phosphamide moiety affects the biological property of the resultant molecule. ID_{50} values were determined from plots of mean cell counts after 72 h. Assays were carried out in triplicate, with appropriate controls.

The above findings are of interest in that they represent a novel series of nucleoside cyclophosphamide analogues, with good biological activity; however, whether they have unique and desirable pharmacologic properties in vivo remains to be determined.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The UV spectra were recorded on a Beckman-25 spectrophotometer, and the NMR spectra were taken on a Bruker 270 HX spectrometer at 270 MHz (Me_4Si). The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-5′-amino-5′-deoxythymidine (6). A solution of bis(2-chloroethyl)-phosphoramidic dichloride (0.54 g, 2.07 mmol) in 5 mL of ethyl acetate was added to a magnetically stirred mixture of 5′-amino-5′-deoxythymidine (1; 0.50 g, 2.07 mmol) and triethylamine (0.42 g, 4.15 mmol) in 15 mL of DMF. The reaction mixture was stirred for 48 h at room temperature. The insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was crystallized from 95% EtOH–Et_2O to yield 0.42 g (42%) of product. The compound effervesced above 120 °C: UV (EtOH) λ_{max} 267 nm (ϵ 7820), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 1.81 (s, 3, C-5 CH_3), 2.10 (m, 2, C-2′ H), 3.15–3.35 (m, 6, C-5′ H, C-CH_2-N), 3.69–3.80 (m, 5, C-4′ H, Cl-CH_2-C), 4.30 (m, 1, C-3′ H), 6.13 (m, 1, C-1′ H), 7.54 (s, 1, C-6 H), 9.41 (br s, 1, 5′-NH), 11.34 (br s, 1, 3-NH). Anal. ($C_{14}H_{20}Cl_2N_4O_5P \cdot 0.5H_2O$) C, H, N.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-5′-(methylamino)-5′-deoxythymidine (7). A solution of bis(2-chloroethyl)phosphoramidic dichloride (1.02 g, 3.92 mmol) in 10 mL of ethyl acetate was reacted with 5′-(methylamino)-5′-deoxythymidine (2; 1.00 g, 3.92 mmol) in DMF (15 mL) and triethylamine (0.79 g, 7.84 mmol) according to the method described above to give 0.79 (46%) of 7. Compound 7 started to soften at 105 °C and effervesced above 130 °C: UV (EtOH) λ_{max} 266 nm (ϵ 8680), λ_{min} 238 nm; NMR (Me_2SO-d_6) δ 1.81 (s, 3, C-5 CH_3), 2.07 (m, 2, C-2′ H), 2.50 (s, 3, C-5′ N-CH_3), 3.16–3.33 (m, 6, C-5′ H, C-CH_2-N), 3.64–3.73 (m, 5, C-4′ H, Cl-CH_2-C), 4.13 (m, 1, C-3′ H), 6.20 (m, 1, C-1′ H), 7.63 (s, 1, C-6 H), 11.33 (br s, 1, 3-NH). Anal. ($C_{15}H_{22}Cl_2N_4O_5P$) C, H, N.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-3′,5′-diamino-3′,5′-dideoxythymidine (8). A solution of bis(2-chloroethyl)phosphoramidic dichloride (0.32 g, 1.25 mmol) in ethyl acetate (10 mL) was added to a solution of 3′,5′-diamino-3′,5′-dideoxythymidine (3; 0.30 g, 1.25 mmol) and triethylamine (0.25 g, 2.50 mmol) in 10 mL of DMF. The reaction mixture was stirred for 48 h at room temperature. Compound 8 was isolated in the same manner as mentioned previously to afford 0.22 g (41%): mp 220 °C (dec); UV (EtOH) λ_{max} 267 nm (ϵ 8960), λ_{min} 234 nm; NMR (Me_2SO-d_6) δ 1.81 (s, 3, C-5 CH_3), 2.15 (m, 2, C-2′ H), 3.33–3.49 (m, 7, C-4′ H, C-5′ H, C-CH_2-N), 3.63–3.69 (m, 5, C-3′ H, Cl-CH_2-C), 4.33 (s, 1, C-5′ NH), 4.60 (s, 1, C-3′ NH), 6.19 (t, 1, C-1′ H, $J_{1',2'a}$ = 3.98 Hz, $J_{1',2'b}$ = 6.19 Hz), 7.45 (s, 1, C-6 H), 11.30 (s, 1, 3-NH). Anal. ($C_{14}H_{21}Cl_2N_5O_4P$) C, H, N.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-5′-amino-2′,5′-dideoxy-5-iodouridine (9). A solution of bis(2-chloroethyl)phosphoramidic dichloride (0.74 g, 2.83 mmol) in 15 mL of ethyl acetate was added to a suspension of 5-iodo-5′-amino-2′,5′-dideoxyuridine (4; 1.00 g, 2.83 mmol) in DMF (30 mL) and triethylamine (0.57 g, 5.66 mmol). The reaction mixture was stirred at room temperature for 6 days. The insoluble solid was removed by filtration. Ether was added to the filtrate until it turned cloudy. The solution was then kept at −20 °C for several days, during which time the compound crystallized out. The product was collected by filtration, washed with ether, and dried to afford 0.79 g (52%). Compound 9 softened above 140 °C and decomposed at 189 °C: UV (EtOH) λ_{max} 283 nm (ϵ 7470), λ_{min} 245 nm; NMR (Me_2SO-d_6) δ 2.11 (m, 1, C-2′ H_a), 2.35 (m, 1, C-2′ H_b), 3.61–3.82 (m, 11, C-4′ H, C-5′ H, C-CH_2-N, Cl-CH_2-C), 4.30 (m, 1, C-3′ H), 6.04 (t, 1, C-1′ H, $J_{1',2'a}$ = 6.63 Hz, $J_{1',2'b}$ = 7.08 Hz), 8.00 (s, 1, C-6 H), 9.75 (br s, 1, C-5′ NH), 11.73 (br s 1, 3-NH). Anal. ($C_{13}H_{17}Cl_2IN_4O_5P$) N; C: calcd, 29.02; found, 29.72. H: calcd, 3.18; found, 3.95.

