern United States and produces the musty-odor compound 2-methylisoborneol (MIB) (*1*). MIB is rapidly absorbed into the flesh of catfish and imparts a "musty" flavor to the catfish, rendering them unpalatable and unmarketable. In the United States, musty "off-flavor" problems in the channel catfish production industry can cost producers as much as $25–65 million annually, depending on the frequency of *O. perornata* blooms (*2*). One management approach for the mitigation of musty off-flavor in farm-raised channel catfish is the application of chemicals to production ponds to kill or prevent the growth of undesirable cyanobacteria. Currently, only copper-based compounds, such as copper sulfate and chelated copper compounds, and the herbicide diuron [under limited-use registration by the U.S. Environmental Protection Agency (USEPA)] are approved by the USEPA for use in fish production ponds. Copper-based products and diuron are limited in their usefulness in controlling cyanobacteria due to their accumulation in the environment, lack of selectivity toward noxious cyanobacteria, and the small margin of safety between phytotoxic concentrations and ichthyotoxic concentrations (*1*).

As part of a research program to discover natural product-based algicides for the selective control of cyanobacteria (blue-green algae), a large number of natural products and their analogues have been evaluated (*3, 4*). Among the compounds tested so far, optimum activity and selectivity were shown by 9,10-anthraquinone and 2-methyl-9,10-anthraquinone (*3*). However, due to their extremely low solubility in water these two compounds were found to be ineffective as selective algicides to control the abundance of *O. perornata* in catfish production ponds.

In an effort to increase the utility of anthraquinones as potential selective algicides, several structural modifications to the anthraquinone nucleus were carried out to retain the activity and increase the solubility in water. This study describes the synthesis of a large number of water-soluble 9,10-anthraquinone analogues (**Figure 1**) and the evaluation of these analogues for their toxicity toward *O. perornata* using a laboratory bioassay.

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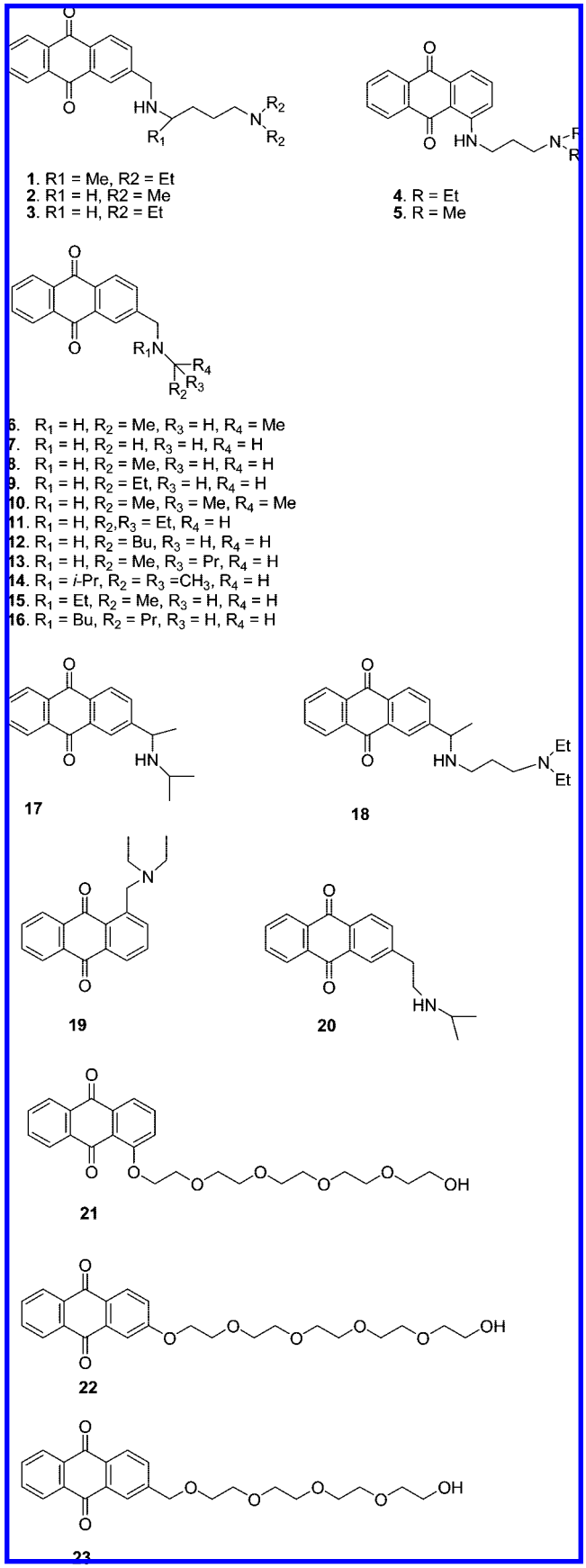

Figure 1. Structures of compounds **1**−**23**.

MATERIALS AND METHODS

General Synthesis. Melting points (uncorrected) were recorded on an Electrothermal 9100 instrument. UV spectra were obtained in $CHCl_3$, using a Hewlett-Packard 8452A spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on Varian Mercury-400BB (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR), or Bruker Avance DRX-500 (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) spectrometers, run in $CDCl_3$ with TMS as an internal standard. High-resolution MS (HRMS) were obtained by direct probe using Bruker Bioapex-FTMS with electro-spray ionization.

