tendency to correlate all of the data moderately successfully.

Experimental Section

p-Methoxybenzyl chloride (Aldrich) was separated from a calcium carbonate stabilizer before use (the purified product contained traces of *p*-methoxybenzaldehyde). Corresponding ethyl and methyl ethers were prepared by standard methods and were shown to give the same HPLC response at 266 nm as *p*-methoxybenzyl alcohol.

Dry solvents were either Fisons commercial grade (acetone, dioxane, methanol) or were freshly dried and distilled before use: acetonitrile (from P_2O_5); dimethyl sulfoxide (from CaH_2); ethanol (from $Mg(OEt_2)$) and trifluoroethanol (from P_2O_5). MeOD (99.5+ %D) was from Aldrich.

Chromatographic and kinetic methods were as described previously.[7b]

Acknowledgment. We are grateful to the SERC (U.K.) for the award of a studentship to (S.J.N.) and for an equipment grant, to the British Council in Seoul for a travel grant (I.S.K.), to K. T. Liu and J. P. Richard for preprints, to M. F. Ruasse and R. A. More O'Ferrall for comments on the interpretation of the data on solvent isotope effects, and to H. C. Harris, D. N. Kevill, and H. Langhals for helpful discussions.

Substituent Effects upon the Peak Potentials and Reductive Cleavage Rate Constants of Hydroxy- and Methoxy-Substituted 9,10-Anthraquinones in 50% Aqueous CH_3CN: Do They Correlate?

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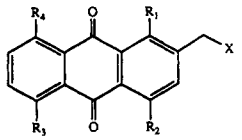
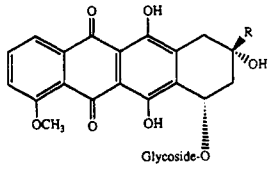
A variety of hydroxy- and methoxy-substituted 2-(acetoxymethyl)-9,10-anthraquinones (**2a–7a**) were reduced electrochemically and with dithionite ($S_2O_4^{2-}$) in 50% aqueous CH_3CN buffers over a wide pH range. Good to excellent yields of their corresponding reductive cleavage products, the substituted 2-methyl-9,10-anthraquinones **2b–7b**, were obtained from most of these anthraquinone acetates, but only at higher pH. Rate constants for the reaction of 2-(acetoxymethyl)-9,10-anthraquinone (**1a**) with excess dithionite ranged from 1.0×10^{-4} s^{-1} at pH values less than 7 to 4.0×10^{-4} s^{-1} at a pH of 10, demonstrating that loss of acetate occurs in the rate-determining step and that cleavage occurs slower via the anthrahydroquinone of **1a** than the conjugate base of the anthrahydroquinone. Substituent effects upon the reductive cleavage process were determined by measuring rate constants for those acetates that react cleanly with dithionite at pH 8. These effects, which are rationalized on the basis of resonance theory and intramolecular H bonding, correlate fairly well with the peak potentials (E_p) of the reductive cleavage products of these acetates. Thus, electron-donating substituents on an anthraquinone acetate not only make it more difficult to reduce resulting in a more negative E_p but also enhance the rate of acetate cleavage in the corresponding anthrahydroquinone.

We recently reported that the electroreduction of 2-(acetoxymethyl)-9,10-anthraquinone (**1a**) and its methoxy derivatives **2a**, **4a**, and **6a** to their corresponding 2-methyl-9,10-anthraquinones **1b**, **2b**, **4b**, and **6b**, respectively, in aqueous media is pH dependent.[1] Since much higher conversions were obtained at higher pH (> 7), we concluded that this electroreductive cleavage process proceeds through the sequence of reactions shown in Scheme I for **1a**. Reduction of acetate **1a** leads to its anthrahydroquinone **8**, which at pH < 7, undergoes cleavage slowly to give the protonated, vinylogous quinone methide **9**. At higher pH, equilibrium shifts toward the conjugate base **10**, which is converted to the quinone methide **11** at a higher rate.



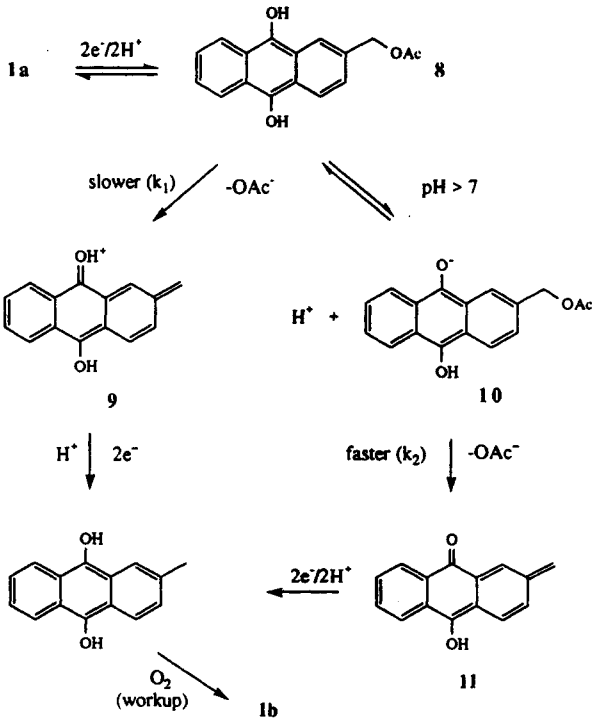
1: $R_1 = R_2 = R_3 = R_4 = H$
2: $R_1 = OCH_3$, $R_2 = R_3 = R_4 = H$
3: $R_1 = OH$, $R_2 = R_3 = R_4 = H$
4: $R_1 = R_4 = OCH_3$, $R_2 = R_3 = H$
5: $R_1 = R_4 = OH$, $R_2 = R_3 = H$
6: $R_1 = R_4 = H$, $R_2 = R_3 = OCH_3$
7: $R_1 = R_4 = H$, $R_2 = R_3 = OH$