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2-Methylanthraquinone Derivatives as Potential Bioreductive Alkylating Agents

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Hypoxic cells of solid tumors are an obstacle to effective cancer therapy. Since hypoxic cells remote from the tumor blood supply may have a greater capacity for reductive reactions than well-oxygenated cells, we have prepared a series of anthraquinone prodrugs which may be capable of generating a reactive quinonemethide species following enzymatic reduction to the hydroquinone and loss of the substituent on the methylene group in the 2 position. The synthesized 2-methyl-substituted anthraquinone derivatives have first half-wave reduction potentials of −0.52 to −0.56 V at pH 7.0, which are the lowest oxidation–reduction potentials of quinone bioreductive alkylating agents synthesized by this laboratory to date. Tests of the cytotoxicity of these agents to oxygenated and chronically hypoxic EMT6 tumor cells in culture demonstrated that 2-(hydroxymethyl)anthraquinone, 2-[(*N*-methylcarbamyl)-methyl]anthraquinone, 2-[[(*p*-toluenesulfonyl)oxy]methyl]anthraquinone, and 2-(methoxymethyl)anthraquinone were significantly more toxic to hypoxic cells than to their normally aerated counterparts. The findings demonstrate differences between various leaving groups in the 2 position for the expression of differential cytotoxicity.

Significant advances toward the cure of human cancer by chemotherapy have been achieved primarily with cytotoxic agents directed toward proliferating cells. Thus, certain rapidly growing cancers, such as acute lymphocytic leukemia and Hodgkin's disease, can respond dramatically to existing chemotherapy, and cure of both localized and disseminated malignancies are not uncommon. The more slow-growing solid tumors which represent the majority

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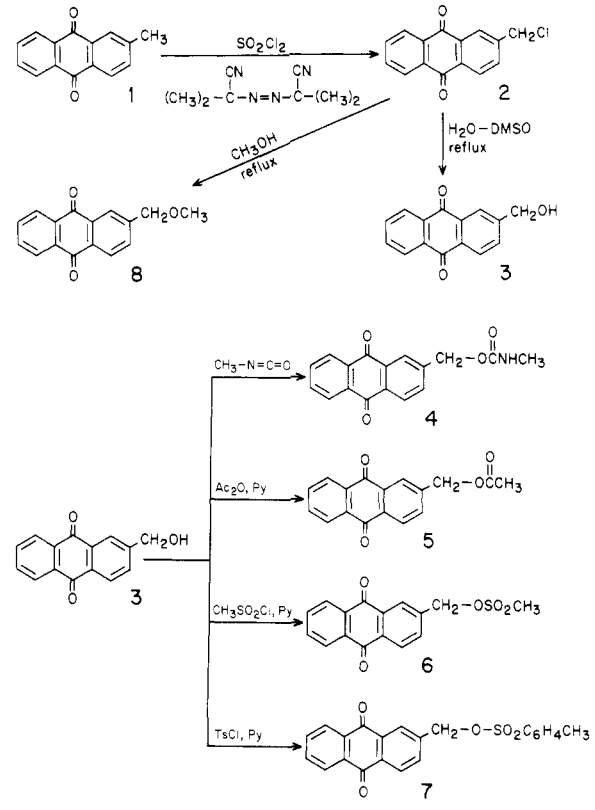
of human cancers, in general, respond relatively poorly to existing chemotherapeutic agents, and curative treatment by any therapeutic modality or combination of modalities is uncommon in patients presenting with extensive disease.[1]