General Procedure for the Preparation of Phosphate Salts of Alkylamino-9,10-anthraquinone Analogues. A mixture of appropriate haloanthraquinone [2-chloromethylanthraquinone or 2-bromomethylanthraquinone for compounds **1**−**3** and **6**−**16**; 2-chloroanthraquinone or 2-bromoanthraquinone for compounds **4** and **5**; 2-(1′-bromoethyl) anthraquinone for compounds **17** and **18**; 1-bromomethylanthraquinone for compound **19**] (1 mmol) and an excess amount of requisite amine (5 mL), in the absence (for compounds **4** and **5**) or presence (for compounds **1**−**3**, **6**−**19**) of cosolvent acetonitrile (8 mL), was stirred at refluxing temperature for up to 1 h (for compounds **1**−**3**, **6**−**19**) or at 100 °C for 2 h (for compounds **4** and **5**). The cosolvent was evaporated and the reaction mixture was partitioned between HCl (1 M, 50 mL) and dichloromethane (50 mL). The aqueous layer was then basified (pH 10) with 20% sodium hydroxide solution and extracted with ethyl acetate. This organic layer was washed with water, dried over anhydrous sodium sulfate, and evaporated. The product obtained was dissolved in methanol and then precipitated as a phosphate salt by treating with phosphoric acid. The precipitate was filtered off and dried under vacuum.

*2-[N-(1′-Methyl-4′-N,N-diethylaminobutyl)aminomethyl]anthraquinone (***1***).* Physical and spectroscopic data were reported earlier (*7*).

*2-[N-(3′-N,N-Dimethylaminopropyl)aminomehyl]anthraquinone (***2***).* ^1H NMR δ 1.68 (2H, quintet, pentet, $J = 6.9$ Hz, 2′-CH_2), 2.20 (6H, s, N(CH_3)$_2$), 2.31 (2H, t, $J = 7.0$ Hz, 3′-CH_2), 2.67 (2H, t, $J = 6.9$ Hz, 1′-CH_2) 3.93 (2H, s, 2-CH_2), 7.74–7.77 (3H, m, 3,6,7-H), 8.19–8.27 (4H, m, 1,4,5,8-H); HRMS, *m/e* 323.1727 (M + H^+, $C_{20}H_{22}N_2O_2$, calcd 323.1738).

2-[N-(3′-N,N-Dimethylaminopropyl]aminomehyl]anthraquinone Diphosphate. Anal.: C, 46.61; H, 5.30; N, 5.27; P, 11.78%. Calcd for $C_{20}H_{22}N_2O_2 \cdot 2H_3PO_4$: C, 46.34; H, 5.44; N, 5.40; P, 11.95%.

*2-[N-(3′-N,N-Diethylaminopropyl)aminomehyl]anthraquinone (***3***).* NMR δ 0.88 (6H, t, $J = 7.2$ Hz, −N−($CH_2C\underline{H}_3$)$_2$), 1.57 (2H, m, 2′-CH_2), 2.37 (4H, t, $J = 7.1$, −N−($C\underline{H}_2CH_3$)$_2$), 2.42 (2H, t, $J = 7.2$ Hz, 3′-CH_2), 2.57 (2H, t, $J = 6.7$ Hz, 1′-CH_2), 3.79 (2H, s, 2-CH_2), 7.61 (3H, m, 3,6,7-H), 8.02–8.11 (4H, m, 1,4,5,8-H); HRMS, *m/e* 351.2038 (M + H^+, $C_{22}H_{27}N_2O_2$, calcd 351.2067).

2-[N-(3′-N,N-Diethylaminopropyl)aminomehyl]anthraquinone Diphosphate. Anal.: C, 48.22; H, 5.84; N, 4.97; P, 11.45%. Calcd for $C_{22}H_{27}N_2O_2 \cdot 2H_3PO_4$: C, 48.36; H, 5.90; N, 5.13; P, 11.34%.

*1-[N-(3′-N,N-Diethylaminopropyl)amino]anthraquinone (***4***).* NMR δ 0.96 (6H, t, $J = 7.1$ Hz, N−($CH_2C\underline{H}_3$)$_2$), 1.57 (2H, quintet, $J = 6.9$ Hz, 2′-CH_2) 2.47 (6H, q, $J = 7.1$ Hz, 3′-CH_2, N−($C\underline{H}_2CH_3$)$_2$), 3.17 (2H, q, $J = 6.7$ Hz, 1′-CH_2), 6.84 (1H, d, $J = 8.2$ Hz, 2-H), 7.31 (1H, t, $J = 8.2$ Hz, 3-H), 7.37 (1H, d, $J = 8.1$ Hz, 4-H), 7.57 (2H, m, 6, 7-H), 8.07 (2H, m, 5, 8-H), 9.55 (1H, t, N$\underline{H}$); HRMS, *m/e* 336.1872 (M + H^+, $C_{21}H_{24}N_2O_2$, calcd 336.1838).

1-[N-(3′-N,N-Diethylaminopropyl)amino]anthraquinone Phosphate. Anal.: C, 57.68; H, 6.45; N, 6.41; P, 7.07%. Calcd for $C_{21}H_{24}N_2O_2 \cdot H_3PO_4$: C, 58.06; H, 6.26; N, 6.45; P, 7.13%.

