Electroreductive Cleavage of Substituted 9,10-Anthraquinones in 50% Aqueous THF Buffers: A pH-Dependent Process

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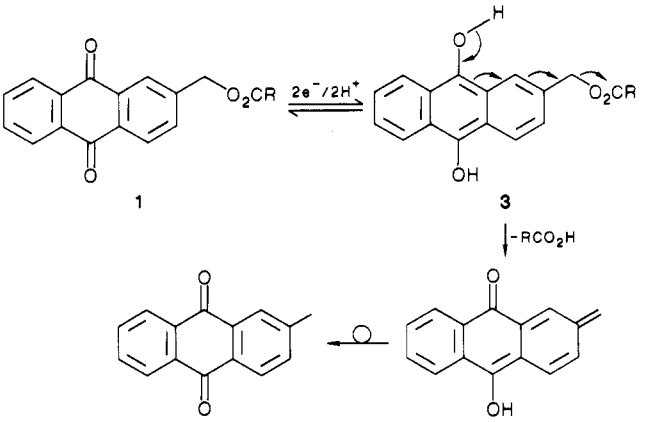
A variety of substituted 9,10-anthraquinones with acetate leaving groups (**4a–7a**) were reduced at a glassy carbon electrode in 50% aqueous THF prepared from aqueous buffers at pH 6, 7, and 8. Cyclic voltammograms of these compounds exhibited a single reduction wave with E_p values of −450 to −530 mV (vs Ag/AgCl/0.10 M Cl^-) at pH 7. E_p shifted to more negative values with increasing pH (45–50 mV/pH unit) consistent with a $2e^-/2H^+$ reduction process which converts **4a–7a** to their corresponding anthrahydroquinones. Constant potential reduction of acetates **4a–7a** at −800 mV at pH 6 or 7 gave n values of 2.2–2.4. Air oxidation of the catholytes, a procedure that converts anthrahydroquinones to anthraquinones, led to a 56–89% recovery of the acetates and 7–32% yields of the reductive cleavage products **4b–7b**. In contrast, electroreduction of **4a–7a** at pH 8 gave much higher yields (50–73%) of **4b–7b** with n values of 3.5–3.8. This pH-dependent process suggests that **4a–7a** cleave much slower via their intermediate anthrahydroquinones than the conjugate bases of their anthrahydroquinones, which are present in relatively high concentration at higher pH. NaOH titration curves of the anthrahydroquinones of **5a** and **5b** support this mechanistic picture.

Reductive cleavage of substituted 9,10-anthraquinones has emerged as an important process in several areas. In 1977, Kemp and Reczek reported their preparation of 2-methylene-9,10-anthraquinone (Maq) esters of several amino acids and peptides.[1] Reduction of these compounds (**1**, in which RCO_2 is an amino acid or peptide) with sodium dithionite ($Na_2S_2O_4$) in aqueous dioxane or by photolysis in isopropyl alcohol containing *N*-methylmorpholine produced RCO_2H and 2-methyl-9,10-anthraquinone (**2**) in nearly quantitative yields. The sequence of reactions in Scheme I was proposed to account for these results. In a more recent study, Maq esters of a carboxylic acid and carbamic acid (**1**, in which RCO_2 is a carboxylate or carbamate) were electrochemically reduced in a 50% aqueous tetrahydrofuran (THF) buffer at pH 7.[2] The anthrahydroquinones (**3**) produced in this manner did not undergo cleavage, however, even after long periods of time (1–2 h). This result suggests that **3** may not be the actual species that cleaves in Kemp and Reczek's experiments. Although the R groups are not the same in the two studies, it seems unlikely that minor differences in leaving groups would significantly affect the rate of cleavage.

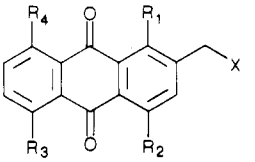
Bioreductive cleavage of the antitumor anthracyclines, which have structures reminiscent of substituted 9,10-anthraquinones, has been proposed as a possible mechanism whereby these drugs function as antineoplastic agents.[3–5] There is uncertainty in the literature though regarding the exact species that undergoes in vivo cleavage of the glycosidic residue. Koch and co-workers[3b] have

Scheme I



provided evidence for an anthrahydroquinone intermediate whereas other workers[3c,4,5] favor a semianthraquinone.