The clonogenic hypoxic cells in solid tumors are an obstacle to effective cancer treatment, for such malignant cells may have prolonged cell-cycle times[2] or may be blocked in their progression through the cycle.[3] Therefore, most hypoxic cells would be expected to be resistant to chemotherapeutic agents requiring cellular replication. Hypoxic cells, however, appear to be capable of entering the proliferative cellular pool and thereby cause tumor recurrence in situations in which therapy has induced significant remission.[1] Hypoxic tumor cells may also exhibit resistance to chemotherapeutic agents due to pharmacodynamic considerations. Thus, appropriate concentrations of drugs that have physiochemical properties not conducive to diffusion into tumor tissue, or that are unstable or metabolized rapidly, may not reach chronically hypoxic cells located in regions of severe vascular insufficiency.

Since hypoxic cells are remote from the vascular supply of a tumor mass and, therefore, are deficient in oxygen which has limited diffusion capability due both to the hydrophilicity and the rapid metabolism of oxygen, it was hypothesized that hypoxic cells might have a greater capacity for reductive reactions than their normally oxygenated counterparts and that this characteristic of hypoxic cells might be exploited by developing chemotherapeutic agents which exerted cytotoxic activity after reductive activation. The first bioreductive alkylating agents designed and synthesized by this laboratory were a series of benzo- and naphthoquinones.[4-10] These compounds were visualized to be reduced preferentially by hypoxic neoplastic cells to generate highly reactive quinonemethides which alkylate critical components of these cells. The naturally occurring bioreductive alkylating agent mitomycin C was shown by Iyer and Szybalski[11] and by Schwartz[12] to require reduction by an NADPH-dependent enzyme system to exert alkylating activity. We have demonstrated that mitomycin C is bioactivated to alkylating species under anaerobic conditions by liver microsomes and nuclei, and by sarcoma 180 and EMT6 tumor cell sonicates, and that this antibiotic causes preferential kill of hypoxic tumor cells in vitro.[13,14] The enzyme system(s) involved in the reductive bioactivation was inhibited by both oxygen and carbon monoxide, suggesting the involvement of a cytochrome P-450 mediated reaction.

Scheme I



Studies on the relationship between the oxidation–reduction potential of benzo- and naphthoquinones and their effectiveness as antitumor agents revealed a positive correlation between the first half-wave reduction potential and antineoplastic efficacy, with agents having the lowest reduction potential being the most effective.[6,15,16] Furthermore, since Cosby et al.[17] demonstrated that (a) 2,3-bis(chloromethyl)-1,4-naphthoquinone, which has a half-wave reduction potential of −0.29 V, is capable of being bioactivated by mitochondrial electron-transport processes, a phenomenon that would be expected to decrease the therapeutic potential of these agents through reductive activation by oxygenated cells, and (b) mitomycin C, with a reduction potential of −0.40 V, is not reduced by mitochondrial electron transport, it appeared that the design and synthesis of quinones with even lower reduction potentials were warranted. For this reason we have prepared and evaluated in vitro against hypoxic and oxygenated EMT6 mammary tumor cells several anthraquinone derivatives with the potential to function as bioreductive alkylating agents.

Chemistry. A series of anthraquinone derivatives containing halide (**2**), alcohol (**3**), carbamate (**4**), acetate (**5**), sulfonates (**6** and **7**), and ether (**8**) substituents in the