*1-[N-(3′-N,N-Dimethylaminopropyl)amino]anthraquinone (***5***).* NMR δ 1.80 (2H, quintet, $J = 7$ Hz, 2′-CH_2), 2.20 (6H, s, N($C\underline{H}_3$)$_2$), 2.35 (2H, t, $J = 7.0$ Hz, 3′-CH_2), 3.22 (2H, q, $J = 6.8$ Hz, 1′-CH_2), 6.89 (1H, d, $J = 8.2$ Hz, 2-H), 7.35 (1H, t, $J = 8.1$ Hz, 3-H), 7.41 (1H, d, $J = 6.6$ Hz, 4-H), 7.60 (2H, m, 6, 7-H), 8.10 (2H, m, 5, 8-H), 9.58 (1H, t, N$\underline{H}$); HRMS, *m/e* 308.1541 (M + H^+, $C_{19}H_{20}N_2O_2$, calcd 308.1525).

1-[N-(3′-N,N-Diethylaminopropyl)amino]anthraquinone Phosphate. Anal.: C, 55.98; H, 5.84; N, 6.77; P, 7.48%. Calcd for $C_{19}H_{20}N_2O_2 \cdot H_3PO_4$: C, 56.16; H, 5.70; N, 6.89; P, 7.62%.

*2-[N-(1′-Methylethyl)aminomethyl]anthraquinone (***6***).* Physical and spectroscopic data were reported earlier (*7*).

*2-[(Methylamino)methyl]anthraquinone (***7***).* NMR δ 2.48 (3H, s, N−C<u>H</u>$_3$), 3.92 (2H, s, 2-C<u>H</u>$_2$), 7.76–7.80 (3H, m, 3,6,7-H), 8.22–8.29 (4H, m, 1,4,5,8-H); HRMS, *m/e* 252.0997 (M + H$^+$, $C_{16}H_{13}NO_2$, calcd 252.1024).

2-[(Methylamino)methyl]anthraquinone Monophosphate. Anal.: C, 55.36; H, 4.67; N, 3.94; P, 9.08%. Calcd for $C_{16}H_{13}NO_2 \cdot H_3PO_4$: C, 55.02; H, 4.62; N, 4.01; P, 8.87%.

*2-[(Ethylamino)methyl]anthraquinone (***8***).* NMR δ 1.12 (3H, t, *J* = 7.1 Hz, NCH$_2$C<u>H</u>$_3$), 2.26 (2H, q, *J* = 7.1 Hz, NC<u>H</u>$_2$CH$_3$), 3.90 (2H, s, 2-C<u>H</u>$_2$), 7.70–7.73 (3H, m, 3,6,7-H), 8.13–8.22 (4H, m, 1,4,5,8-H); HRMS, *m/e* 266.1153 (M + H$^+$, $C_{17}H_{16}NO_2$, calcd 266.1181).

2-[(Ethylamino)methyl]anthraquinone Monophosphate. Anal.: C, 56.49; H, 5.22; N, 3.89; P, 8.24%. Calcd for $C_{17}H_{16}NO_2 \cdot H_3PO_4$: C, 56.20; H, 4.99; N, 3.86; P, 8.53%.

*2-[N-(Propyl)aminomethyl]anthraquinone (***9***).* NMR δ 0.93 (3H, t, *J* = 7.0 Hz, NHCH$_2$CH$_2$C<u>H</u>$_3$), 1.56 (2H, hextet, *J* = 6.9 Hz, NHCH$_2$C<u>H</u>$_2$CH$_3$), 2.62 (2H, t, *J* = 6.9 Hz, NHC<u>H</u>$_2$CH$_2$CH$_3$), 3.95 (2H, s, 2-C<u>H</u>$_2$), 7.77–7.79 (3H, m, 3,6,7-H), 8.22–8.30 (4H, m, 1,4,5,8-H); HRMS, *m/e* 280.1321 (M + H$^+$, $C_{18}H_{18}NO_2$, calcd 280.1259).

2-[N-(Propyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 57.19; H, 5.45; N, 3.82; P, 7.98%. Calcd for $C_{18}H_{18}NO_2 \cdot H_3PO_4$: C, 57.30; H, 5.36; N, 3.71; P, 8.21%.

*2-[N-(1′,1′-Dimethylethyl)aminomethyl]anthraquinone (***10***).* NMR δ 1.19 (9H, s, NC(CH$_3$)$_3$) 3.90 (2H, s, 2-C<u>H</u>$_2$), 7.77–7.82 (3H, m, 3,6,7-H), 8.23–8.31 (4H, m, 1,4,5,8-H); HRMS, *m/e* 294.1483 (M + H$^+$, $C_{19}H_{20}NO_2$, calcd 294.1494).

2-[N-(1′,1′-Dimethylethyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 58.11; H, 5.83; N, 3.52; P, 7.69%. Calcd for $C_{19}H_{19}NO_2 \cdot H_3PO_4$: C, 58.31; H, 5.67; N, 3.58; P, 7.91%.

*2-[N-(Cyclopropyl)aminomethyl]anthraquinone (***11***).* NMR δ 0.43 (4H, m, −C<u>H</u>$_2$−C<u>H</u>$_2$−), 2.17 (1H, m, NHC<u>H</u>), 4.01 (2H, s, 2-C<u>H</u>$_2$), 7.75–7.81 (3H, m, 3,6,7-H), 8.23–8.32 (4H, m, 1,4,5,8-H); HRMS, *m/e* 278.1155 (M + H$^+$, $C_{18}H_{16}NO_2$, calcd 278.1181).

2-[N-(Cyclopropyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 57.74; H, 5.09; N, 3.51; P, 8.21%. Calcd for $C_{18}H_{16}NO_2 \cdot H_3PO_4$: C, 57.60; H, 4.83; N, 3.73; P, 8.25%.