a: X = OAc
b: X = H
c: X = OH
d: X = Br



12 a: R = $COCH_3$
b: R = $COCH_2OH$

Scheme I



Our interest in these compounds is based in part upon their structural similarity to anthracyclines such as dau-

(1) Blankespoor, R. L.; Hsung, R.; Schutt, D. L. *J. Org. Chem.* **1988**, *53*, 3032.

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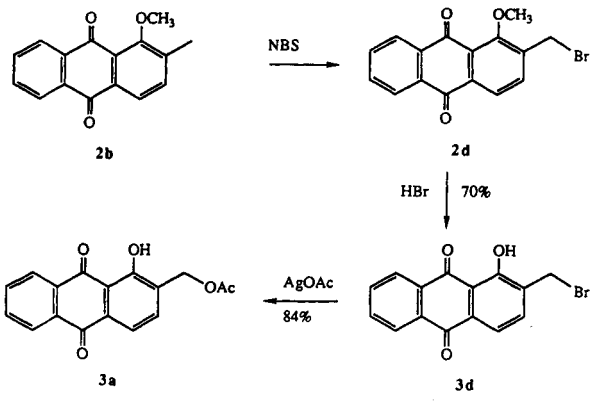
Scheme II



norubicin (**12a**) and adriamycin (**12b**), which are substituted anthraquinones that function as antineoplastic agents.[2-6] One mechanism that has been proposed in the literature to account for their cytotoxicity involves an in vivo reduction followed by cleavage of the benzylic glycoside at C-10,[7] a process that parallels the sequence of reactions in Scheme I. The vinylogous quinone methide that is formed in this manner[3,7d] is then believed to alkylate a biomolecule such as DNA. Although this "bioreductive alkylation" mechanism[8] still lacks experimental support in the case of the anthracyclines, it appears to be on a more solid footing for mitomycin C,[9] an antineoplastic agent that possesses a benzoquinone. Even if this mechanism is not the basis for the antineoplastic properties of the anthracyclines, it seems likely that they are metabolized in part via bioreductive deglycosidation, as has been noted recently by Brand and Fisher.[10]

Unfortunately, a serious limitation in utilizing anthracyclines as antitumor agents is that they display cumulative and dose-dependent cardiotoxicity.[11a,b] The presence of anthracyclines and their reduction products in heart tissue gives rise to reactive oxygen species such as O_2^-, H_2O_2, and OH^-.[11c,d] Although there is uncertainty in the literature regarding the precise mechanism that is involved, there is growing evidence that this cardiotoxicity arises from a redox cycle involving oxygen and reduced forms of the anthracyclines.[12] It appears then that the mode of action of anthracyclines as antitumor agents is mechanistically distinct from their mode of action as cardiotoxic agents. This is an important observation since it suggests that, in principle, it should be possible to design drugs that display maximum antitumor behavior with minimal cardiotoxicity.

One of our goals at the outset of this work was to test this hypothesis by constructing a variety of hydroxy- and methoxy-substituted anthraquinones that would undergo reductive cleavage and thereby serve in a limited sense as models for the anthracyclines. A knowledge and understanding of their reductive peak potentials, which would give a measure of their tendency to undergo reaction with oxygen, and the rate constants of their reductive cleavage reactions would hopefully provide a better basis for the rational design of anthraquinone-type antitumor agents that would exhibit maximal antitumor qualities and minimal cardiotoxicity. The series of anthraquinones **1–7** were prepared for this purpose and their reduction and reductive cleavage reactions are the subject of this article.

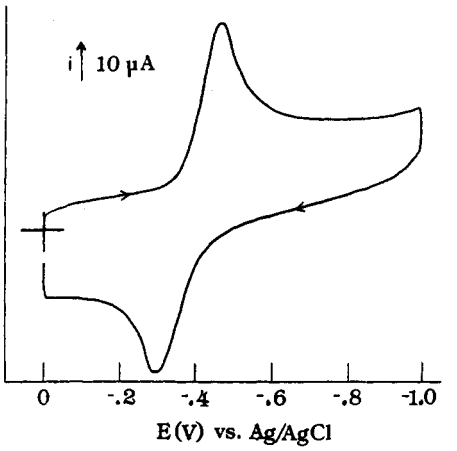


Figure 1. Cyclic voltammogram of 0.33 mM **6b** in 50% aqueous CH_3CN buffer at a sweep rate (ν) of 100 mV s^{-1}.

