pH Dependence of the Mechanism of Hydrolysis of Benzo[a]pyrene-*cis*-7,8-diol 9,10-Epoxide Catalyzed by DNA, Poly(G), and Poly(A)

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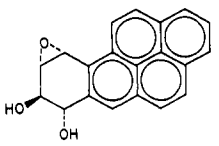
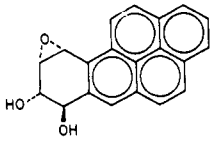
Abstract: The rates of reaction of (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[α]pyrene (**DE-1**, in which the 7-hydroxyl group and epoxide oxygen are cis) in solutions of varied DNA, Poly(A), and Poly(G) concentrations were determined as a function of pH. The rate data were consistent with a mechanism in which **DE-1** forms a physical complex with the polynucleotide, and this physical complex than reacts both by a pathway whose rate is first-order in respect to hydronium ion concentration (k_{cat}^{H} route) and by a second pathway whose rate is independent of hydronium ion concentration (k_{cat}^{0} route). Product studies showed that >95% of the products formed from both the k_{cat}^{H} and k_{cat}^{0} reactions were tetraols resulting from cis and trans hydration of the epoxide, and <5% of covalent binding of the diol epoxide to the polynucleotides occurred. The DNA- and Poly(A)-catalyzed hydrolyses of **DE-1** are similar to those of **DE-2** in that the physically bound diol epoxide reacts significantly faster (>50 fold) than free diol epoxide by the acid-catalyzed routes ($k_{cat}^{H} \gg k_{H^+}$) and moderately faster (<5) by the spontaneous pathway ($k_{cat}^{0} > k_0$). Poly(G) is a significantly better catalyst than either DNA or Poly(A) for both k_{cat}^{H} and k_{cat}^{0} reactions. At pH ca. 7, however, the physical **DE-1**·DNA complex reacts mainly by the k_{cat}^{0} reaction, whereas the physical **DE-2**·DNA complex reacts mostly by the k_{cat}^{H} reaction.

The carcinogenic activities of various polycyclic aromatic hydrocarbons have been attributed to their metabolism to bay region diol epoxides,[1] which have been proposed to act as the ultimate carcinogenic reagents by reacting with critical cellular macromolecules such as DNA.[1c,2] Whereas the major bay region diol epoxide metabolite of benzo[a]pyrene ((+)-**DE-2**) is a potent carcinogen,[3] the diasteriomeric isomer in which the benzylic 7-hydroxyl group and epoxide oxygen are cis ((+)-**DE-1**) is not significantly carcinogenic.[3,4]



(+) DE-1

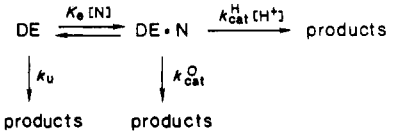


(+) DE-2

It is known that both **DE-1** and **DE-2** bind covalently to DNA in vivo and in vitro.[2,5,6] The reaction rates of **DE-2** are markedly accelerated in aqueous DNA solutions,[6b,c,7] and under such conditions >90% of the diol epoxide undergoes hydrolysis to tetraols and <10% covalently binds to DNA. At pH < 7, the mechanism of reaction of **DE-2** with DNA involves a preequilibrium association of the diol epoxide to DNA followed by a reaction of noncovalently bound diol epoxide in a reaction that is kinetically first-order in hydronium ion concentration.[7] In kinetically equivalent mechanisms, the proton donating species may be H_3O^+, a protonated nucleic acid base, a protonated phosphodiester group, or any combination of the above acids.

We have now examined the kinetics of reaction of cis diol epoxide **DE-1** in aqueous solutions containing DNA, Poly(G), and Poly(A). Although the predominant reaction of the physical complex between **DE-2** and DNA is first-order in respect to hydronium ion concentration at physiological pH,[7] the reaction of the complex between **DE-1** and DNA takes place mainly by a mechanism whose rate is *independent* of hydronium ion concentration at this same pH. The mechanism is kinetically related to the "spontaneous" or "k_0" reactions of **DE-1**, **DE-2**,[8] and certain other epoxides[9] in aqueous solutions at higher pH values, and we refer to it as the "k_{cat}^{0}" mechanism.