We recently prepared a variety of substituted 9,10-anthraquinones with acetate leaving groups (**4a–7a**) and examined their electroreductive cleavage in DMF solution.[6] In this paper we extend these studies to aqueous THF buffers.



4: $R_1 = R_2 = R_3 = R_4 = H$
5: $R_1 = OMe$, $R_2 = R_3 = R_4 = H$ **a**: $X = O_2CCH_3$
6: $R_1 = R_4 = OMe$, $R_2 = R_3 = H$ **b**: $X = H$
7: $R_1 = R_4 = H$, $R_2 = R_3 = OMe$

Results and Discussion

Cyclic Voltammetry of Anthraquinones 4–7 in 50% Aqueous THF. Two problems were encountered in using 100% aqueous buffers in this study. First, anthraquinones **4-7** have limited solubility in water. Second, and more importantly, these compounds adsorb unto the surface of glassy carbon, an ideal electrode material for aqueous

(1) Kemp, D. S.; Reczek, J. *Tetrahedron Lett.* **1977**, 1031.
(2) Blankespoor, R. L.; Lau, A. N. K.; Miller, L. L. *J. Org. Chem.* **1984**, *49*, 4441.
(3) (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) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 2504. (c) Komiyama, T.; Oki, J.; Inui, T. *J. Antibiot.* **1979**, *32*, 1219. (d) Ramakrishnan, K.; Fisher, J. *J. Am. Chem. Soc.* **1983**, *105*, 7187. (e) Sinha, B. K. *Biochem. Pharmacol.* **1981**, *30*, 2626. (f) Donehower, R. C.; Meyers, C. E.; Chabner, B. A. *Life Sci.* **1979**, *25*, 1.
(4) Recent references in which extensive literature citations are given include: (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.
(5) (a) Pan, S.-S.; Pedersen, L.; Bachur, N. R. *Mol Pharmacol.* **1981**, *19*, 184. (b) Bachur, N. R.; Gordon, S. L.; Gee, M. V.; Kon, H. *Proc. Natl. Acad. Sci. U.S.A* **1979**, *76*, 954. (c) Komiyama, T.; Oki, T.; Inui, T.; Takeuchi, T.; Umeyawa, H. *Gann* **1979**, *70*, 403.
(6) Blankespoor, R. L.; Schutt, D. L.; Tubergen, M. B.; De Jong, R. L. *J. Org. Chem.* **1987**, *52*, 2059.

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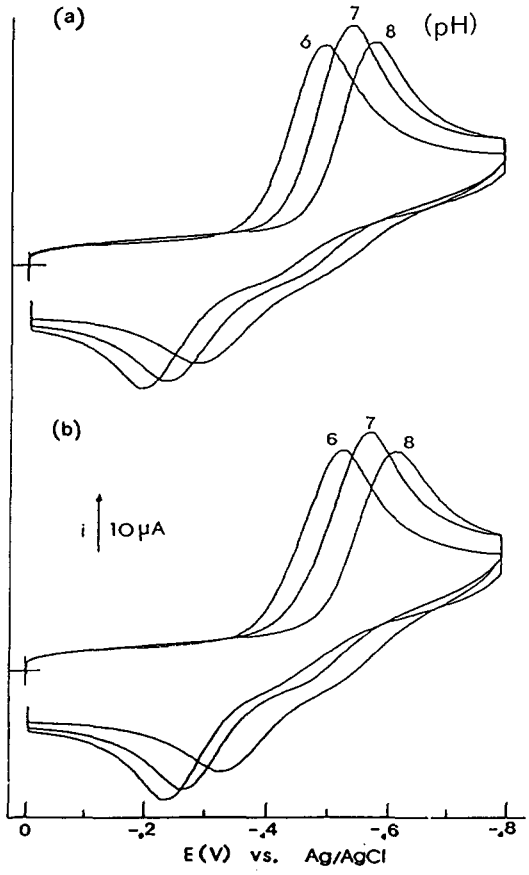


Figure 1. (a) Cyclic voltammograms of 1.0 mM **6a** in 50% aqueous THF buffer at a sweep rate (ν) of 100 mV s^{-1}. (b) Cyclic voltammograms of 1.0 mM **6b** in 50% aqueous THF buffer at a sweep rate of 100 mV s^{-1}.

electrolytes. Both of these problems were overcome by employing 50% aqueous THF buffers containing 0.10 M KCl and 0.050 M phosphate. Concentrations of up to 3 mM could be realized and plots of i_p vs $\nu^{1/2}$ (i_p = peak current and ν = scan rate) from cyclic voltammograms of **4-7** were nearly linear, demonstrating that the electrode processes were under diffusion, not adsorption control.