*2-[N-(Pentyl)aminomethyl]anthraquinone (***12***).* NMR δ 0.78 (3H, t, *J* = 6.7 Hz, 5′-CH$_3$), 1.19 (4H, m, 3′- and 4′-CH$_2$), 1.44 (2H, m, 2′-CH$_2$), 2.53 (2H, t, *J* = 7.4 Hz, 1′-CH$_2$), 3.82 (2H, s, 2-C<u>H</u>$_2$), 7.63–7.68 (3H, m, 3,6,7-H), 8.06–8.14 (4H, m, 1,4,5,8-H); HRMS, *m/e* 308.1619 (M + H$^+$, $C_{20}H_{22}NO_2$, calcd 308.1645).

2-[N-(Pentyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 59.53; H, 6.25; N, 3.66; P, 7.46%. Calcd for $C_{20}H_{22}NO_2 \cdot H_3PO_4$: C, 59.26; H, 5.97; N, 3.46; P, 7.64%.

*2-[N-(1′-Methylbutyl)aminomethyl]anthraquinone (***13***).* NMR δ 0.89 (3H, t, *J* = 7.1 Hz, 4′-CH$_3$) 1.08 (3H, d, *J* = 6.2 Hz, 1′-CH$_3$), 1.33 (2H, m, 3′-CH$_2$), 1.46 (2H, m, 2′-CH$_2$), 2.68 (1H, *J* = 6 Hz, 1′-NHC<u>H</u>), 3.89, 3.97 (2H, 2×AB doublets, *J* = 14.2 Hz, 2-C<u>H</u>$_2$), 7.74–7.77 (3H, m, 3,6,7-H), 8.20–8.28 (4H, m, 1,4,5,8-H); HRMS, *m/e* 308.1619 (M + H$^+$, $C_{20}H_{22}NO_2$, calcd 308.1645).

2-[N-(1′-Methylbutyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 59.11; H, 5.86; N, 3.37; P 7.51%. Calcd for $C_{20}H_{22}NO_2 \cdot H_3PO_4$: C, 59.26; H, 5.97; N, 3.46; P, 7.64%.

*2-[N,N-(Diisopropyl)aminomethyl]anthraquinone (***14***).* NMR δ 1.03 (12H, d, *J* = 6.5 Hz, N-CH(C<u>H</u>$_3$)$_2$) 3.03 (2H, septet, *J* = 6.5 Hz, N-C<u>H</u>(CH$_3$)$_2$), 3.78 (2H, s, 2-C<u>H</u>$_2$), 7.75–7.88 (3H, m, 3,6,7-H), 8.21−8.31 (4H, m, 1,4,5,8-H); HRMS, *m/e* 322.1767 (M + H$^+$, $C_{21}H_{24}NO_2$, calcd 322.1802).

2-[N,N-(Diisopropyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 60.22; H, 6.20; N, 3.39; P, 7.51%. Calcd for $C_{21}H_{24}NO_2 \cdot H_3PO_4$: C, 60.14; H, 6.25; N, 3.34; P, 7.39%.

*2-[N,N-(Diethyl)aminomethyl]anthraquinone (***15***).* NMR δ 1.03 (6H, t, *J* = 7.1 Hz, −N(CH$_2$C<u>H</u>$_3$)$_2$), 2.52 (4H, t, *J* = 7.1 Hz, −N(C<u>H</u>$_2$CH$_3$)$_2$) 3.67 (2H, s, 2-CH$_3$) 7.71–7.80 (3H, m, 3,6,7-H), 8.17–8.24 (4H, m, 1,4,5,8-H); HRMS, *m/e* 294.1473 (M + H$^+$, $C_{19}H_{20}NO_2$, calcd 294.1488).

2-[N,N-(Diethyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 58.12; H, 5.69; N, 3.71; P, 7.72%. Calcd for $C_{19}H_{20}NO_2 \cdot H_3PO_4$: C, 58.31; H, 5.67; N, 3.58; P, 7.91%.

*2-[N,N-(Dibutyl)aminomethyl]anthraquinone (***16***).* NMR δ 0.83 (6H, t, *J* = 7.2 Hz, 4′-(C<u>H</u>$_3$)$_2$) 1.27 (4H, m, 3′-(CH$_2$)$_2$), 1.43 (4H, m, 2′-(CH$_2$)$_2$), 2.41 (4H, t, *J* = 7.4 Hz, 1′-(CH$_2$)$_2$), 3.65 (2H, s, 2-CH$_2$), 7.69–7.72 (3H, m, 3,6,7-H), 8.15–8.22 (4H, m, 1,4,5,8-H); HRMS, *m/e* 350.2077 (M + H$^+$, $C_{23}H_{27}NO_2$, calcd 350.2118).

2-[N,N-(Dibutyl)aminomethyl]anthraquinone Monophosphate. Anal.: C, 61.84; H, 6.57; N, 3.33; P, 7.11%. Calcd for $C_{23}H_{27}NO_2 \cdot H_3PO_4$: C, 61.74; H, 6.76; N, 3.13; P, 6.92%.