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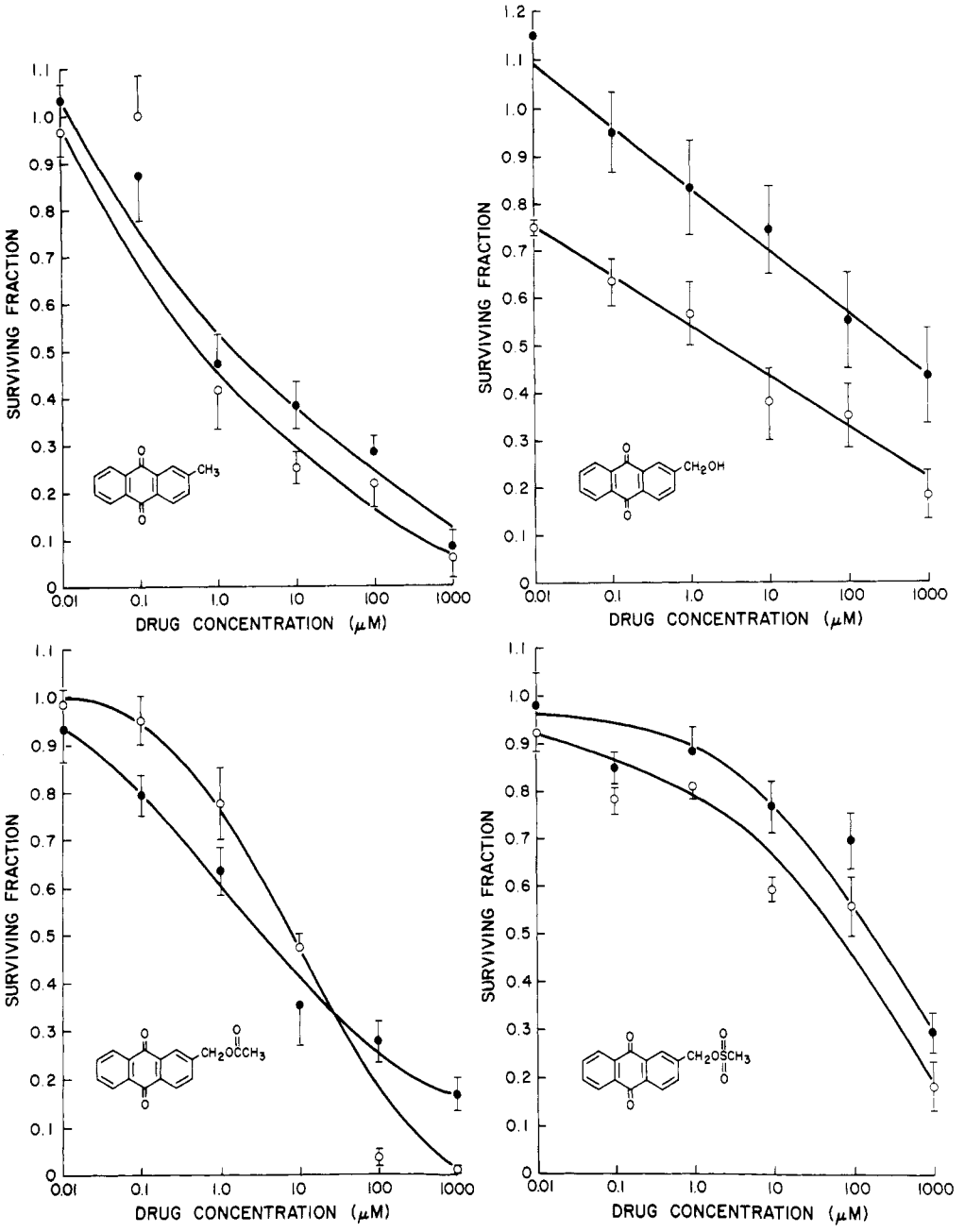


Figure 1. Survival of aerobic (●) and hypoxic (○) EMT6 tumor cells treated for 1 h with various concentrations of 2-methylanthraquinone (1) (top left), 2-(hydroxymethyl)anthraquinone (3) (top right), 2-(acetoxymethyl)anthraquinone (5) (bottom left) and 2-[[(methanesulfonyl)oxy]methyl]anthraquinone (6) (bottom right).

2 position has been synthesized (Scheme I). Treatment of 2-methylanthraquinone (1) with an excess of sulfuryl chloride in the presence of a catalytic amount of 2,2′-azobis(2-methylpropionitrile) under refluxing temperature afforded the 2-(chloromethyl) analogue **2**, which is the key intermediate for the preparation of other anthraquinone derivatives. Refluxing **2** in a mixture of water and Me_2SO gave the alcohol (**3**). Treatment of **3** with methyl isocyanate in CH_2Cl_2 at 70 °C or Ac_2O in pyridine at 3 °C yielded the carbamate (**4**) and the acetate (**5**), respectively. The sulfonates (**6** and **7**) were obtained by reacting **3** with the corresponding sulfonyl chlorides in pyridine at 3 °C. The ether (**8**) was prepared by refluxing **2** in MeOH.

Biological Evaluation. Measurement of the surviving fractions of aerated and chronically hypoxic EMT6 tumor cells treated in culture with various concentrations of the anthraquinones for 1 h was used to determine the specificity of these agents for hypoxic cells. The curves are plotted with the surviving fraction of cells on a linear scale to emphasize the differential cytotoxicities of these compounds to oxygenated and hypoxic cells at very low drug concentrations. The survival of chronically hypoxic cells (i.e., cells maintained under 95% N_2/5% CO_2 for 4 h prior to the addition of drug) exposed to some of the anthraquinone derivatives for 1 h was significantly lower than that of normally aerated cells treated with these agents under similar conditions. Thus, as Figures 1 and 2 and the ED_{50} values shown in Table I indicate, compounds **3** and **7**, the alcohol and the toluenesulfonate, required greater than 100-times more drug to kill 50% of the aerobic cells than the corresponding percentage of hypoxic cells. Similarly, compounds **2**, **4**, and **8** required between 30- and 50-fold more drug to kill 50% of the aerobic cells than the hypoxic cells. In contrast, the concentrations of compounds **1**, **5**, and **6** needed to obtain 50% survival of aerobic and hypoxic cells were nearly the same. It is possible that the 2-(hydroxymethyl) compound (**3**) exerts its cytotoxicity following intracellular conjugation to the gluc-