Unless stated otherwise, all of the pH values reported in this paper are of the aqueous buffers prior to mixing with THF. The *apparent* pH following mixing is 0.6–0.7 higher and is probably not the actual pH as noted in earlier studies on mixed solvents.[7]

The electrochemistry of quinones in aqueous media has been thoroughly investigated.[8] One voltammetric wave is generally observed for the quinone/hydroquinone couple corresponding to a $2e^-/2H^+$ process. Cyclic voltammograms of **4–7** exhibit a single reduction wave and a very broad anodic wave upon scan reversal as shown in Figure 1 for **6a** and **6b** at pH of 6, 7, and 8. The peak potential, E_p, shifts to more negative values with increasing pH (45–50 mV/pH unit), which is consistent with a $2e^-/2H^+$ process that converts **4b–7b** to their corresponding anthrahydroquinones.

Spectroscopic evidence for anthrahydroquinone formation was obtained by electrochemically reducing a 0.50 mM solution of **5b** in 50% aqueous THF buffer at pH 6 under argon and recording the UV–vis spectrum of the catholyte in the absence of oxygen. The spectrum of 1-methoxy-2-methyl-9,10-anthracenediol (**8**), acquired in this manner,

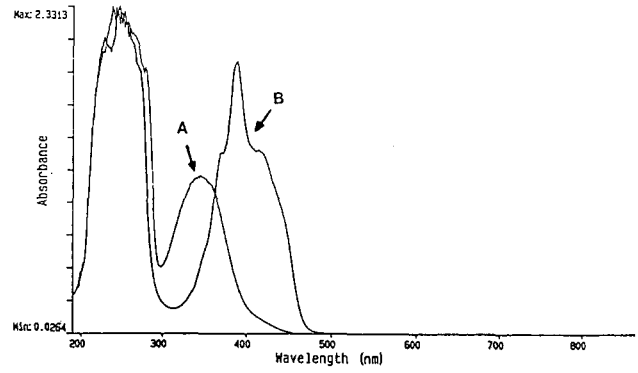


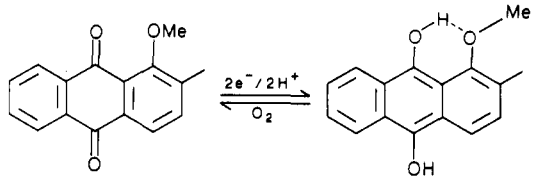
Figure 2. UV–vis spectra of **5b** (curve A) and 8 (curve B) in 50% aqueous THF prepared from an aqueous buffer at pH 6. Upon exposure to oxygen, curve B gradually transformed into curve A with an isosbestic point at 361 nm.

Table I. Cathodic Peak Potentials Measured by Cyclic Voltammetry (mV)[a]

anthraquinone	E_p[b]	anthraquinone	E_p[b]
4a	-516	**6a**	-553
4b	-554	**6b**	-570
5a	-489	**7a**	-525
5b	-495	**7b**	-561

[a] pH 7.0 buffer (0.10 M KCl, 0.050 M phosphate). [b] Reference is Ag/AgCl; sweep rate = 100 mV s^{-1}.

is shown in Figure 2 and is similar to the one reported for 9,10-dimethoxyanthracene.[9] Exposure of this solution to oxygen resulted in the conversion of **8** to **5b** (eq 1), which was monitored spectroscopically (Figure 2).



(1)

Peak potentials from the cyclic voltammograms of anthraquinones **4–7** in pH 7 buffer are given in Table I. A comparison of E_p values for **4a–7a** with those for **4b–7b** shows that replacement of hydrogen with the acetoxy group increases the peak potential (e.g., makes E_p less negative) as would be expected for an electron-withdrawing group. The effect of introducing methoxy groups unto the 9,10-anthraquinone is more complex. A comparison of **4b** and **5b** shows that the first methoxy group *increases* the peak potential by 59 mV, whereas the second methoxy group decreases E_p for **6b** and **7b** by 75 and 66 mV, respectively. The trends in the **4a–7a** series are the same, but less pronounced. One possible explanation for the overall effect of the first methoxy group is that its electron donation, which would be expected to decrease E_p, is more than offset by stabilization of the anthrahydroquinone through H bonding as shown in structure **8**. The second methoxy group then decreases E_p by electron donation which is not offset by additional H bonding.