Scheme I



Results

Plots of the pseudo-first-order rate constants (k_{obsd}) for reaction of racemic **DE-1** vs. the concentration of DNA in aqueous solutions ($\mu = 0.1$) exhibited saturation kinetics and are provided in Figure 1. These rate data are consistent with those expected for the

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(1) For reviews, see: (a) Miller, E. C. *Cancer Res.* **1978**, *38*, 1479. (b) Jerina, D. M.; Lehr, R. E.; Yagi, H.; Hermandez, O.; Dansette, P. M.; Wislocki, P. G.; Wood, A. W.; Chang, R. L.; Levin, W.; Conney, A. H. In *In Vitro Metabolic Activation in Mutagenesis Testing*; de Serres, F. J., Fouts, J. R., Bend, J. R. Philpot, R. M., Eds; Elsevier: Amsterdam, 1976; p 159. (c) Dipple, A.; Moschel, R. C.; Bigger, C. A. H. In *Chemical Carcinogens*, 2nd ed.; Searle, C. E., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984; Vol. 1, p 41.

(2) (a) *Chemical Carcinogens and DNA*; Grover, P. L. Ed.; CRC Press: Boca Raton, FL, 1979. (b) Neidle, S. *Nature (London)*, *1980*, *283* (*5743*), 135.

(3) (a) Buening, M. K.; Wislocki, P. G.; Levin, W.; Yagi, H.; Thakker, D. R.; Akagi, H.; Koreeda, M.; Jerina, D. M.; Conney, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5358. (b) Slaga, T. J.; Bracken, W. B.; Gleason, C.; Levin, W.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1979**, *39*, 67.

(4) The synthetic (−) enantiomers of both **1** and **2** are only weakly carcinogenic, ref 3.

(5) Koreeda, M.; Moore, D. D.; Wislocki, P. G.; Levin, W.; Conney, A. H.; Yagi, H.; Jerina, D. M. *Science (Washington, DC)* **1978**, *199*, 778.

(6) (a) Weinstein, I. B.; Jeffrey, A. M.; Jennette, K. W.; Blobstein, S. H.; Harvey, R. G.; Kasai, H.; Nakanishi, K. *Science* (Washington, DC) **1976**, *193*, 592. (b) Kootstra, A.; Haas, B. L.; Slaga, T. J. *Ibid.* **1980**, *94*, 1432. (c) Geacintov, N. E.; Yoshida, H.; Ibanez, V.; Harvey, R. G. *Biochem.* **1982**, *21*, 1964. (d) King, K. W.; Osborne, M. R.; Brookes, P. *Chem. Biol. Interact.* **1979**, *24* (*3*), 345.

(7) Michaud, D. P.; Gupta, S. C.; Whalen, D. L.; Sayer, J. M.; Jerina, D. M. *Chem. Biol. Interact.* **1983**, *44*, 41.

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(9) (a) Kasperek, G. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1972**, *94*, 198. (b) Ross, A. M.; Pohl, T. M.; Piazza, K.; Thomas, M.; Fox, R.; Whalen, D. L. *Ibid.* **1982**, *104*, 1658.