*2-[1′-(Isopropylamino)ethyl]anthraquinone (***17***).* NMR δ 0.97, 1.02 (6H, 2×d, *J* = 6.3 Hz, NHCH(C<u>H</u>$_3$)$_2$), 1.36 (3H, d, *J* = 6.6 Hz, 2′-CH$_3$), 2.60 (1H, septet, *J* = 6.2 Hz, NHC<u>H</u>(CH$_3$)$_2$), 4.06 (1H, q, *J* = 6.6 Hz, 1′-CH), 7.72–7.78 (3H, m, 3,6,7-H), 8.19–8.28 (4H, m, 1,4,5,8-H); HRMS, *m/e* 294.1512 (M + H$^+$, $C_{19}H_{20}NO_2$, calcd 280.1494).

2-[1′-(Isopropylamino)ethyl]anthraquinone Monophosphate. Anal.: C, 58.11; H, 5.76; N, 3.37; P, 7.71%. Calcd for $C_{19}H_{19}N_2O_2 \cdot H_3PO_4$: C, 58.31; H, 5.67; N, 3.58; P, 7.91%.

*2-[1′-{3″-(N,N-Diethylamino)propylamino}ethyl]anthraquinone (***18***).* NMR δ 0.94 (6H, t, *J* = 7.1 Hz, −N(CH$_2$C<u>H</u>$_3$)$_2$), 1.35 (3H, d, *J* = 6.6 Hz, 2′-CH$_3$) 1.58 (2H, quintet, *J* = 6.9 Hz, 2″-CH$_2$), 2.34–2.56 (8H, m, 1″, 3″-CH$_2$, −N(C<u>H</u>$_2$CH$_3$)$_2$), 3.89 (1H, q, *J* = 6.5 Hz, 1′-CH), 7.71–7.77 (3H, m, 3,6,7-H), 8.17–8.24 (4H, m, 1,4,5,8-H); HRMS, *m/e* 364.2168 (M + H$^+$, $C_{23}H_{28}N_2O_2$, calcd 364.2151).

2-[1′-{3″-(N,N-Diethylamino)propylamino}ethyl]anthraquinone Diphosphate. Anal.: C, 49.16; H, 5.87; N, 4.87; P, 11.36%. Calcd for $C_{23}H_{28}N_2O_2 \cdot 2H_3PO_4$: C, 49.29; H, 6.11; N, 5.00; P, 11.05%.

*1-(N,N-Diethylaminomethyl)anthraquinone (***19***).* NMR δ 1.08 (6H, t, *J* = 7.0 Hz, N(CH$_2$C<u>H</u>$_3$), 2.61 (4H, q, *J* = 7.0 Hz, N(C<u>H</u>$_2$CH$_3$), 4.23 (2H, s, 1-CH$_2$), 7.72–7.76 (4H, m, 2,3,6,7-H), 8.25–8.38 (3H, m, 4,5,8-H); HRMS, *m/e* 294.1476 (M + H$^+$, $C_{19}H_{20}NO_2$, calcd 294.1494).

1-(N,N-Diethylaminomethyl)anthraquinone Monophosphate. Anal.: C, 58.54; H, 5.82; N, 3.60; P, 7.76%. Calcd for $C_{19}H_{19}N_2O_2 \cdot H_3PO_4$: C, 58.31; H, 5.67; N, 3.58; P 7.91%.

Preparation of 2-Ethylaminoanthraquinone Analogues. *2-(Hydroxyethyl)anthraquinone.* A mixture of 2-methylanthraquinone (2.2 g, 10 mmol), paraformaldehyde (150 mg, 5 mmol), and KOH (50 mg) in DMSO (25 mL) was heated at 110–120 °C under nitrogen for 96 h. Three more portions of paraformaldehyde (150 mg, 5 mmol each) were added at 24 h intervals. The reaction mixture was poured into water and then filtered. The precipitate obtained was chromatographed over silica gel and elution with dichloromethane:hexane (1:1) yielded the unreacted 2-methylanthraquinone. Further elution with dichloromethane/methanol (99:1) yielded 2-(hydroxyethyl)anthraquinone (700 mg): NMR δ 3.05 (2H, t, *J* = 7.2 Hz, C<u>H</u>$_2$CH$_2$OH), 3.99 (2H, dt, *J* = 7.2, 1.2 Hz, CH$_2$C<u>H</u>$_2$OH), 7.66 (1H, br d, *J* = 8.0 Hz, H-3), 7.77–7.80 (2H, m, 6,7-H), 8.14 (1H, br s, 1-H), 8.21 (1H, d, *J* = 8.0 Hz, 4-H), 8.26–8.30 (2H, m, 5,8-H); HRMS, *m/e* 253.0865 (M + H$^+$, $C_{16}H_{13}O_3$, calcd 253.0855).

Synthesis of 2-(Bromoethyl)anthraquinone. A mixture of 2-(hydroxyethyl)anthraquinone (400 mg, 1.6 mmol), triphenylphosphine (620 mg, 2.4 mmol), and carbon tetrabromide (660 mg, 2 mmol) in dichloromethane (15 mL) was stirred at 0 °C. After 1 h, solvent was evaporated and the residue was chromatographed over silica gel. Elution with ethyl acetate/hexanes (5:95) yielded 2-(bromoethyl)anthraquinone, which was crystallized from chloroform/hexanes to yield pale yellow crystals (310 mg): NMR δ 3.34 (2H, t, *J* = 7.2 Hz, C<u>H</u>$_2$CH$_2$Br), 3.65 (2H, t, *J* = 7.2 Hz, CH$_2$C<u>H</u>$_2$Br), 7.64 (1H, dd, *J* = 8.0, 1.6 Hz, H-3), 7.75–7.80 (2H, m, 6,7-H), 8.14 (1H, d, *J* = 1.6 Hz, 1-H), 8.26 (1H, d, *J*=6.4 Hz, 4-H), 8.25–8.31 (2H, m, 5,8-H); HRMS, *m/e* 315.0023 (M + H$^+$, $C_{16}H_{12}BrO_2$, calcd 315.0027).