Table I. Cathodic Peak Potentials (mV) Measured by Cyclic Voltammetry in 50% Aqueous CH_3CN[a]

anthraquinone	E_p[b]	anthraquinone	E_p[b]
1a	−460 ± 4	**4b**	−497 ± 8
1b	−486 ± 6	**5a**	−448 ± 20
2a	−447 ± 5	**5b**	−524 ± 15
2b	−455 ± 9	**6a**	−480 ± 6
3a	−431 ■ 5	**6b**	−470 ± 9
3b	−501 ± 4	**7a**	−465 ± 20
4a	−473 ± 8	**7b**	−515 ± 4

[a] pH 6.0 buffer (0.10 M KCl, 0.050 M phosphate). [b] Reference is Ag/AgCl; sweep rate = 100 mV s^{-1}; a range of values is given based upon a minimum of three measurements.

Results and Discussion

Synthesis of Anthraquinones 3a, 5a, and 7a. The sequence of reactions that was employed in the preparation of these anthraquinones is outlined in Scheme II for **3a**. Since direct bromination of **3b** with NBS gave poor yields of **3d**, presumably due to the inhibitory effect of the phenol upon the free radical bromination process, it was necessary to start with the methoxy-substituted anthraquinone **2b**,

(2) (a) Moore, H. W.; West, K. F.; Srinivasacher, K.; Czerniak, R. In *Structure-Activity Relationships of Anti-Tumor Agents*; Reinhoudt, D. N., Connors, J. A., Pinedo, H. M., van de Poll, K. W., Eds.; Martinus Nijhoff: Boston, 1983; pp 93–110. (b) Moore, H. W.; Czerniak, R.; Hamdan, A. *Drugs Exp. Clin. Res.* **1986**, *12*, 475–494.
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(7) Recent references in which extensive literature citations are given include the following: (a) Arcamone, F. *Med. Res. Rev.* **1984**, *4*, 153. (b) Bouma, J.; Beijen, J. H.; Bult, A.; Vanderberg, W. J. M. *Pharm. Weekbl., Sci. Ed.* **1986**, *8*, 109. (c) Lown, J. W. *Adv. Free Radical Biol. Med.* **1985**, *1*, 225. (d) Gaudiano, G.; Egholm, M.; Haddadin, M. J.; Koch, T. H. *J. Org. Chem.* **1989**, *54*, 5093.
(8) (a) Kirkpatrick, D. L.; Johnson, K. E.; Sartorelli, A. C. *J. Med. Chem.* **1986**, *29*, 2048. (b) Wilson, I.; Wardman, P.; Lin, T. S.; Sartorelli, A. C. *Chem.-Biol. Interact.* **1987**, *61*, 229.
(9) (a) Tomasz, M.; Lipman, R.; McGuinness, B. F.; Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 5892. (b) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science (Washington, D.C.)* **1987**, *235*, 1204. (c) Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1987**, *109*, 1576. (d) Sartorelli, A. C. *Biochem. Pharmacol.* **1986**, *35*, 67. (e) Pan, S.-S.; Iracki, T.; Bachur, N. R. *Mol. Pharmacol.* **1986**, *29*, 622.
(10) Brand, D. J.; Fisher, J. F. *J. Org. Chem.* **1990**, *55*, 2518.
(11) (a) Bonadonna, G.; Monfardini, S. *Lancet* **1969**, 837. (b) Smith, B. *Br. Heart J.* **1969**, *31*, 607. (c) Lown, J. W.; Chen, H. H.; Plambeck, A.; Acton, E. M. *Biochem. Pharmacol.* **1982**, *31*, 575. (d) Iwamoto, Y.; Hansen, I. L.; Porter, T. H.; Folkers, K. *Biochem. Biophys. Res. Commun.* **1974**, *58*, 633.

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which was converted to **2d** with NBS. Demethylation of **2d** was accomplished by treatment with 48% HBr. The resulting **3d** gave the desired acetate **3a** in a high yield, using AgOAc.

Cyclic Voltammetry of Anthraquinones 1–7 in 50% Aqueous CH_3CN. Cyclic voltammograms (CVs) of **1** and the methoxy-substituted anthraquinones **2**, **4**, and **6** in 50% aqueous THF buffers were reported in our earlier work.[1] In this study 50% aqueous CH_3CN buffers were employed since the hydroxy-substituted anthraquinones **3**, **5**, and **7** are more soluble in this medium. The pH values given in this article are of the aqueous buffers prior to mixing with CH_3CN. As has been noted in earlier investigations on mixed solvents,[13] the *apparent* pH following mixing (i.e., 0.6–0.7 higher for 50% aqueous CH_3CN) is most likely not the actual pH.

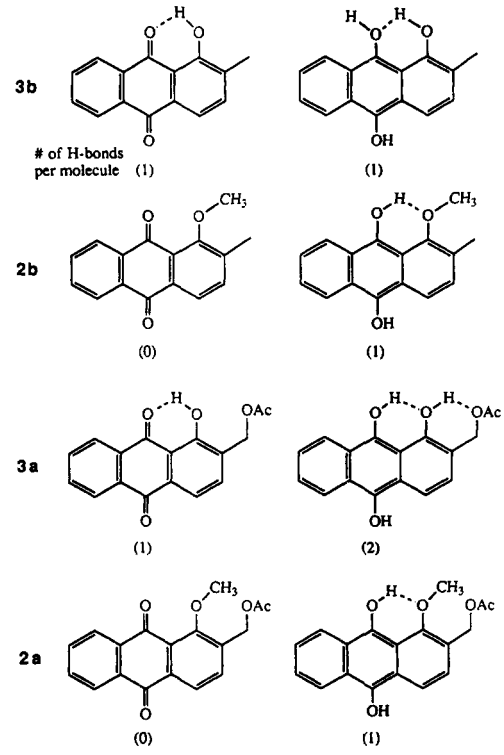
CVs of anthraquinones **1–7** in 50% aqueous CH_3CN buffer exhibit a single cathodic wave, as shown in Figure 1 for **6b**, using a glassy carbon (GC) electrode, resulting from reduction to their anthrahydroquinones.[1,14] The peak potential (E_p) for this quasi-reversible wave shifts to more negative values with increasing pH (45–50 mV/pH unit), which, based upon the Nernst equation, is consistent with a $2e^-/2H^+$ process. Plots of i_p vs $\nu^{1/2}$ (i_p = peak current and $\nu^{1/2}$ = scan rate) deviate only slightly from linearity with increasing scan rate, demonstrating that the electrode processes are under diffusion, not adsorption, control.[15] The E_p values are quite reproducible provided that the GC electrode is pretreated prior to each scan.