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Table I. Values of K_e, k_{cat}^H, and k_{cat}^0 for Reaction of **DE-1** and **DE-2** at 25 °C in Solutions Containing DNA, Poly(A), and Poly(G)

compd	N	K_e (M^{-1})	k_{cat}^H (M^{-1} s^{-1})	k_{cat}^0 (s^{-1})
DE-1	DNA[a]	$1.07 \pm 0.13 \times 10^3$	$3.06 \pm 0.26 \times 10^4$	$3.30 \pm 0.18 \times 10^{-2}$
	Poly(A)[b]	$2.23 \pm 0.27 \times 10^2$	$2.98 \pm 0.30 \times 10^4$	$3.67 \pm 0.20 \times 10^{-2}$
	Poly(G)[c,d]	$<2.5 \times 10^2$	$<6.3 \times 10^5$	<0.20
			($k_{H^+} = 5.5 \times 10^2$ (M^{-1} s^{-1})[e]	($k_0 = 8.9 \times 10^{-3}$ s^{-1})[e]
DE-2[f]	DNA[g]	2.40×10^3	8.55×10^4	1.4×10^{-3}
	Poly(A)[c]	$3.16 \pm 0.48 \times 10^2$	$5.46 \pm 0.51 \times 10^4$	$4.32 \pm 0.33 \times 10^{-4}$
	Poly(G)[c,d]	$<2.5 \times 10^2$	$>9.2 \times 10^5$	>0.037
			($k_{H^+} = 1.4 \times 10^3$ M^{-1} s^{-1})[e]	($k_0 = 3.0 \times 10^{-4}$ s^{-1})[e]

[a] $\mu = 0.1$ (NaCl), 4% dioxane. [b] $\mu = 0.1$ (KCl), 2% dioxane. [c] $\mu = 0.1$ (KCl), 4% dioxane. [d] For **DE-1**, $k_{cat}^H K_e = 1.57 \pm 0.17 \times 10^8$ M^{-2} s^{-1} and $k_{cat}^0 K_e = 49.0 \pm 0.36$ M^{-1} s^{-1}; for **DE-2**, $k_{cat}^H K_e = 2.31 \pm 0.23 \times 10^8$ M^{-2} s^{-1} and $k_{cat}^0 K_e = 9.2 \pm 0.2$ M^{-1} s^{-1}. [e] Estimated for 4% dioxane ($\mu = 0.1$) from rate data in water ($\mu = 0.1$) and 10% dioxane–90% water ($\mu = 0.1$), ref 8. [f] Data for reaction of **DE-2** with DNA are from ref 7. The parameters for reaction of **DE-2** in Poly(A) solutions are calculated from that rate data of ref 7 and additional rate data collected at pH 8.0 and 9.0. [g] $\mu = 0.1$ (NaCl), 0.5% dioxane.

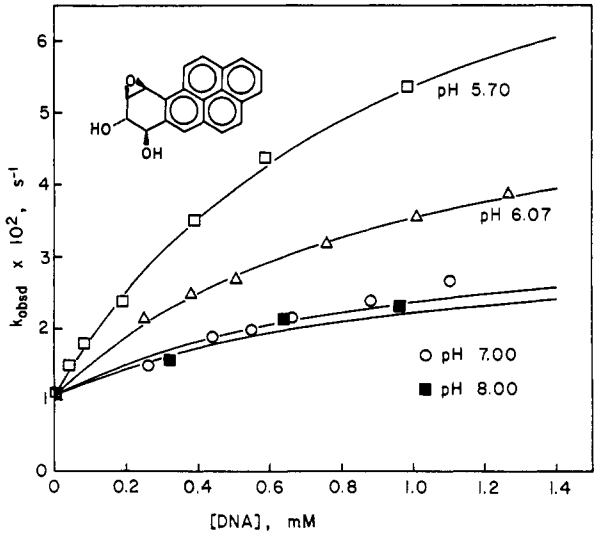

Figure 1. Plots of k_{obsd} for hydrolysis of **DE-1** vs. concentration of DNA in water solutions, 4% dioxane, $\mu = 0.1$ (NaCl), 25.0 ± 0.1 °C. The solid lines are theoretical curves calculated from eq 1 and parameters given in Table I. DNA concentrations are in nucleotide units.