Electroreduction of 4a–7a in 50% Aqueous THF. Solutions of anthraquinones **4a–7a** (1–3 mM) in 50% aqueous THF buffers at pH 6, 7, and 8 were reduced at carbon felt at -800 mV under argon. The catholytes, containing the resulting anthrahydroquinones, were air oxidized and analyzed by HPLC using a reverse-phase column. The results are summarized in Table II.

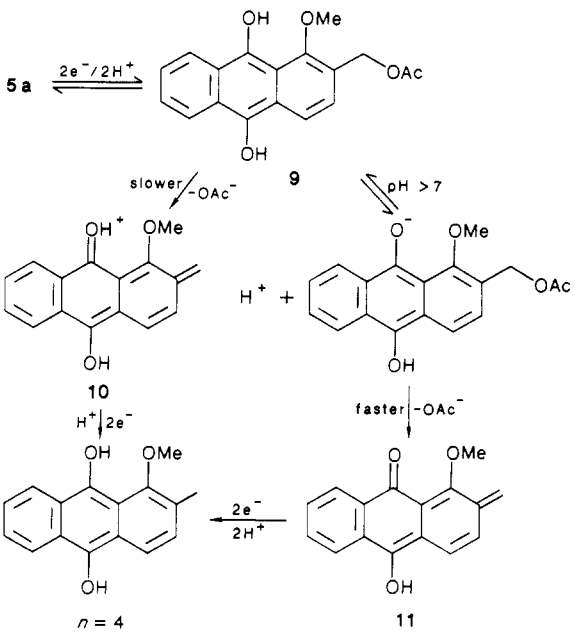
(7) Bates, R. G. *Pure Appl. Chem.* **1969**, *18*, 419.
(8) Chambers, J. Q. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: London, 1974; Chapter 14.
(9) Carlson, S. A.; Hercules, D. M. *Anal. Chem.* **1973**, *45*, 1794.

Table II. Products from the Constant Potential Reduction of Anthraquinones 4a–7a in 50% Aqueous THF[a]

anthraquinone	products (% yield)[b] pH 6.0	pH 7.0	pH 8.0
4a	**4a** (89)	**4a** (64)	**4a** (9)
	4b (9)	**4b** (11)	**4b** (68)
5a	**5a** (78)	**5a** (67)	**5a** (3)
	5b (10)	**5b** (10)	**5b** (73)
6a	**6a** (75)	**6a** (56)	**6a** (36)
	6b (23)	**6b** (32)	**6b** (50)
7a	**7a** (75)	**7a** (87)	**7a** (6)
	7b (9)	**7b** (7)	**7b** (61)

[a] Applied potential is −800 mV. Working electrode is carbon felt. Reference electrode is Ag/Ag Cl (0.10 M Cl^-). [b] HPLC analysis using a reverse-phase column.

Scheme II



At pH 6 and 7, electroreduction of **4a–7a** leads to only low yields (7–32%) of their corresponding cleavage products, **4b–7b**, and the precursors are recovered in high yield (56–89%). In contrast, electroreduction in pH 8 buffer produces **4b–7b** in considerably higher yields (50–73%). The n values obtained from these electrolyses varied from 2.2–2.4 for the lower conversions to 3.5–3.8 for the higher ones.

The pH dependence of the electroreductive cleavage of **4a–7a** can be accounted for by the sequence of reactions given in Scheme II for **5a**. At lower pH **5a** is reduced to its anthrahydroquinone **9**, which slowly cleaves to the protonated vinylogous quinone methide **10**. Reduction of **10** produces the anthrahydroquinone of **5b**, giving an overall n value of 4. At higher pH **9** is in equilibrium with its conjugate base, a negatively charged intermediate that cleaves more rapidly, giving the quinone methide **11**. Quinone methides derived from the structurally related anthracyclines have been trapped with nucleophiles[3d] and observed spectroscopically.[10]

The ECE processes in Scheme II are apparently slow relative to the time frame of our cyclic voltammetry experiments. The cyclic voltammograms in Figure 1 for 1.0 mM solutions of **6a** and **6b** have similar peak widths and peak currents, even at pH 8. If the ECE processes were

(10) Boldt, M.; Gaudiano, G.; Koch, T. H. *J. Org. Chem.* **1987**, *52*, 2146.