Peak Potentials of 1–7. E_p values from the CVs of anthraquinones **1–7** in the 50% aqueous CH_3CN buffer at pH 6 are given in Table I. This pH was chosen to examine the substituent effects upon E_p since follow-up cleavage reactions of the anthrahydroquinones derived from acetates **1a–7a** are slower at lower pH. Irreversible homogeneous chemical reactions following reversible electron transfer at the electrode, often referred to as E_rC_i processes, can significantly alter E_p.[16] E_p shifts to less negative potentials as the rate of the chemical reaction increases. The electrochemistry of the acetates **1a–7a** actually represents an ECE process since the products from the chemical reaction (e.g., **9** and **11** from acetate **1a**) are electrochemically reduced at potentials similar to those of their acetate precursors. The electrochemistry of **1b–7b** is obviously much less complex since their anthrahydroquinones do not react.

Table I shows that the substituent effects on E_p are quite significant, giving rise to a nearly 100 mV difference between **3a** and **5b**. A number of observations and comments can be made concerning the data. First, each 2-(acetoxymethyl)anthraquinone that is hydroxy substituted (i.e., **3a**, **5a**, or **7a**) has a less negative potential than the corresponding methoxy-substituted one (i.e., **2a**, **4a**, or **6a**). The situation is exactly reversed for the hydroxy-substituted anthraquinones that have a methyl group at the 2-position. These anthraquinones (i.e., **3b**, **5b**, and **7b**) have more negative potentials than their methoxy analogues (i.e., **2b**, **4b**, and **6b**). Undoubtedly, the origin of these opposing trends is complex.

One factor that could be involved is intramolecular H bonding. There is evidence that suggests that intramolecular H bonding occurs in some of these compounds. Support for this postulate comes from the observation that the hydroxy-substituted anthraquinones have much longer retention times than their methoxy analogues on reverse-phase HPLC columns using aqueous methanol as the eluting solvent. Furthermore, in thin-layer chromatography (TLC) of anthraquinones **2–7** using aprotic solvents such as CH_2Cl_2, the hydroxy-substituted ones have higher R_f values than their methoxy analogues. The apparently lower polarity of the hydroxy-substituted anthraquinones in both HPLC and TLC would be difficult to rationalize without intramolecular H bonding.

Scheme III



Given that intramolecular H bonding is important in some of these anthraquinones, we can now attempt to explain why a hydroxy-substituted anthraquinone has a more or less negative E_p than its methoxy analogue depending upon whether it possesses an acetoxymethyl or methyl group at the 2-position. In Scheme III the number of intramolecular H bonds is given for **2** and **3** and their corresponding anthrahydroquinones. Consider anthraquinones **2b** and **3b**. **3b** and its reduced form have one such H bond. In contrast, **2b** has one less stabilizing H bond than its reduced form, suggesting that **2b** should be more easily reduced, which is the case. The situation regarding acetates **2a** and **3a** is not the same. Here each of the reduced forms has one more H bond than its acetate and, as a result, **2a** is not more easily reduced than **3a**. In fact, the reverse is observed.

A second observation regarding the data in Table I is that with the exception of **6**, the introduction of an acetoxy group on the methyl group at the 2-position results in a less negative E_p. A couple of factors could be responsible for this. One that was noted earlier is the cleavage reaction following reduction of the acetates that shifts E_p to less negative potentials. Another factor is the electron-withdrawing effect of an acetoxy group. Since an sp^3 center separates the acetoxy group from the anthraquinone, its effect would have to be transmitted inductively and/or through space, not via resonance.

The last observation we wish to make regarding the data in Table I deals with the effect of introducing either hydroxy or methoxy groups on the anthraquinone. With

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(14) Chambers, J. Q. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: London, 1974; Chapter 14.
(15) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods, Fundamentals and Applications*; Wiley: New York, 1980; pp 218, 522.
(16) Reference 15, pp 451–3.