reaction mechanism outlined in Scheme I, where DE refers to diol epoxide, N is DNA (nucleic acid), and DE·N is a physical complex between DE and N.[7,10] The rate constant k_u is that for reaction of diol epoxide in the absence of nucleic acid and for **DE-1** is approximately equal to the spontaneous rate constant k_0 over the pH range listed in Figure 1.[8] The rate data were fit to the equation

$$k_{obsd} = (k_4 + (k_{cat}^H[H^+] + k_{cat}^0)K_e[N])/(1 + K_e[N]) \quad (1)$$

where k_{cat}^H is the second-order rate constant for the acid-catalyzed reaction of the physically bound epoxide, k_{cat}^0 is the apparent first-order rate constant for the "spontaneous" reaction of physically bound epoxide, and K_e is the apparent equilibrium constant for association of diol epoxide and nucleic acid.

Plots of k_{obsd} vs. Poly(A) concentrations ($\mu = 0.1$, KCl) were similar to those shown in Figure 1, and values of K_e, k_{cat}^H, and k_{cat}^0 obtained from weighted nonlinear least-squares fits of the data to eq 1 are provided in Table I for the reaction of **DE-1** with both DNA and Poly(A). Previously published parameters for reaction of **DE-2** in DNA and Poly(A) solutions are also provided for comparisons.

It can be readily seen in Figure 1 that the limiting rates for reaction of **DE-1** in DNA solutions are pH dependent in the pH 5–6 range. However, almost no change in limiting rate occurs between pH 7–8, and, therefore, there is a change of mechanism for the reaction of the DE·DNA association complex as the pH of the solution is increased from 5 to 7. From the data of Table I, it can be calculated that at pH 7, >90% of **DE-1** that is physically complexed with both DNA and Poly(A) reacts by the k_{cat}^0 mechanism. In contrast, only about 14% of the complex between DNA and **DE-2** reacts by the k_{cat}^0 mechanism at this same pH.

Table II. Product Distributions from Hydrolysis of **DE-1** in 4% Dioxane–Water Solutions Containing DNA, Poly(A), and Poly(G)[a]

N	[N] (mM)	pH	cis hydratn (%)	trans hydratn (%)
		4.0	86.9	13.1
DNA[b]	1.0	5.70	86.7	13.3
	1.0	7.00	86.3	13.7
Poly(A)[c]	2.0	5.72	83.9	16.1
	2.0	6.98	90.4	9.6
Poly(G)[c]	2.0	5.73	84.0	14.0
	2.0	7.00	90.5	9.5

[a] Total yield of tetraols, as determined by quantitative HPLC experiments, was >98% in each experiment. [b] $\mu = 0.1$ (NaCl). [c] $\mu = 0.1$ (KCl).

Because of the diastereomeric relationship between the physical complexes of the enantiomers of **DE-1** and DNA, it would be reasonable to expect that the values of K_e, k_{cat}^H, and k_{cat}^0 for reaction of **(+)-DE-1** with DNA might be different from those for reaction of **(−)-DE-1** with DNA. If these differences were large enough, then the reactions of racemic **DE-1** in DNA solutions should exhibit biphasic kinetics. However, it was our observation that the reactions of racemic **DE-1** in DNA, Poly(A), and Poly(G) solutions followed pseudo-first-order kinetics within experimental error under the conditions of our experiments. Therefore, the values of K_e, k_{cat}^H, and k_{cat}^0 for reaction of both enantiomers of **DE-1** with nucleic acids must be rather similar. To provide additional information in this respect, we have also determined the rate constants for reaction of **(+)-** and **(−)-DE-1** in solutions containing DNA and compared these rate constants with those of **(±)-DE-1** under the same conditions. At pH 5.85 in solutions with DNA concentrations ranging from 0.22 to 0.88 mM and at pH 6.75 in 0.66 mM DNA solution, the rate constants for reaction of **(+)-DE-1** agreed with those for reaction of **(−)-DE-1** with an average deviation of less than 10%. These data demonstrate that the apparent binding constant K_e and limiting rate constants for reaction of the (+) and (−) enantiomers of **DE-1** with DNA are within our experimental error the same.

