Change of Rate Limiting Step in General Acid-Catalyzed Benzo[*a*]pyrene Diol Epoxide Hydrolysis

Bin Lin,[†] Nafisa Islam,[†] Steve Friedman,[†] H. Yagi,[‡] Donald M. Jerina,[‡] and Dale L. Whalen*,[†]

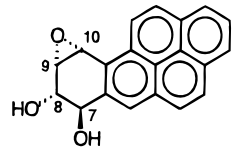
Contribution from the Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore, Maryland 21250, and Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892

Received August 25, 1997. Revised Manuscript Received February 10, 1998

Abstract: The rates of reaction of (±)-7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (**1**) in 1:9 dioxane−water buffer solutions containing primary amines whose pK_a values span the range of 5.4−10.7 have been determined. For those amines with pK_a values < ca. 8, only the acid form (RNH_3^+) gives rise to a kinetic term for reaction with **1**. The rate-limiting step of this reaction is general acid-catalyzed epoxide ring opening to yield a discrete α-hydroxycarbocation, followed by a fast reaction of this intermediate with solvent. The intermediate α-hydroxycarbocation is sufficiently stable so that its reactions with external nucleophiles and bases compete with its reaction with solvent. For those amines with pK_a > ca. 8, both acid and base forms of the buffer react with **1**. The magnitude of the kinetic term in the base form of the amine (RNH_2) increases with amine pK_a and is attributed to nucleophilic addition of the amine to the epoxide group. Curvatures in plots of the kinetic term due to buffer (k_{buff}) as a function of the mole fraction of buffer acid for substituted ammonium ions with pK_a > 8 are interpreted in terms of a change in rate-limiting step of the general acid-catalyzed pathway from epoxide ring opening at low amine base concentrations to reaction of amine base acting as either a general base or nucleophile with an α-hydroxycarbocation at higher amine base concentrations. *Thus, epoxide ring opening of **1** in buffer solutions of the more basic amines is a reversible reaction*. Rate and product studies of the reaction of **1** in acid solutions (pH 5.5) and in Tris buffer solutions containing sodium azide show that azide ion is effective in trapping the α-hydroxycarbocation intermediate, subsequent to its rate-limiting formation by reaction of **1** with either H^+ or Tris-H^+. These results demonstrate that the intermediate formed from epoxide ring opening of **1** with acids has a sufficient lifetime so that its reaction with azide ion competes with its reaction with solvent.

Introduction

Metabolism of the environmental carcinogen, benzo[*a*]pyrene, leads in part to a mixture of diastereomeric bay-region diol epoxides, one with the benzylic hydroxyl group cis to the epoxide group and the second with the benzylic hydroxyl group trans to the epoxide (**1**, a "**DE-2**" stereoisomer).[1] These diol epoxides react with DNA, proteins, and other biomolecules, and it is thought that **1** is the metabolite primarily responsible for the carcinogenic activity of benzo[*a*]pyrene.[2] A recent study implicates **1** as a reagent responsible for causing mutations in human lung tissue via covalent modification of guanine residues at mutational hotspots in the *p53* gene.[3] Covalent binding of (+)-**1** to DNA occurs primarily through bond formation between C-10 of **1** and the exocyclic amino group of guanine residues.[4] Thus, a clear understanding of the factors that control the reactions of epoxide metabolites with amino functional groups is particularly important.



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Diol epoxide **1** reacts readily with nucleophiles and acidic reagents. In water solutions, it hydrolyzes to tetrols by hydronium ion-catalyzed[5−7] and spontaneous[6] mechanisms. In addition, the hydrolysis of **1** exhibits pronounced general acid

[†] University of Maryland, Baltimore County.

[‡] National Institutes of Health.