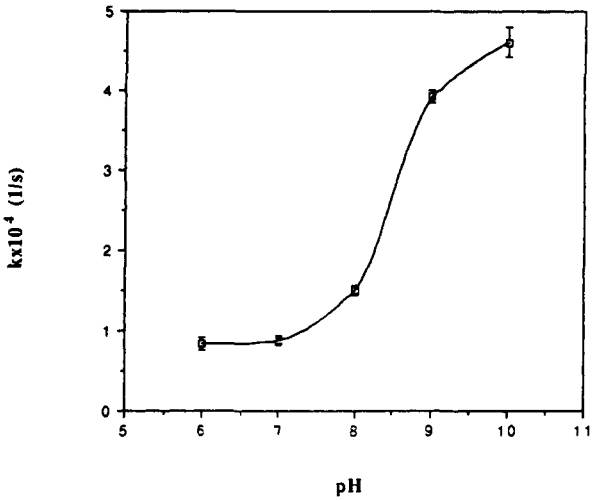


Figure 2. Plot of rate constants from the reaction of acetate **1a** with excess $S_2O_4^{2-}$ vs the pH of the 50% aqueous buffer.

several exceptions the general trend is that introducing one such group (i.e., **1** → **2** or **3**) results in a less negative potential, whereas the introduction of two such groups (i.e., **1** → **4**–**7**) results in a more negative potential. The electronic effect of either a methoxy or hydroxy group on an anthraquinone would be expected to increase the electron density via resonance and thereby make the anthraquinone more difficult to reduce, resulting in a more negative E_p. Since the opposite effect is observed, we suggest that the electronic effect is more than offset by stabilization of the anthrahydroquinone through H bonding. The introduction of a second hydroxy or methoxy group then decreases E_p further by electron donation, which is not offset by additional H bonding.

It is important to note that a CV of oxygen in the 50% aqueous CH_3CN buffer at pH 6 shows a completely irreversible wave with E_p = −650 mV for its reduction. Since all of the anthraquinones in this study have E_p values more positive than −650 mV, they would be expected to form anthrahydroquinones that react with oxygen. The reaction of anthrahydroquinones with oxygen is well known and has been monitored spectroscopically.[1]

pH Effects upon the Reductive Cleavage of Acetate 1a. In our earlier work we found qualitative experimental support for the sequence of reactions in Scheme I.[1] The primary reaction pathway at higher pH (> 7) appeared to involve the conjugate base of the anthrahydroquinone, rather than the anthrahydroquinone itself. We decided to obtain quantitative support for this picture by measuring the rate constants for the reductive cleavage process in buffers at different pH. This was accomplished by reacting **1a** with a large excess of sodium dithionite in the absence of oxygen and using HPLC to monitor the reaction. The results are shown in Figure 2. The rate constant increases from 1.0×10^{-4} s^{-1} at pH values less than 7 to 4.6×10^{-4} s^{-1} at a pH of 10.

These data not only are consistent with the mechanism in Scheme I but also rule out the tautomerization of the quinone methide as the rate-determining step. Since this enol–keto-type tautomerization would be either acid or base catalyzed, the rate constant would be the smallest in the vicinity of pH 7 and increase in either direction from that point. The observation then that the rate constant increases only in the direction of higher pH supports a mechanism in which loss of acetate from the anthraqhydroquinone (H_2A) or its conjugate base (HA^-) is the rate-determining step. If this mechanistic picture is correct, the overall rate of the cleavage step is $k_1[H_2A] + k_2[HA^-]$. If pK_{a1} for H_2A is 9, which is a reasonable estimate, then, at pH 6, $[H_2A]/[HA^-] = 1000$ and $k_{obs} \approx k_1$. From the observed rate constants at pH 6 and pH 9 (Figure 2), one can conclude that k_2/k_1 is no more than 4. Actually, this would be an upper limit since any cleavage occurring at pH 9 via the dianion A^{2-} would make this ratio even smaller.

Table II. Products from the Constant Potential Reduction of Anthraquinones 1a–7a in 50% Aqueous Buffers[a]

anthraquinone	products (% yield)[b]			ref
	pH 7.0	pH 8.0	pH 9.0	
1a[c]	**1a** (64)	**1a** (9)		1
	1b (11)	**1b** (68)		
2a[c]	**2a** (67)	**2a** (3)		1
	2b (10)	**2b** (73)		
3a[d]		**3b** (8)[e]	**3b** (16)	this work
4a[d]	**4a** (56)	**4a** (4)		this work
	4b (32)	**4b** (82)		
	4c (trace)	**4c** (10)		
5a[d]		**5a** (15)[e]	**5a** (trace)	this work
		5b (25)	**5b** (34)	
			5c (22)	
6a[c]	**6a** (87)	**6a** (6)		1
	6b (7)	**6b** (61)		
7a[d]	**7a** (91)	**7a** (6)	**7a** (trace)	this work
	7b (5)	**7b** (67)	**7b** (80)	
		7c (5)	**7c** (10)	

[a] Applied potential is −800 mV. Working electrode is carbon felt. Reference electrode is Ag/AgCl (0.10 M Cl^-). [b] HPLC analysis using a C-18 reverse-phase column. [c] Buffer is 50% aqueous THF containing 0.10 M Cl^- and 0.050 M phosphase. [d] Buffer is 50% aqueous CH_3CN containing 0.10 M Cl^- and 0.05 M phosphate. [e] Other products are formed that have not been identified, including a relatively insoluble solid that adheres to the electrode surface.

Electroreduction of Acetates 1a–7a in 50% Aqueous Buffers at Different pHs. Solutions of acetates **3a**, **4a**, **5a**, and **7a** (0.5–2.0 mM) in 50% aqueous CH_2CN buffers at several pHs were reduced at carbon felt at −800 mV under argon. The catholytes were exposed to oxygen to convert the product anthrahydroquinones to anthraquinones prior to analysis with HPLC using a reverse-phase column. The results from the electroreduction of these acetates, as well as those from **1a**, **2a**, and **6a** reported earlier,[1] are summarized in Table II.