Our finding that the apparent physical binding constant K_e and limiting rate constants k_{cat}^H and k_{cat}^0 are similar for reaction of both enantiomers of **DE-1** with DNA is somewhat surprising and parallels the results from hydrolysis of **(+)-** and **(−)-DE-2** in DNA solutions.[7] The value of K_e for binding **DE-1** to DNA is slightly less than half that for binding of **DE-2** to DNA[11] and suggests

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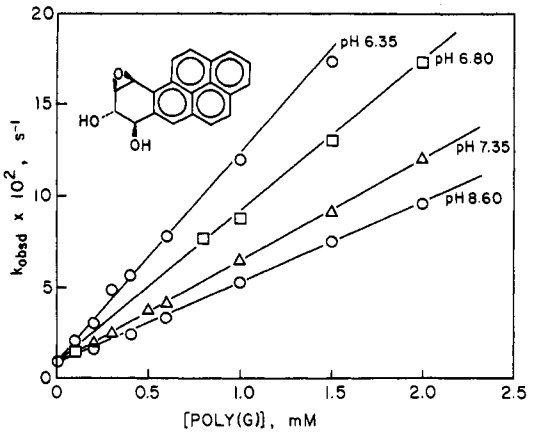


Figure 2. Plots of k_{obsd} for hydrolysis of **DE-1** vs. concentration of Poly(G) in water solutions, 4% dioxane, μ = 0.1 (KCl), 25.0 ± 0.1 °C. Poly(G) concentrations are in nucleotide units.

that the relative stereochemistry of the substituent groups in the angular ring has some effect on binding. But this effect is rather small, and the absolute configuration of the groups appears to have even less effect.

Comparison of the data from Table I with previously published data[8] for the hydrolysis of **DE-1** and **DE-2** shows striking similarities for their reactions in solution and when physically bound to DNA. For example, **DE-1** and **DE-2** both undergo acid-catalyzed hydrolysis and "spontaneous" hydrolysis in solution (k_{H^+} and k_0, respectively). They also both undergo acid-catalyzed and "spontaneous" reactions when physically bound to DNA (k^H_{cat} and k^0_{cat}, respectively). The actual mechanism for hydrolysis of **DE-1** and **DE-2** in either case is, therefore, dependent on the pH of the solution. **DE-2** is more reactive than **DE-1** in the acid-catalyzed reactions (k_{H^+} and k^H_{cat}) and less reactive than **DE-1** in the "spontaneous" reactions (k_0 and k^0_{cat}).

The rates of reaction of **DE-1** and **DE-2** in solutions containing Poly(G) have also been determined, and plots of k_{obsd} for reaction of **DE-1** vs. [Poly(G)] are provided in Figure 2. These rate profiles, unlike those from DNA and Poly(A) solutions obtained under similar conditions, do not exhibit saturation kinetics. If the assumption is made that the rate expression given in eq 1 also holds for the Poly(G) reactions, then for k_{obsd} to increase linearly with [Poly(G)], $K_e[N] \ll 1$. The slopes of the rate profiles of Figure 2 (for **DE-1**) are then equal to $(k^0_{cat}K_e + k^H_{cat}K_e[H^+])$. From a plot of these slopes vs. $[H^+]$, values of $k^H_{cat}K_e$ and $k^0_{cat}K_e$ are determined and are provided for both **DE-1** and **DE-2** in footnote d of Table I. The plots of k_{obsd} vs. [Poly(G)] in Figure 2 show no detectable curvature for [Poly(G)] < 0.002 M. If it is assumed that the limiting rate constants are at least twice the rate constants for 0.002 M Poly(G) solutions, then it can be estimated that $k^H_{cat} > 2K_ek^H_{cat}[N]$ and $k^0_{cat} > 2K_ek^0_{cat}[N]$, where $[N]$ = 0.002 M. The minimum values of k^H_{cat} and k^0_{cat} thus calculated are provided in Table I. Maximum values of K_e are also calculated from the above equations and listed in Table I.