*2-(Isopropylaminoethyl)anthraquinone (***20***).* A mixture of 1-bromoethylanthraquinone (300 mg) and isopropylamine (1 mL) in acetonitrile (10 mL) was refluxed for 6 h. The reaction mixture was evaporated under vacuum and the residue was sonicated with chloroform and then filtered to yield 2-[ethylamino-*N*-(1′-methylethyl)]-9,10-anthraquinone hydrobromide as an off-white crystalline compound. This compound was partitioned between 0.5 M sodium hydroxide and ethyl acetate, and the organic layer was dried and evaporated to yield **20**: NMR δ 1.03 (6H, d, *J* = 6.4 Hz, NCHC<u>H</u>$_3$), 2.81 (1H, septet, *J* = 6.4 Hz), 2.93 (4H, s, C<u>H</u>$_2$C<u>H</u>$_2$N), 7.60 (1H, dd, *J* = 8.0, 1.6 Hz, H-3), 7.73–7.80 (2H, m, 6,7-H), 8.10 (1H, d, *J* = 1.6 Hz, 1-H), 8.19 (1H, d, *J* = 6.4 Hz, 4-H), 8.20–8.28 (2H, m, 5,8-H); HRMS, *m/e* 294.1511 (M + H$^+$, $C_{19}H_{20}NO_2$, calcd 294. 1494).

2-(Isopropylaminoethyl)anthraquinone Monophosphate. The residue obtained was dissolved in methanol (10 mL), treated with H_3PO_4 (1 mL), diluted with ether (10 mL), and left overnight at 4 °C. The crystalline product was filtered off and dried to yield an off-white solid. Anal.: C, 58.07; H, 5.85; N, 3.38; P, 7.86%. Calcd for $C_{19}H_{19}NO_2 \cdot H_3PO_4$: C, 58.31; H, 5.67; N, 3.58; P, 7.91%.

General Procedure for the Preparation of 1- and 2-Pentaethyleneglyco-9,10-anthraquinone. A mixture of 1-chloroanthraquinone (2.5 g) or 2-chloroanthraquinone and potassium carbonate (2.5 g) in pentaethyleneglycole (15 mL) was heated for 3 h at 140 °C, poured into cool water, and filtered. The resulting solid was chromatographed over silica gel and elution with dichloromethane:methanol (96:4) to yield 1- or 2-[2-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy] anthraquinone. This compound was crystallized from ether to yield yellow crystalline compounds.

*1-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]anthraquinone (**21**).* NMR δ 3.44 (2H, m, 10′-CH_2), 3.50–3.58 (12H, m, 4′,5′,6′,7′,8′,9′-CH_2), 3.73 (2H, m, 3′-CH2), 3.88 (2H, t, $J = 4.9$ Hz, 2′-CH_2), 4.16 (2H, t, $J = 4.7$ Hz, 1′-CH_2), 7.20 (1H, br d, $J = 8.4$ Hz, 2-H), 7.51 (1H, br t, $J = 8.0$ Hz, 3-H), 7.58 (2H, m, 6,7-H), 7.73 (1H, dd, $J = 7.7$, 0.8 Hz, 4-H), 8.01 (1H, dd, $J = 7.6$, 1.4 Hz, 8-H) 8.04 (1H, dd, $J = 7.8$, 1.5 Hz, 5-H); HRMS, *m/e* 445.1867 ($M + H^+$, $C_{24}H_{29}O_8$, calcd 445.1862).

*2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]anthraquinone (**22**).* NMR δ 3.21 (3H, s, $-OCH_3$), 3.39 (2H, m, 8′-CH_2), 3.46–3.62 (10H, m, 3′,4′,5′,6′,7′-CH_2), 3.77 (2H, t, $J = 4.6$ Hz, 2′-CH_2), 4.11 (2H, t, $J = 4.6$ Hz, 1′-CH2), 7.04 (1H, dd, $J = 8.5$, 2.6 Hz, 3-H), 7.42 (1H, d, $J = 2.5$ Hz, 1-H), 7.56 (2H, m, 6,7-H), 7.95 (1H, d, $J = 8.6$ Hz, 4-H), 8.01 (2H, m, 5,8-H); HRMS, *m/e* 445.1856 ($M + H^+$, $C_{24}H_{29}O_8$, calcd 445.1862).

*2-Methylene-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]anthraquinone (**23**).* A mixture of 2-chloromethylanthraquinone (2.5 g) and barium hydroxide (5 g) in pentaethyleneglycol (15 mL) was sonicated for 10 min and then stirred for 3 h at room temperature. The reaction mixture was poured into cool water and filtered. The solid obtained was chromatographed over silica gel and then eluted with dichloromethane/ethanol (96:4) to yield 2-methylene-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]anthraquinone. This compound was crystallized from ether to yield an off-white crystalline compound: NMR δ 3.60 (2H, m, 6′-CH_2), 3.67–3.72 (14H, m, 1′,2′,3′,4′,5′,6′,7′-CH_2), 4.73 (2H, s, 2-CH_2), 7.75–7.85 (3H, m, 3,6,7-H), 8.24–8.32 (4H, m, 1,4,5,8-H); HRMS,*m/e* 415.1768 ($M + H^+$, $C_{23}H_{27}O_7$, calcd. 415.1756).