Note that **1a** and the methoxy-substituted acetates **2a**, **4a**, and **6a** undergo electroreductive cleavage to their corresponding 2-methyl-substituted anthraquinone derivatives **1b**, **2b**, **4b**, and **6b**, respectively, in high yield at pH 8. The origin of the small amount of alcohol **4c**, which also forms in the electroreduction of **4a**, remains unclear to us. Since acetate **4a** is recovered nearly quantitatively from the same buffer under the conditions used in the electrolysis, hydrolysis of the acetate can be ruled out as a pathway to alcohol **4c**. Formation of the alcohol by nucleophilic attack of water on the intermediate vinylogous quinone methide (Scheme I) also seems unlikely based upon previous work on related anthraquinones in DMF electrolytes.[17] Formation of **4c** via an acyl transfer process involving the conjugate base of an anthrahydroquinone is certainly a possible mechanism, but one for which we have no experimental support.

Table II shows that electroreduction of the hydroxy-substituted acetates gives mixed results. Acetate **3a** gives poor yields of **3b** (8–16%) even in buffers with a pH as high as 9. HPLC analysis of the catholyte shows the presence of at least eight other substances in low yield. In addition

(17) Blankespoor, R. L.; Lau, A. N. K.; Miller, L. L. *J. Org. Chem.* **1984**, *49*, 4441.

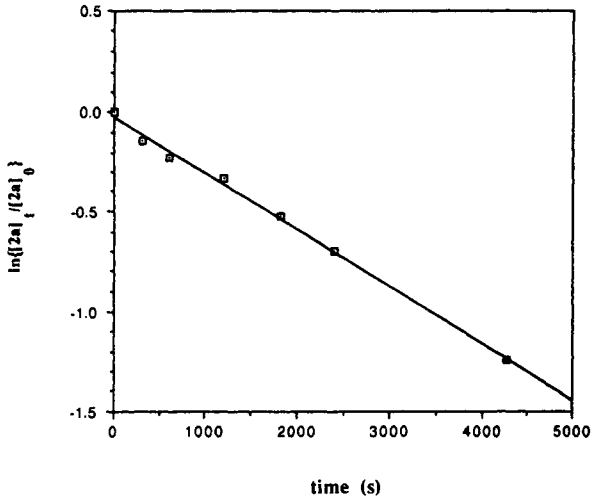


Figure 3. First-order plot of the data obtained from the reaction of acetate **2a** with excess $S_2O_4^{2-}$ in 50% aqueous CH_3CN at pH 8. Slope = k = 2.84 × 10^{-4} s^{-1}. R = 0.997.

Table III. Rate Constants for the Reductive Cleavage of 2-(Acetoxymethyl)-9,10-anthraquinones in 50% Aqueous CH_3CN Buffer at pH 8[a]

anthraquinone	10^4 k (s^{-1})[b]
1a	1.50 ± 0.07
2a	2.86 ± 0.03
4a	4.68 ± 0.36
6a	3.23 ± 0.01
7a	5.14 ± 0.18

[a] Sodium dithionite ($Na_2S_2O_4$) is the reducing agent. [b] 25.0 °C.

to these methanol-soluble products, a significant amount of insoluble material is formed that adheres to the carbon felt electrode. No attempt was made to identify any of the side products. In contrast to **3a**, acetate **7a** gives electroreductive cleavage product **7b** in a high yield of 80% with a small amount of alcohol **7c**. Anthraquinone **5a** lies in between these two acetates in terms of complexity of reaction mixtures, giving 34% and 22% of **5b** and **5c**, respectively. Control experiments show that none of these three hydroxy-substituted acetates react appreciably with the buffer in the time frames used in the electroreductions.

Rate Constants for the Reductive Cleavage of Selected Acetates Using Sodium Dithionite as Reducing Agent. Each of the acetates **1a–7a** was allowed to react with a 10-fold excess of sodium dithionite ($Na_2S_2O_4$) in 50% aqueous CH_3CN buffer at pH 8 for several hours or until reaction went to completion. The results closely paralleled those from the electrochemical reductions. All of the acetates gave high yields of their corresponding 2-methyl-substituted derivatives except **3a** and **5a**, which gave complex mixtures containing **3b** and **5b**, respectively, in low yields. Therefore, rate constants for these two acetates were not measured. Figure 3 shows a first-order plot of **2a**.

The rate constants for the five acetates that undergo reductive cleavage rather cleanly and in high yield are shown in Table III. The rate constant increases with the introduction of either a hydroxy or methoxy group on the anthraquinone, which is consistent with the electron-donating effect of these groups. Thus, as the anthraquinone becomes more electron-rich, the loss of OAc^- is facilitated, giving a larger k (i.e., k_{obs}). Not only does the number of the electron-donating substituents affect k but also their position on the anthraquinone as shown by comparing the rate constants for **4a** and **6a**. In **4a**, but not **6a**, both methoxy groups are in direct conjugation to the reaction site, as demonstrated in structure **13**, resulting in a larger k.