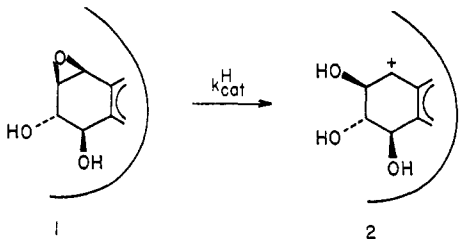
In our previous publication[7] on the reactions of **DE-2** in DNA and Poly(A) solutions, a k^0_{cat} reaction for **DE-2** with DNA was detected from the kinetic data. A k^0_{cat} reaction for **DE-2** with Poly(A) was not detected from the rate data, which was collected at pH 6.05–6.57. If the ratio of k^H_{cat}/k^0_{cat} for reaction of **DE-2** in Poly(A) solutions were similar to that for reaction of **DE-2** in DNA solutions, then it could be predicted that a k^0_{cat} reaction for **DE-2** with Poly(A) should be detectable at higher pH. We have, therefore, examined the rates of reaction of **DE-2** in Poly(A) solutions at pH 8.0 and 9.0 and have observed the limiting rate constants to be greater than those predicted if only the k^H_{cat} reaction were operative. The rate data for the reaction of **DE-2** with Poly(A) throughout the pH range 6.05–9.00 were fit to eq 1, where $k_u = k_{H^+}[H^+] + k_0$ for hydrolysis of free **DE-2**. A value of 4.3 ± 0.3 × 10^{-4} s^{-1} was determined for k^0_{cat} and is listed in Table I for **DE-2**.

From HPLC experiments, >98% of the products from reaction of **DE-1** in DNA solutions at both pH 5.7 and 7.0 result from cis and trans hydration to yield tetraols in the ratio ca. 86:14, respectively. Therefore, the k^H_{cat} and k^0_{cat} reactions result in very little covalent binding of the diol epoxide to DNA to yield stable adducts, and the tetraol product distributions are very similar to those from the k_{H^+} and k_0 reactions.[8] Covalent binding of **DE-1** or **DE-2** to Poly(A) or Poly(G) was not detected either, although formation of small amounts of adducts or very unstable adducts cannot be ruled out. The k^0_{cat} reactions of **DE-1** with Poly(A) and Poly(G) resulted in slightly more cis hydration than the k^H_{cat} reactions, but overall the product distributions from the k_{H^+}, k_0, k^H_{cat}, and k^0_{cat} reactions (regardless of the structure of the polynucleotide) are very similar.

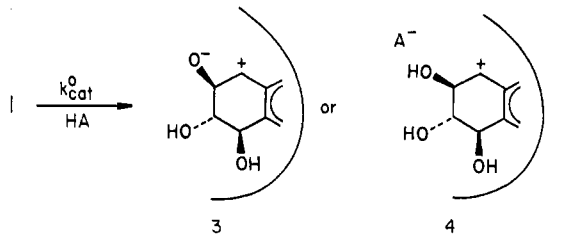
In the k^H_{cat} reaction, the identity of the proton donor is uncertain. Hydronium ion and any other species whose concentration is proportional to H_3O^+ concentration, such as a protonated nucleic acid base or protonated phosphodiester group, are potential candidates. Although the concentrations of the latter groups would be expected to be rather low, the observation that general acid-catalyzed hydrolysis of epoxides by phosphoric acid in solutions at pH some 4–5 units above the pK_a of phosphoric acid[12] suggests that such groups cannot be ruled out as potential proton donors. The mechanism for the k^H_{cat} reaction therefore appears to be similar to that for the k_{H^+} reaction in that the epoxide must be protonated before or during the rate-limiting step(s). The stereochemistries of the tetraol products suggest that a benzylic carbocation is an intermediate in the reaction. A possible mechanism is depicted in Scheme II, in which the physically bound **DE-1** (**1**) reacts to form a physically bound intermediate carbocation **2** in the rate-limiting step. Cationic intermediates such as **2** are generally accepted as the electrophilic reagents that are responsible for the covalent binding of epoxides to DNA.