Bioassay of Water-Soluble Anthraquinone Analogues. An isolate of the cyanobacterium *O. perornata* was obtained from a water sample collected from a Mississippi catfish pond (*5*). An isolate of the green alga *S. capricornutum* was obtained from Dr. J. C. Greene, USEPA, Corvallis, OR, and *S. capricornutum* was used as a representative of green algae in the bioassay to determine selective toxicity of the compounds. Green algae (Division Chlorophyta) are the preferred type of phytoplankton in catfish aquaculture ponds due to their lack of production of earthy and musty off-flavor compounds and because they are better oxygenators of the water compared to cyanobacteria (*6*). Each culture was maintained separately in continuous, steady-state growth using the conditions outlined in Schrader et al. (*7*) to provide a source of cells growing at a fairly constant rate.

The rapid bioassay of Schrader et al. (*7*) was used to evaluate the water-soluble anthraquinone analogues for selective toxicity toward *O. perornata*. Stock solutions of each pure compound were made in deionized water at concentrations of 4.0, 40.0, 400.0, 4000.0, 40000.0, 400000.0, and 4000000.0 nM. Stock solutions of water-soluble anthraquinones were micropipetted into wells (50 μL of anthraquinone solution per well) in a 96-well microplate (Costar Corp., Cambridge, MA) containing 150 μL of either cyanobacterial or unialgal culture material from continuous cultures. Deionized water was added to control wells (50 μL per well). Final test concentrations were 1.0, 10.0, 100.0, 1000.0, 10000.0, 100000.0, and 1000000.0 nM. Three replications were used for each concentration of each pure compound and for the control; bioassay experiments were repeated. Microplates were placed in a growth chamber held at 29 ± 1 °C and were illuminated continuously by fluorescent lights (40 W, cool white) at a photon flux density of 21−27 μEinstein/m^2/s. Absorbance measurements of each well were measured at 650 nm at 24 h intervals for 4 days using a Packard model SpectraCount microplate photometer (Packard Instrument Co., Meriden, CT). Mean values and standard deviations of absorbance measurements were calculated and graphed to determine the lowest-observed-effect concentration (LOEC) and lowest-complete-inhibition concentration (LCIC). The 96 h IC_{50} (50% inhibition concentration) values of the most promising compounds (*8*) were compared to the 96-h IC_{50} of anthraquinone, with determination of 95% confidence limits for each value.

Table 1. Bioassay Results of Anthraquinone Analogues

	test organism			
	O. perornata		*S. capricornutum*	
compd	LOEC[a] (nM)	LCIC[b] (nM)	LOEC (nM)	LCIC (nM)
1	10	100	10000	10000
2	100	1000	10000	10000
3	100	100	10000	10000
4	10000	10000	100000	100000
5	1000	1000	100000	100000
6	10	100	10000	100000
7	1000	1000	10000	10000
8	1000	1000	10000	10000
9	1000	1000	10000	10000
10	1000	1000	1000	10000
11	1000	1000	10000	100000
12	1000	1000	100000	100000
13	100	100	100000	100000
14	1000	1000	100000	1000000
15	100	100	10000	100000
16	1000	1000	100000	1000000
17	1000	1000	100000	100000
18	10000	10000	100000	100000
19	100	1000	10000	10000
20	1000	1000	10000	100000
21	1000	10000	>100000	>100000
22	10000	100000	>100000	>100000
23	100	100000	>100000	>100000

[a] Lowest-observed-effect concentration (lowest concentration to inhibit growth). [b] Lowest-complete-inhibition concentration.

RESULTS AND DISCUSSION

In previous studies, a few water-soluble anthraquinone analogues were evaluated for their algicidal activity (*3*). Anthraquinone-2-sulfonic acid, the anionic (sodium) salt of anthraquinone-2-sulfonic acid and anthraquinone-1,5-disulfonic acid disodium salt have high water solubility. These compounds had significantly low algicidal activity compared to 9,10-anthraquinone. Bioassay results indicated that water-soluble analogue **1** is highly effective and selective toward *O. perornata* (*8*). The side chain of this compound is the same side chain in the antimalarial drug chloroquine and contains an asymmetric center. These results guided us to prepare and evaluate water-soluble cationic salt analogues of 9,10-anthraquinone. The condensation of 1-chloro- or 2-chloromethylanthraquinone with 3-(diethylamino)propylamine, 3-(dimethylamino)propylamine, or 2-amino-5-(diethylamino)pentane yielded a series of diaminoanthraquinone analogues (**2–5**). The treatment of a methanolic solution of these amines with phosphoric acid (85%) yielded highly water-soluble dicationic anthraquinone analogues. Their algicidal activities are presented in **Table 1**. Compounds **2** and **3** also had high water solubility, however, less activity than **1**. Compounds **4** and **5**, in which the side chain is directly attached to the anthraquinone ring, were less active. Because of the reported mutagenic activity of 2-aminoanthraquinone, its analogues were not prepared.