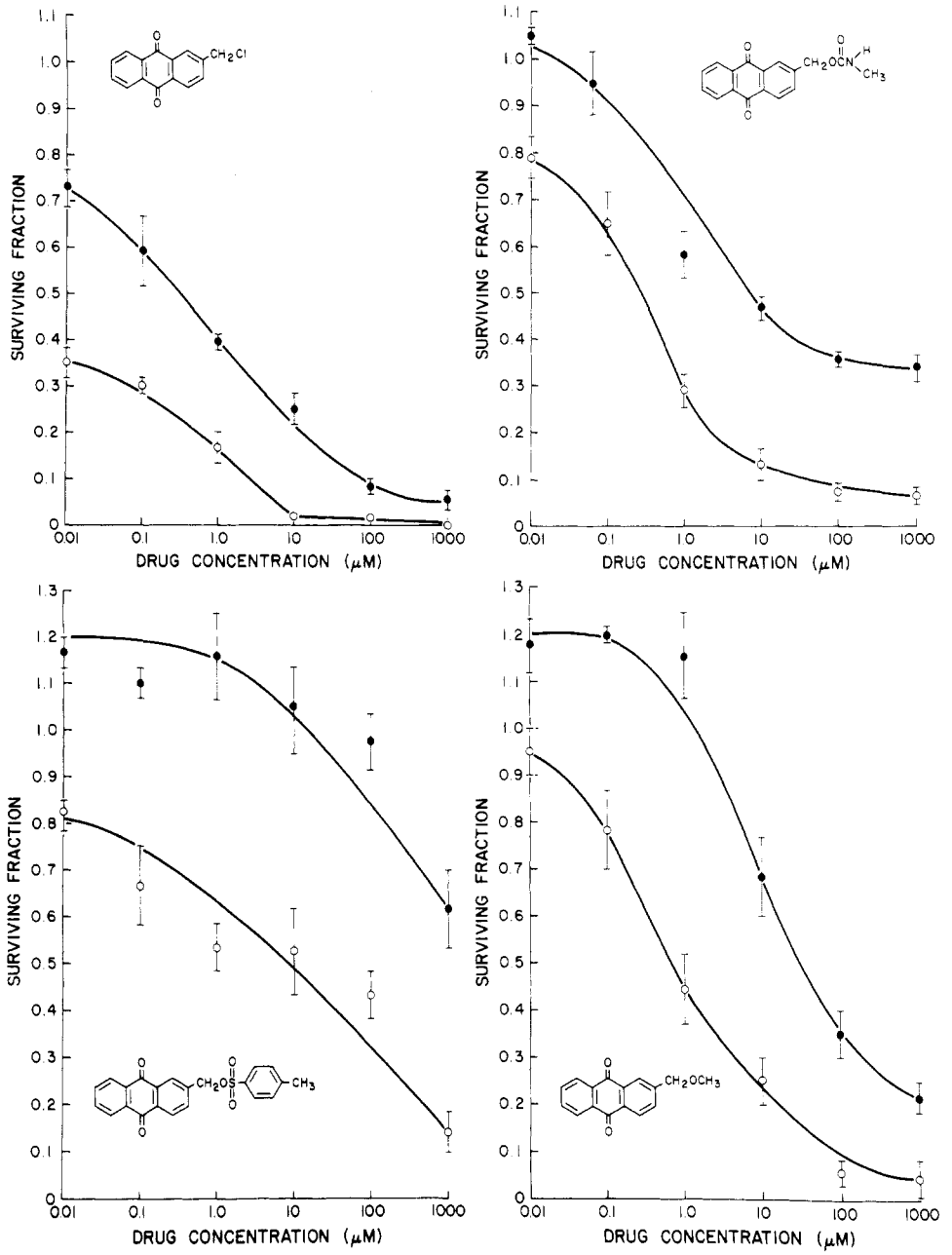


Figure 2. Survival of aerobic (●) and hypoxic (○) EMT6 tumor cells treated for 1 h with various concentrations of 2-(chloromethyl)anthraquinone (**2**) (top left), 2-methylanthraquinone *N*-methylcarbamate (**4**) (top right), 2-[[(*p*-toluenesulfonyl)oxy]methyl]anthraquinone (**7**) (bottom left), and 2-(methoxymethyl)anthraquinone (**8**) (bottom right).

uronidate, sulfate, or phosphate, reduction of the quinone moiety, and departure of the conjugating group to form a reactive quinonemethide.