In view of the fact that the k^0_{cat} reaction of **DE-1** with DNA yields essentially the same mixture of tetraol products as the k_{H^+} and k^H_{cat} reactions, it is possible that the same carbocation is an intermediate. Several potential mechanisms for this reaction are

Scheme II



Scheme III



(11) The equilibrium constants for association of **DE-1** and **DE-2** to DNA have also been determined by spectroscopic methods. (a) ref 6c. (b) Shahbaz, M.; Geacintov, N. E.; Harvey, R. G. *Biochem.* **1986**. The apparent association constants reported (4600 and 11 700 M^{-1} for **DE-1** and **DE-2**, respectively) are considerably larger than the K_e values determined by the kinetic method and reported in this paper. The lower values reported here are due at least in part to the greater ionic strength employed in this study and in ref 7. In each case, however, the binding constant for **DE-1** is approximately half that for **DE-2**.

(12) (a) Rogers, D. Z.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 4713. (b) Becker, A. R.; Janusz, J. M.; Bruice, T. C. *Ibid.* **1979**, *101*, 5679. (c) Sayer, J. M.; Yagi, H.; Croisy-Delcey, M.; Jerina, D. M. *Ibid.* **1981**, *103*, 4970.

outlined in Scheme III. One possibility is that physically bound **DE-1** (**1**) undergoes benzyl C–O bond cleavage to yield **3** in the rate-limiting step.[13] Subsequent protonation of **3** would yield the carbocationic species **2**. Another possibility is that water or a neutral nucleic acid base (HA) acts as a general acid catalyst in directly yielding the carbocation **4**. Various general acids with pK_a values up to 10 have been shown to be efficient catalysts in the hydrolysis of both **DE-1** and **DE-2**.[14] The pK_a for deprotonation of guanosine monophosphate at N-1 is 9.5,[15] and, therefore, this group in DNA, for example, can potentially serve as a general acid catalyst in the k_{cat}^0 reaction.

Another mechanism for the k_{cat}^0 reaction that is kinetically equivalent to those outlined in Scheme III is a nucleophilic displacement reaction in which a nucleophilic group of an unionized nucleic acid base adds to neutral epoxide. We have observed, however, that nucleophilic reactions of amines such as tris(hydroxymethyl)aminomethane (Tris) and $HOCH_2CH_2NH_2$ with **DE-1** and **DE-2** are much less favorable than their general acid-catalyzed hydrolyses by the conjugate acids Tris-H^+ and $HOCH_2CH_2N^+H_3$, which have pK_a values of 8.2 and 9.5, respectively. Reactions of **DE-1** and **DE-2** with DNA might, therefore, be expected to occur more readily by the general acid catalysis mechanism than by the nucleophilic addition mechanism in the k_{cat}^0 reaction. Another argument against the latter mechanisms is that >98% of the products are tetraols, and, therefore, all nucleophilic addition products would have to be extremely labile. Although this possibility cannot be ruled out, it appears unlikely, because a number of covalent adduct types are sufficiently stable to withstand DNA degradation and isolation techniques.[6a,16]

Conclusion

Several important features can be summarized from the data of Table I: (1) DNA and Poly(A) provide significant accelerations of the acid-catalyzed hydrolysis (>50-fold, $k_{cat}^H > k_{H^+}$) and a modest acceleration of the spontaneous hydrolysis (<5, $k_{cat}^0 > k_0$) for *both* **DE-1** and **DE-2**; (2) Poly(G) is a significantly better catalyst than either DNA or Poly(A) for both the k_{cat}^H and k_{cat}^0 mechanisms for hydrolysis of **DE-1** and **DE-2**; and (3) at pH ca. 7, the physical complex between **DE-1** and DNA reacts largely by the k_{cat}^0 mechanism whereas from that of the physical complex between **DE-2** and DNA reacts mainly by the k_{cat}^H mechanism.