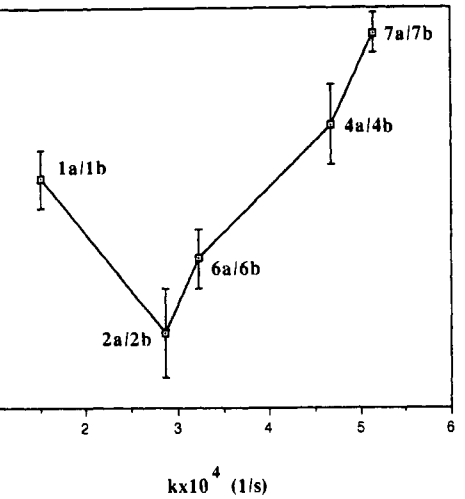
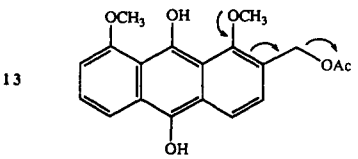


Figure 4. Plot of the peak potentials (E_p) of anthraquinones **1b**, **2b**, **4b**, **6b**, and **7b** (Table I) vs the rate constants from the reaction of acetates **1a**, **2a**, **4a**, **6a**, and **7a**, respectively, with $S_2O_4^{2-}$ in 50% aqueous CH_3CN at pH 8 (Table III).



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Correlation between E_p and the Rate Constants for Reductive Cleavage. Since substituents that increase the electron density of an anthraquinone, making E_p more negative, would also be expected to increase the rate of the reductive cleavage process, the question that naturally arises is how well do these two distinct properties correlate? Ideally, one would answer this question by plotting the rate constants of the acetates in Table III versus their E_p values in Table I. This was done, but the correlation is poor. This should not be surprising, though, since the E_p values of these acetates are not only sensitive to substituent effects but also to follow-up cleavage reactions.

A better probe then to use in answering this question is to plot the rate constants of the acetates in Table III with the E_p values of their corresponding 2-methyl derivatives **1b**, **2b**, **4b**, **6b** and **7b** in Table I. This is done in Figure 4, and with the exception of the first data point for acetate **1a/1b**, the correlation is fairly good with k increasing with decreasing E_p in accordance with expectations. One possible explanation for the poor correlation of the data from **1a/1b** is that **1b** is the only one in the group that forms an anthrahydroquinone that cannot form an intramolecular H bond, which could result in a more negative E_p.

Summary and Conclusions

In this work we have shown that the effects of methoxy and hydroxy substituents on the E_p of the reduction of an anthraquinone are significant and complex. The electron-donating effect of either group, which would be expected to make E_p more negative, can be partially or completely offset by intramolecular H bonding in the anthrahydroquinones that are produced. The effect of one or two methoxy or hydroxy substituents on the reductive cleavage process of anthraquinones with an acetoxymethyl group at the 2-position is more predictable, resulting in higher rates for most of the anthraquinones studied. In general, the rate constants for acetates that possess one

or two of these substituents increase as the E_p values of their corresponding reductive cleavage products, the 2-methyl-substituted anthraquinones, become more negative. This work shows then that electron-donating substituents that increase the rate of the reductive cleavage process of 2-(acetoxymethyl)anthraquinones also make the anthraquinone more difficult to reduce and thereby generate anthrahydroquinones that react with oxygen more favorably. Any relevance this study may have on the medicinal use of anthracyclines will depend, at least in part, upon a better understanding of how these drugs function as antitumor agents and display their cardiotoxic properties. This study does suggest, though, that it may be difficult to design anthraquinone-containing drugs that display both maximum antitumor behavior via bioreductive cleavage and minimal cardiotoxicity.

Experimental Section

General. Melting points were determined in open capillary tubes with a Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. IR spectra were obtained with a Sargent-Welch Pye Unicam 3-200 IR or Mattson Galaxy 4020 FT-IR spectrometer. Mass spectra were recorded on a Finnigan OWA Model 1020 GC-MS instrument. HPLC analyses were made with a Rainin Model 81-NM pump, Rainin C-18 reverse-phase column, and Milton Roy 3100 detector, using methanol–water mixtures as the eluting solvent. Proton and C-13 NMR spectra were recorded on a Bruker 250 MHz AC-E spectrometer.

Electrochemical Measurements. Electrochemical experiments were performed with a Princeton Applied Research (PAR) potentiostat, Model 273, in conjunction with a PAR 175 universal programmer. Voltammograms were recorded on a Linseis LY 18100 x–y recorder. All potentials in the text are referred to Ag/AgCl (0.10 M KCl).

Cyclic Voltammetry. A 25 mL, three-necked flask served as a one-compartment cell. The working electrode was a glassy carbon (GC) disk (A = 0.090 cm^2) set in a Teflon-coated tube. Prior to measurements on each solution, this electrode was cleaned and polished with 0.30- and 0.050-μm α alumina (Buehler), wiped with a tissue, and sonicated in water for 2–4 min. Pretreatment of the GC electrode was patterned after the method of Blaedel and Jenkins.[18] Preconditioning of the electrode consisted of cycling the potential 15 times between +1.5 and −1.5 V at a scan rate of 100 mV s^{-1} in pH 7 aqueous buffer (0.10 M KCl, 0.050 M phosphate), which was neither stirred nor deoxygenated. Pretreatment was accomplished in the same buffer by holding the potential at +1.5 V for 2 min, then at −1.5 V for 1 min, and repeating the sequence twice. Following pretreatment the potential was cycled four times between +1.5 and −1.5 V and then several times between 0 and −1.0 V.