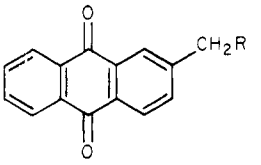
Examination of the shapes of the survival curves (Figures 1 and 2) suggested that the effectiveness of the carbamate (**4**) against aerobic cells reached a maximum at a concentration of about 100 μM. In contrast, the differential cytotoxicity of this agent to hypoxic EMT6 cells appeared to increase with increasing concentration, and the ratio of the ED_{50} for aerobic to hypoxic cells was 125. As can be seen in Figure 2, 2-[[(*p*-toluenesulfonyl)oxy]methyl]anthraquinone (**7**) showed no toxicity to aerobic cells at concentrations up to 10 μM, yet at a concentration of 10 μM 50% of the hypoxic cells are killed by this agent.

Mitomycin C, the most efficacious of the quinone bioreductive alkylating agents tested by this laboratory to date, has a first half-wave reduction potential of −0.40 V in the test system used in this study. On the other hand, adriamycin, which may act as a bioreductive alkylating agent under anaerobic conditions, has a reduction potential of −0.59 V. The anthraquinone derivatives described in this report have reduction potentials ranging from −0.52 to −0.56 V (Table I); these are the lowest oxidation–reduction potentials for quinone bioreductive alkylating agents that we have synthesized thus far, and these agents would not be expected to be capable of being reduced by the mitochondrial electron-transport system.

The magnitude of the differential kill of oxygenated and hypoxic cells expressed by the anthraquinone derivatives varied significantly with the character of the leaving group substituted on the carbon-2 methyl (Figures 1 and 2). These differences in differential kill may reflect (a) the rate at which the various leaving groups form the hypothesized intermediate anthraquinonemethide or (b) differences in the intracellular distribution of the various anthraquinones.

Recently, Cheng and his co-workers reported the synthesis of a series of amino-substituted anthraquinone derivatives with significant antineoplastic activity. The cy-

Table I. First Half-Wave Reduction Potentials and ED_{50} Values for the Bioreducible Anthraquinone Derivatives



compd	R	$E_{1/2}$, V[a]	ED_{50}, μM[b] aerobic	hypoxic	ratio (aerobic/hypoxic)
1	H	−0.56	1.7	0.54	3.2
2	Cl	−0.52	0.31	<0.012	>26
3	OH	−0.56	348	2.5	139
4	$OCONHCH_3$	−0.53	8.1	0.27	30
5	$OCOCH_3$	−0.52	3.6	9.0	0.4
6	OMs	−0.53	180	69.6	2.6
7	OTs	−0.52	>1000	9.0	>111
8	OCH_3	−0.53	30	0.6	50
mitomycin C		−0.40	0.7	0.001	700
adriamycin		−0.59	0.012	<0.0001	>120

[a] Measurements were carried out in 0.05 M phosphate buffer containing 0.1 M KCl at pH 7.0 in water–EtOH (1:1, v/v) using a saturated calomel reference electrode. [b] The concentration, in μM, at which the surviving fraction of cells was 0.50 under the test conditions employed.

totoxic action of these compounds appears to be the result of intercalation between base pairs of the DNA double helix.[20,21] Thus, the anthraquinone analogues described in the present report may have the potential to act as both bioreductive alkylating agents and intercalating agents and, thereby, be considerably different in their spectrum of antitumor action than previously synthesized quinones of the bioreductive alkylating class.

Experimental Section

Biological Methods. The techniques used for propagating and handling of EMT6 mouse mammary tumor cells in culture have been detailed previously.[22-24] The cells were grown as monolayers in 25-cm^2 plastic culture flasks in Waymouth's medium supplemented with 15% bobby calf serum. To produce hypoxia, flasks were fitted with sterile rubber sleeve serum stoppers and exposed to a 95% nitrogen/5% CO_2 atmosphere for 4 h at 37 °C before drug treatment. Parallel flasks were maintained in 95% air/5% CO_2. Drugs were added to each flask in 25 μL of acetone by injection through the rubber stopper without breaking the hypoxia. The cells were exposed to each agent for 1 h under hypoxia or normal aeration and then washed with 3 mL of sterile phosphate-buffered 0.9% NaCl, suspended by treatment with 0.05% trypsin for 15 min, and plated at three dilutions in replicate dishes in Waymouth's medium supplemented with 15% bobby calf serum, and cell survival was measured by colony formation. Colonies grow to a readily countable size in 8 to 10 days. No difference existed between the survival of untreated cells or vehicle-treated cells maintained under the aerobic or hypoxic conditions employed, and the plating efficiencies for these control situations was 65 to 80%. Each drug was tested in at least three separate experiments.