The equlibrium constants for association of both **DE-1** and **DE-2** to DNA are ca. 5–10 times larger than the corresponding association constants for Poly(A), but the limiting rate constants k_{cat}^H and k_{cat}^0 for **DE-1** and k_{cat}^H for **DE-2** are very similar for DNA and the homopolymer Poly(A). The larger values of k_{cat}^H and k_{cat}^0 for reaction of **DE-1** and **DE-2** with Poly(G) are consistent with the observation that the abilities of natural DNA and synthetic polynucleotides to catalyze the hydrolysis of **DE-2** increase with increasing (guanine + cytosine) composition.[17]

Experimental Section

Solvents and Materials. 5′-Polyadenylic acid (Poly(A)) and 5′-polyguanilic acid (Poly(G)), as their potassium salts, and DNA were purchased from Sigma Chemical Co., St. Louis, MO. **DE-1** ((±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and its enantiomers) and **DE-2** ((±)-7β-8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene) were prepared by published procedures.[18] DNA stock solutions were filtered through filter-aid, and the DNA concentrations of the filtrates, expressed as mononucleotide concentrations, were determined from their absorptions at 260 nm (ϵ = 6300).[19] Dioxane was distilled from sodium prior to use.

Kinetic Procedures. For each kinetic run, approximately 5–20 μL of a stock solution of **DE-1** or **DE-2** in dioxane was added to 2.0 mL of reaction solution in the thermostatted cell compartment (25.0 ± 0.2 °C) of a Gilford Response spectrophotometer. The reactions were monitored at 348 nm, and pseudo-first-order rate constants were calculated by nonlinear regression analysis of the absorbance vs. time data. The kinetic solutions contained 2 × 10^{-4} M of 3-[*N*-morpholino]-2-hydroxypropanesulfonic acid (MOPSO), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 2-[*N*-morpholino]ethanesulfonic acid (MES), or 2-[*N*-cyclohexylamino]ethanesulfonic acid (CHES) buffers to maintain constant pH.

Product Studies. Aliquots of a stock solution of **DE-1** were added to 1.0 mL of water at pH 4.0 for 1.5 m (ca. 10 half-lives). The pH of the solution was then adjusted to 7.0, and aliquots of naphthaleneethanol or cinnamyl alcohol were added to serve as standards. The solutions were then analyzed directly by HPLC on a Waters C18 Radial Pak column, with 55% methanol–45% water as the eluting solvent, 1.5 mL/m. The tetraol products and standard compounds were detected by their absorbance at 254 nm; retention times for tetraol from trans hydration, 10.4 m; tetraol from cis hydration, 17.1 m; naphthaleneethanol, 15.1 m; cinnamyl alcohol, 7.5 m. The total yield of tetraols from **DE-1** was assumed to be 100% from hydrolysis of **DE-1** at pH 4 in the absence of polynucleotide.

The above procedures were repeated for reaction of **DE-1** in 2.0 mM Poly(A) solutions at pH 5.72 and 6.98, 2.0 mM Poly(G) solutions at pH 5.73 and 7.00, and 1.0 mM DNA solutions at pH 5.70 and 7.00. Reactions were allowed to proceed for ca. 5–7 half-lives, aliquots of standards were added, and the solutions were analyzed directly by HPLC. Naphthaleneethanol was used as standard for the Poly(A) and Poly(G) reactions, and cinnamyl alcohol was used as standard for the DNA reactions. The yields of tetraols were determined by comparing the areas of their HPLC peaks with the areas of the peaks due to the standard compounds added. The results are summarized in Table II.

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