General Procedure for Constant Potential Electrolyses in 50% Aqueous CH_3CN Buffers. The procedure reported in our earlier work was used.[1]

Preparation of Buffer Solutions. In the preparation of 1 L of a 50% aqueous CH_3CN buffer, 0.10 mol of KCl and 0.050 mol of KH_2PO_4 was added to approximately 475 mL of HPLC-grade water (Aldrich) and the pH was adjusted to the desired value with the addition of KOH. To this buffer in a 1-L volummetric flask was added 500 mL of HPLC-grade CH_3CN (Fisher). Sufficient water was added to obtain a final volume of 1 L. The *apparent* pH of the final solution was 0.6–0.7 higher than the pH of the aqueous component.

General Procedure for Kinetic Measurements. To 0.50–1.00 mg of the anthraquinone (i.e., **1a**, **2a**, **4a**, **6a**, or **7a**) in a three-necked round-bottom flask fitted with a mechanical stirrer, a septum, and a pressure equalizing separatory funnel was added approximately 40 mL of the desired 50% aqueous CH_3CN buffer. After the addition of 5 mL of the buffer solution to the separatory funnel, both buffer solutions in the apparatus were thoroughly deoxygenated using argon. A large excess of sodium dithionite (20–40 mg) was added to the separatory funnel. With the three-necked flask in a constant-temperature water bath at 25 °C, the buffer solution in the separatory funnel containing sodium dithionite was added all at once to rapidly convert the anthraquinone to its anthrahydroquinone and thereby establish the start of the reductive cleavage process. Using a syringe, 2-mL aliquots of the reaction mixture were removed at various times and immediately quenched by the addition of two drops of 30% H_2O_2, which rapidly oxidizes anthrahydroquinones to their corresponding anthraquinones. Plots of ln {[reactant]$_t$/[reactant]$_0$} vs time yielded straight lines with slopes equal to $-k$.

1-Hydroxy-2-(bromomethyl)-9,10-anthraquinone (3d). A mixture of **2d** (195 mg, 0.588 mmol) in 50 mL of concentrated aqueous HBr (48%) was heated in an oil bath at 140 °C for 3 h. After cooling, the reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL). The extracts were combined, washed with 80 mL of H_2O and 10 mL of saturated $NaHCO_3$, and dried over Na_2SO_4. Removal of solvent in a rotary evaporator gave 165 mg of a light red solid, which was chromatographed on silica gel and eluted with CH_2Cl_2 to give 129 mg (69%) of **3d**. An analytically pure sample was obtained by treating a hot heptane/toluene solution of **3d** with charcoal prior to recrystallization: mp 193–4 °C; IR (Nujol) 1640, 1590, 1420, 1292, 1253, 1213, 1155, 1027, 980, 790, 766, 720 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.64 (s, 2 H), 7.76–7.88 (m, 4 H), 8.29–8.35 (m, 2 H), 13.17 (s, 1 H); ^{13}C NMR (250 MHz, $CDCl_3$) δ 189, 182, 161, 139, 138, 137, 135, 134, 133, 132, 128, 127, 119, 116, 27; MS, m/e (relative intensity) 318 (3), 316 (3), 238 (18), 237 (100), 209 (5), 152 (12), 151 (4), 76 (5). Anal. Calcd for $C_{15}H_9O_3Br$: C, 56.81; H, 2.86; Br, 25.20. Found: C, 56.96; H, 3.06; Br, 24.89.

1-Hydroxy-2-(acetoxymethyl)-9,10-anthraquinone (3a). A mixture of **3d** (235 mg, 0.741 mmol), AgOAc (778 mg, 4.65 mmol), and 60 mL of $CH_3CO_2H/CHCl_3$ (2:1 v/v) was heated to reflux for 4 h. After cooling, the silver salts were removed by filtration and washed with acetone. The filtrate was combined with 150 mL of H_2O and extracted with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2 extracts were combined, washed with 100 mL of H_2O, and dried over Na_2SO_4. Removal of solvent in rotary evaporator gave 200 mg of a yellow solid. Recrystallization from heptane/toluene following treatment with charcoal gave 151 mg of yellow needles. Chromatography of the residue resulting from the removal of solvent from the mother liquor on silica gel followed by elution with CH_2Cl_2 gave an additional 34 mg for a total yield of 185 mg (84%): mp 147–8 °C; IR (Nujol) 1734, 1662, 1630, 1586, 1295, 1220, 1160, 1062, 1020, 971, 895, 853, 822, 786, 745, 710 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.18 (s, 3 H), 5.26 (s, 2 H), 7.68–7.82 (m, 4 H), 8.22–8.27 (m, 2 H); ^{13}C NMR (250 MHz, $CDCl_3$) δ 189, 182, 171, 160, 135 (2), 134, 133 (3), 131, 127, 126, 119, 116, 60, 21; MS, m/e (relative intensity) 268 (19), 253 (68), 238 (100), 237 (28), 152 (25). Anal. Calcd for $C_{17}H_{12}O_5$: C, 68.91; H, 4.08. Found: C, 69.24; H, 4.19.

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Supplementary Material Available: Experimental procedures and characterization data for anthraquinones **5f**, **5d**, **7a**, and **7d** (3 pages). Ordering information is given on any current masthead page.

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