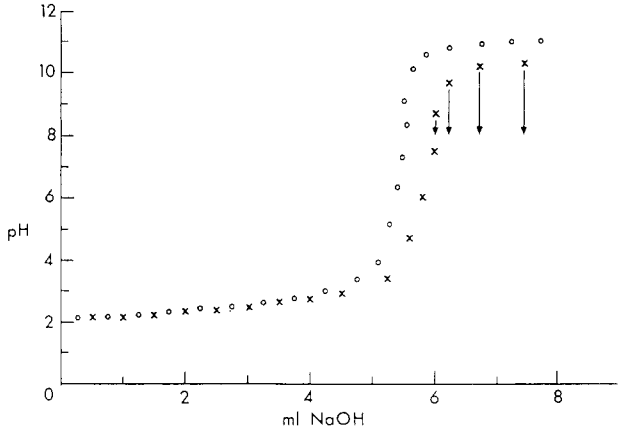


Figure 3. Titration of a 25-mL solution of 0.275 mmol of **5a** (×) or **5b** (○) in 50% aqueous THF (0.050 M HCl, 0.10 M KCl) with 0.103 M NaOH under argon. Arrows show the decrease in pH of the mixed solvent that occurred when the titration was interrupted for 1–2 min at each of these points.

fast under these conditions, the peak currents for **6a** would be considerably higher than those for **6b** since two more electrons would be involved in the reduction process. The current function, $i_p/v^{1/2}$, was nearly constant for **4a–7a** over a wide range of scan rates (v = 20–500 mV/s).

NaOH Titration of the Anthrahydroquinones of 5a and 5b. To obtain additional experimental support for the mechanistic picture in Scheme II solutions of **8** and **9** were prepared and titrated with NaOH in the absence of oxygen. This was accomplished by electrochemically reducing **5b** and **5a**, respectively, in 50% aqueous THF (0.10 M KCl) containing sufficient HCl to keep the medium acidic during the electrolysis and titrating the resulting catholytes under argon with a standardized NaOH solution (same solvent) while monitoring the pH.

The results of the two titrations are given in Figure 3 based upon pH measurements of the *mixed* solvent. As mentioned earlier, solutions of 50% aqueous THF consistently give apparent pH values 0.6–0.7 higher than 100% aqueous solutions containing the same electrolyte. The titration curve for **8** resembles one for a strong acid. This is expected since the mole ratio of HCl to **8** was initially 2.5:1.0. At pH 7.5–8.0, a color change from yellow to wine red occurred presumably due to the formation of the conjugate base of **8**. This color intensified as more NaOH was added.

The titration curve of **9** under identical conditions coincides with the one for **8** up to a pH of approximately 4 and then diverges, giving consistently lower pH readings thereafter. This is the result of cleavage which produces the weak acid CH_3CO_2H. At pH 7.5–8.0 a color change also occurred from yellow to wine red. However, beyond 8 the pH was unstable. After each additional aliquot of NaOH, the color of the solution faded and the pH slowly decreased to approximately 8 as indicated by the arrows. After sufficient NaOH had been added to neutralize the HCl and react with **8**, the titration was stopped, and **5b** was isolated in high yield (80%) from the reaction mixture. Clearly, the titration experiment shows that the conjugate base of **9** is present at pH 8 in 50% aqueous THF, thereby lending support to the proposed mechanism in Scheme II.

A question that remains though is whether or not cleavage of the anthrahydroquinones (e.g., **9** → **10**) is the predominent reaction pathway even at lower pH. If the pK_a of an anthrahydroquinone is 9, then the ratio of anthrahydroquinone to its conjugate base in bulk solvent is 1000 at pH 6. At the electrode surface, which is undoubtedly more basic, the ratio would be considerably less. If

the rate constants for the two reaction pathways differed by a factor of at least 10^3, which is quite possible, then the pathway involving the conjugate base would actually predominate even at pH 6.

Conclusions

This work demonstrates that electroreductive cleavage of substituted anthraquinones in aqueous media is pH dependent since their intermediate anthrahydroquinones cleave much more slowly than the conjugate bases of their anthrahydroquinones. As a result, good yields of cleavage products are only obtained at higher pH where the concentrations of the conjugate bases are relatively high. Although the compounds used in this study are structurally distinct from anthracyclines, particularly with respect to leaving group (e.g., acetate vs glycoside), these results do raise the question of whether or not anthracyclines also undergo bioreductive cleavage via the conjugate bases of their anthrahydroquinones.