Chemistry. Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded at 60 and/or 270 MHz on a Varian T-60 or a Bruker 270 HX spectrometer using Me_4Si as the internal reference. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

2-(Chloromethyl)anthraquinone (2). A mixture of 2-methylanthraquinone (1; 50 g, 0.23 mol), SO_2Cl_2 (364 g, 2.70 mol), and 2,2′-azobis(2-methylpropionitrile) (1.2 g, 7.3 mmol) was refluxed for 1 h. The excess SO_2Cl_2 was removed by distillation in vacuo. The solid residue was collected by filtration, washed several times with petroleum ether (35–60 °C), dried, and recrystallized from DMF to yield 43.2 g (75%) of **2**: mp 165–166 °C; TLC, R_f 0.67 ($CHCl_3$–Et_2O, 4:1, v/v); NMR ($CDCl_3$) δ 4.76 (s, 2 H, 2-CH_2Cl), 7.70–7.97 (m, 3 H, H-1, H-3, and H-4, aromatic), 8.22–8.40 (m, 4 H, H-5, H-6, H-7, and H-8 aromatic). Anal. ($C_{15}H_9ClO_2$) C, H, N.

2-(Hydroxymethyl)anthraquinone (3). A suspension of **2** (5.0 g, 19.5 mmol) in 500 mL of water and 350 mL of Me_2SO was refluxed with vigorous stirring. Upon heating for 4 h, a clear solution was obtained. The reaction mixture was refluxed for an additional 20 h and then cooled to room temperature. The crystalline product was collected by filtration and recrystallized from $CHCl_3$ to give 2.75 g (59%): mp 192–193 °C; (lit.[25] 193 °C); TLC, R_f 0.35 ($CHCl_3$–Et_2O, 4:1, v/v); NMR ($CDCl_3$) δ 1.82 (s, 1 H, OH, D_2O exchangeable), 4.92 (s, 2 H, 2-CH_2), 7.72–7.92 (m, 3 H, H-1, H-3, and H-4, aromatic), 8.25–8.40 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic).

2-Methylanthraquinone *N*-Methylcarbamate (4). Methyl isocyanate (45 mL) was added to a suspension of **3** (1.30 g, 5.46 mmol) in 25 mL of CH_2Cl_2. The reaction mixture was refluxed with stirring for 24 h, and the resulting crystalline material was collected by filtration, washed with ether, and dried to afford 1.42 g (88%) of fine pale yellow needle-like crystals: mp 180–181 °C; TLC R_f 0.44 ($CHCl_3$–Et_2O, 4:1, v/v); NMR ($CDCl_3$) δ 2.92 (d, 3 H, -NH-CH_3), 4.90 (br s, 1H, -NH, D_2O exchangeable), 5.29 (s, 2 H, 2-CH_2), 7.60–7.98 (m, 3 H, H-1, H-3, and H-4, aromatic), 8.16–8.40 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{17}H_{13}NO_4$) C, H, N.

2-(Acetoxymethyl)anthraquinone (5). A solution of **3** (1.12 g, 4.70 mmol) and Ac_2O (5 mL) in 20 mL of pyridine was kept at 3 °C for 24 h. The resulting solid material was filtered, washed with ether, dried, and recrystallized from $CHCl_3$–Et_2O to afford 0.89 g (79%) of pale yellow crystals: mp 152–153 °C; NMR ($CDCl_3$) δ 2.20 (s, 3 H, CH_3), 5.30 (s, 2 H, 2-CH_2), 7.60–7.98 (m, 3 H, H-1, H-3, and H-4, aromatic), 8.20–8.40 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{17}H_{12}O_4$) C, H.

2-[[(Methanesulfonyl)oxy]methyl]anthraquinone (6). Methanesulfonyl chloride (0.38 g, 3.27 mmol) was added slowly with stirring to a cooled solution (ice bath) of **3** (0.60 g, 2.52 mmol)

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in 20 mL of pyridine. The reaction mixture was kept at 3 °C for 24 h, during which the crystalline product precipitated. The crystals were isolated by filtration, washed thoroughly with petroleum ether, dried, and recrystallized from $CHCl_3$ to give 0.42 g (53%): mp 203–206 °C (dec); NMR (Me_2SO-d_6) δ 2.40 (s, 3 H, $-CH_3$), 6.12 (s, 2 H, $-CH_2$), 7.63–8.34 (m, 5 H, H-4, H-5, H-6, H-7, and H-8, aromatic), 9.14–9.34 (m, 2 H, H-1, H-3, aromatic). Anal. ($C_{16}H_{12}O_5S$) C, H, S.