Previous pond studies conducted with compound **1** at an application rate of 2.0 μM (1.15 mg/L) found levels of MIB

Table 2. 96-h IC_{50} Values of Anthraquinone and Compounds **1** and **6**

compd	IC_{50}[a] (nM) for test organism	
	O. perornata	*S. capricornutum*
AQ[b]	79 (4.8)	5012.0 (144.4)
1	63.0 (6.1)	5012.0 (144.4)
6	6.3 (1.2)	5623.0 (842.4)

[a] IC_{50} = 96-h 50% inhibition concentration; 95% confidence limits are in parentheses. [b] AQ = anthraquinone; unpublished observations.

were reduced by 93.6% after 18 h and remained low for 8 days (*8*). However, subsequent pond studies at lower concentrations (≤0.58 mg/L) found that compound **1** was ineffective (*8*). The efficacy of this compound may have been dependent on the amount of suspended sediments in the test ponds. This possibility was inferred on the basis of the dicationic characteristics of compound **1**. Compound **1** may have a tendency to bind to suspended sediments and organic matter in the water column and settle to the bottom of the pond, thereby resulting in the loss of activity toward noxious species of cyanobacteria. Similar effects have been observed with other dicationic aquatic herbicides such as diquat (*9*).

To overcome this potential drawback, a series of monoaminoanthraquinone analogues **6–16** were prepared and evaluated for selective algicidal activity. These analogues were readily prepared by reacting 2-chloromethylanthraquinone with the appropriate amine. The results of the evaluation of these analogues are provided in **Table 1**. The monoamino analogues were less water soluble than the diamino analogues, but their solubilities were higher than the LCIC observed for them. These analogues had similar activities except for compounds **6**, **13**, and **15**, which all had greater toxicity. Of these analogues, compound **6** had the best activity. The LOEC and LCIC observed for this compound were similar to those observed for compound **1**. However, determination of the IC_{50} against *O. perornata* indicated that compound **6** was 10 times more active than **1** and >10 times more active than anthraquinone (**Table 2**).

Three efficacy studies performed with compound **6** using limnocorrals placed in catfish ponds indicated that it was very effective against *O. perornata* (*8*). At rates of 0.3 μM (125 μg/L), **6** dramatically reduced the abundance of *O. perornata* and levels of MIB 2 days after application. During this time, the abundance of nontarget organisms such as green algae and diatoms increased dramatically. These studies showed that compound **6** was also effective against *Anabaena circinalis* and that geosmin (a compound responsible for an earthy off-flavor in catfish) levels were reduced (*8*). Previous studies have identified a strain of *A. circinalis* that was isolated from a river providing municipal drinking water to be a producer of geosmin (*10*). However, there has not yet been verification of geosmin production by an isolate of *A. circinalis* from a catfish pond. Analysis of the pond water and suspended sediments indicated that compound **6** has very low tendency to bind to sediments. The half-life of **6** in the water column of ponds was 19 h, making it much less persistent than diuron (*8*). Compound **6** appears to undergo oxidative deamination to yield anthraquinone-2-carboxylic acid, which has low algicidal activity toward *O. perornata* (unpublished observations). Replacement of one of the protons adjacent to the primary amine group is known to retard oxidative deamination. In an effort to decrease the rate of the conversion, analogues with a methyl group on the 2-methylene group were prepared and evaluated. These compounds were readily prepared by condensing 2-(1′-bromoethyl) anthraquinone with the corresponding amine. Algicidal activity of compounds **17** and **18** are provided in **Table 1**. The introduction of a methyl group to the "2-CH_2" group was found to reduce the algicidal activity.

To investigate the effect of the position of methyl amino group on the algicidal activity, 1-aminomethylanthraquinone analogues were prepared. Bromination of 1-methylanthraquinone with *N*-bromosuccinimide in the presence of azobisisobutyronitrile yielded 1-bromomethylanthraquinone in good quantities. Condensation of 1-bromomethylanthraquinone with secondary amines such as isopropylamine or 2-amino-5-diethylaminopentane yielded a deep-blue compound, which tentatively was identified as the product resulting from the condensation of the secondary amino group with the adjacent carbonyl. The reaction of 1-chloromethylanthraquinone with diethylamine yielded the expected compound **19**. This compound had moderate algicidal activity toward *O. perornata*.

To investigate the effect of the length of the carbon chain on algicidal activity, the 2-ethylaminoanthraquinone analogue was prepared and evaluated. Condensation of 2-methylanthraquinone with paraformaldehyde in the presence of base yielded 2-ethanolanthraquinone. Bromination of 2-ethanolanthraquinone and subsequent condensation with isopropyl amine yielded 2-[ethylamino-*N*-(1′-methylethyl)]-9,10-anthraquinone (**20**). This compound also had only moderate algicidal activity toward *O. perornata*.

A series of neutral water-soluble anthraquinone analogues were prepared by attaching polyethylene glycol of different ethylenedioxy chain lengths. These compounds were prepared by reacting either 1-chloroanthraquinone, 2-chloroanthraquinone, or 2-chloromethylanthraquinone with the respective polyethylene glycol. The water solubility of the analogues increased with the length of the ethylenedioxy chain, and those chains with four or more ethylenedioxy units (e.g., **21**, **22**, and **23**) were found to be substantially water-soluble. Algicidal activities of these compounds are provided in **Table 1**. Although some of these compounds had low LOEC values, the LCIC values were relatively high, indicating that they had only marginal algicidal activity toward *O. perornata*.

Our results indicate that certain water-soluble anthraquinone analogues that were synthesized during this study have high potential for use as selective algicides toward undesirable species of cyanobacteria found in catfish aquaculture ponds. Compound **6** appears to be the most promising among this group of patented quinones (*11*). Additional studies are needed to further evaluate the most promising compounds to determine their environmental fate in catfish ponds, toxicity toward nontarget organisms, and potential accumulation in the flesh of channel catfish.

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