Experimental Section

Materials. Anthraquinones **4–7** were prepared by the literature method.[6] The buffers were prepared by dissolving KCl and KH_2PO_4 into water (deionized and distilled from $KMnO_4$) and adjusting the pH to 6, 7, or 8 with KOH. Sufficient THF (distilled from CaH_2) and some additional water were added to give 50% aqueous THF (0.10 M KCl and 0.050 M phosphate). The pHs of the final solutions were 6.67, 7.68, and 8.66, respectively, and they were stored under N_2 to prevent peroxide formation from THF.

Chromatography. HPLC analyses were performed by using a Waters Associate C-18 Bondapak reverse-phase column, a Varian Vari-Chrom detector, and an Altex Model 100 metering system. The temperature was approximately 25 °C, the eluant was 65:35 methanol–water, the flow rate was 1.15 mL/min, and the wavelength was 260 nm.

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 Cl^-.

Cyclic Voltammetry. A 25-mL, three-necked, round-bottom flask was used to prepare a one-compartment cell. The working electrode was a glassy-carbon disk (A = 0.090 cm^2) set in a Teflon tube. Prior to measurements on each solution this electrode was cleaned, polished with 0.30- and 0.050-μm α alumina (Buehler), wiped with a tissue, sonicated in water, and pretreated by using the method of Blaedel and Jenkins.[11]

(11) Blaedel, W. J.; Jenkins, P. A. *Anal. Chem.* **1975**, *47*, 1337.

General Procedure for Constant Potential Electrolyses in 50% Aqueous THF. A three-compartment cell was used for the electrolyses. The center compartment, containing Carborundum carbon felt (pretreated by soaking in concentrated HNO_3 for 10 min, washing thoroughly with deionized water, and drying in an oven at 100 °C), was separated from the reference electrode on one side and the counter electrode on the other side by a glass frit (medium) and aqueous agar. The counter electrode was a graphite rod in 0.10 M KCl, and the reference compartment contained 0.10 M KCl and Ag/AgCl. Approximately 10 mL of 50% aqueous THF buffer (0.10 M KCl, 0.050 M phosphate) was introduced into the center compartment. After deoxygenation with argon, the background current was measured. The anthraquinone to be reduced was added (5–20 mg), and the resulting yellow solution was again deoxygenated. After the electrolysis was complete (1–3 h), as evidenced by constant current, the contents of the center compartment were transferred to an Erlenmeyer flask where a stream of air was passed through the catholyte for 0.5 h to ensure complete oxidation of anthrahydroquinone to anthraquinone. The resulting mixture was analyzed directly with HPLC following filtration. The catholyte was filtered, diluted with 2 volumes of water, and extracted with CH_2Cl_2 (3 × 15 mL). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and evaporated to dryness in a rotary evaporator. The residue was separated by chromatography on silica gel and elution with CH_2Cl_2. Agreement between HPLC and isolated yields was generally excellent (e.g., within 5%).

Procedure for NaOH Titration of the Anthrahydroquinones of 5a and 5b. A two-compartment cell was used with a glass frit and aqueous agar separating the two compartments. One compartment contained the anode, a graphite rod immersed in 0.10 M KCl. The other compartment was four-necked and fitted with a buret containing 0.103 M NaOH in 50% aqueous THF, a Ag/AgCl reference electrode, a carbon felt working electrode with an argon inlet and outlet, and a pH electrode. A Teflon stir bar, 0.275 mmol of **5a** or **5b**, and 25 mL of 50% aqueous THF (0.050 M HCl, 0.10 M KCl) were introduced into the cathodic compartment. After deoxygenation, the anthraquinone was reduced at −800 mV, giving an n value of 2. The electrical leads were disconnected, and the anthrahydroquinone was titrated immediately under the argon atmosphere. The data is plotted in Figure 3.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the Research Corporation. R.L.B. is grateful to Calvin College for a Calvin Research Fellowship (1986–1987).

Registry No. **4a**, 17241-72-4; **4b**, 84-54-8; **5a**, 107960-76-9; **5b**, 20460-44-0; **6a**, 107960-77-0; **6b**, 107960-75-8; **7a**, 107960-78-1; **7b**, 71013-35-9; **9**, 114492-07-8; **10**, 114492-08-9; **11**, 114492-09-0; 2-(acetoxymethyl)-1-methoxy-9,10-anthracenediol (ion 1−), 114492-10-3; 1-methoxy-2-methyl-9,10-anthracenediol, 114492-11-4.