2-[[(*p*-Toluenesulfonyl)oxy]methyl]anthraquinone (7). A solution of **3** (1.12 g, 4.70 mmol) and *p*-toluenesulfonyl chloride (1.07 g, 5.64 mmol) in 30 mL of pyridine was kept at 3 °C for 24 h. The resulting crystalline material was collected by filtration, washed with water, EtOH, and Et_2O, dried, and recrystallized from $CHCl_3$–DMF to yield 1.24 g (67%) of pale yellow fine needles: mp 203–204 °C; NMR (Me_2SO-d_6) δ 2.30 (s, 3 H, $-CH_3$), 6.12 (s, 2 H, 2-CH_2), 7.10 (d, 2 H, H_a tolyl ring), 7.50 (d, 2 H, H_b tolyl ring), 7.80–8.40 (m, 5 H, H-4, H-5, H-6, H-7, H-8, aromatic), 9.20–9.41 (m, 2 H, H-1, H-3, aromatic). Anal. ($C_{21}H_{16}O_5S$) C, H, S.

2-(Methoxymethyl)anthraquinone (8). 2-(Chloromethyl)-anthraquinone (**2**; 4.4 g, 17.4 mmol) in 250 mL of MeOH was refluxed for 18 days. The solution was concentrated, and the crystalline product was isolated by filtration and recrystallized from DMF to yield 1.94 g (44%): mp 132–134 °C (lit.[25] 134 °C); NMR ($CDCl_3$) δ 2.48 (s, 3 H, OCH_3), 4.60 (s, 2 H, 2-CH_2), 7.52–7.94 (m, 3 H, H-1, H-3, and H-4, aromatic), 8.08–8.40 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic).

Polarography. First half-wave reduction potentials were measured in 0.05 M phosphate buffer prepared in water–EtOH (1:1, v/v), pH 7.0, by differential pulse polarography using a PAR 174A polarographic analyzer. The values were obtained in volts vs. a saturated calomel reference electrode using 0.1 M KCl as the supporting electrolyte.

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8-Hydroxyanthracyclinones from ε-Rhodomycinone

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ε-Rhodomycinone was converted into 8,9-dehydro-ζ-rhodomycinone, which gave a cis diol with osmium tetroxide and a pair of epimeric epoxides with *m*-chloroperbenzoic acid. Acid-catalyzed opening of the epoxides gave the corresponding trans diols. In contrast, acid treatment of the trimethyl ethers of these epoxides gave predominantly a lactone and an η-rhodomycinone derivative, with only small amounts of the diols. None of the new rhodomycinones were active against *Bacillus subtilis*, but 8,9-dehydro-ζ-rhodomycinone was active in the induction of lytic phage in *Escherichia coli*.

Beginning with Brockmann's pioneering studies,[1] research on anthracyclines has developed into a major field. Anthracyclines containing the aglycons aklavinone, ε-pyrromycinone, adriamycinone, carminomycinone, or daunomycinone combined with sugars such as daunosamine, rhodosamine, 2-deoxy-L-fucose, rhodinose, and cinerulose have shown significant antitumor activity.[2] The clinical importance of doxorubicin (adriamycin) has stimulated recent investigations into the preparation of semisynthetic anthracyclinones and anthracyclines.[3]

The production of anthracyclines by *Streptomyces* species is usually accompanied by inactive aglycons. One of these aglycons, ε-rhodomycinone (**1**; Scheme I), was made available to us by Bristol Laboratories for the purpose of preparing modified anthracyclinones.

This paper describes the successful replacement of the 7α-hydroxyl group in **1** by 8α- and 8β-hydroxyl groups to obtain products which represent potentially useful intermediates to novel 8-glycosylated anthracycline analogues. The preparation of compounds of this type by total synthesis was reported recently by other workers.[4]

The 7α-hydroxy group of ε-rhodomycinone was readily removed by catalytic hydrogenolysis to give the known ζ-rhodomycinone (**2**).[5] Treatment of **2** with *p*-toluenesulfonic acid then afforded the 8,9-olefin **4**. Catalytic hydrogenation of **4** gave 9-deoxy-ζ-rhodomycinone **3**. When **4** was treated with osmium tetroxide, a good yield of the cis diol, 8α-hydroxy-ζ-rhodomycinone (**9**), was obtained.

Treatment of the 8,9-olefin **4** with *m*-chloroperbenzoic acid gave a mixture of the α-epoxide **5** (45%) and β-epoxide **7** (30%), plus other substances that were not identified. This mixture was readily separated by chromatography on silica gel. Stereochemistry of the epoxides was assigned according to transformations described below and from their NMR spectra (see Experimental Section). Each epoxide afforded the corresponding trans diol (**10** and **12**) upon treatment with 70% perchloric acid in acetone. In contrast to these results, opening of the corresponding epoxide trimethyl ethers, **6** and **8**, with perchloric acid in acetone afforded little of the corresponding diols. The α-epoxide **6** gave a small amount of the desired trans diol **11**, but the predominant product was γ-lactone **14**. The formation of **14** established the stereochemistry of epoxides **5** and **6**. We were unable to find conditions for opening epoxide **6** that did not afford mainly the lactone. Moreover, we were not able to convert the lactone into **11**.

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