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S0002-7863(97)02982-X CCC: $15.00
Published on Web 04/28/1998

catalysis.[7,8] Functional groups that act as general acids may play an important role in the reactions of epoxides with certain biological macromolecules. In order to better understand the mechanisms of reactions of diol epoxides with amino groups, we have examined the hydrolysis of **1** in buffer solutions of amines with widely varying pK_a values. Rate and product studies provide evidence for a change in rate-limiting step from epoxide ring opening by substituted ammonium ions with low pK_a values to reaction of the base form of the buffer (free amine), acting as a general base or a nucleophile, on an intermediate α-hydroxycarbocation when the pK_a of the amine acid becomes sufficiently high. Only amines whose conjugate acids possess pK_a values > 8 are effective as nucleophiles in epoxide ring opening.

Experimental Section

Materials. (±)-7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (**1**) was prepared by published procedures.[9−11] Dioxane was distilled from sodium prior to use. Amines used to prepare buffer solutions were taken from freshly opened bottles of commercially available amines and were distilled if any discoloration was present. All other reagents were purchased from commercial sources.

Kinetic Procedures. For each kinetic run, approximately 5 μL of a stock solution of **1** in dioxane (ca. 1 mg/mL) was added to 2.0 mL of reaction solution in the thermostated cell compartment (25.0 ± 0.1 °C) of a UV−vis spectrophotometer. Reactions were monitored at 348 nm, and pseudo-first-order rate constants were calculated by nonlinear regression analysis of the absorbance versus time data. Values of k_{obsd} for reaction of **1** in the pH range of 4.5−11.0 in 1:9 dioxane−water solutions (μ = 0.2, $NaClO_4$) were fit to the equation $k_{obds} = k_H[H]^+ + k_o$ to yield values of 1.83 ± 0.15 × 10^3 M^{-1} s^{-1} and 1.03 ± 0.04 × 10^{-4} s^{-1} for k_H and k_o, respectively.[12] For determination of k_H and k_o, approximately 2 × 10^{-3} M MES (2-[*N*-morpholino]ethanesulfonic acid), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), and CHES (2-[*N*-cyclohexylamino]ethanesulfonic acid) buffers were used for pH control for those reaction solutions with pH 5−10. The contribution of the buffer at this concentration to k_{obsd} is small relative to k_o.

Product Studies. Aliquots of 10.0 μL of **1** in dioxane (1 mg/mL) were added to vials containing 2.0 mL of 1:9 dioxane−water solutions at pH 5.5 (μ = 0.2, $NaClO_4$) containing sodium azide in concentrations up to 0.015 M. The vials were capped, shaken, and allowed to stand at room temperature for ca. 20 min (8−10 half-lives). An aliquot of 10.0 μL of 2-(1-naphthyl)ethanol in dioxane (4.1 mg/mL) was then added to each vial to serve an HPLC standard, and the solution was immediately analyzed by HPLC on a reverse phase C_{18} column, with 60% methanol−40% water as the eluting solvent. Products were monitored by UV detection at 254 nm. The retention times for the trans tetrol, cis tetrol, and 2-(1-naphthyl)ethanol standard were 6.5, 8.3, and 10.8 min, respectively, and the retention times for the trans and cis azides were 16.8 and 15.2 min, respectively.[13] The HPLC retention time and UV spectra of the azide product assigned the trans stereochemistry were identical to those of the *trans*-azidohydrin prepared by the method of Lakshman et al.[14] Yields of tetrols were determined by comparing the areas of the tetrol peaks with that of the standard. The yield of azides was assumed to be the difference between the percent yield of tetrols in sodium azide solution compared to that in the absence of sodium azide. The ratio of trans:cis tetrols from the acid-catalyzed hydrolysis of **1** in the absence of sodium azide at pH 5.5 was determined by HPLC to be 94:6.[12]

Product studies for the reaction of **1** in Tris/Tris-$HClO_4$ buffer solutions in 1:9 dioxane−water solutions (0.2 M total buffer concentration, pH 8.34) containing varying concentrations of sodium azide were carried out as outlined in the proceeding paragraph for reaction of **1** at pH 5.5 in solutions of sodium azide, except that the reaction solutions were allowed to stand for 30−60 min (8−10 half-lives) before the standard was added. The pH of these reaction solutions was adjusted to between 5 and 7 before they were analyzed by HPLC. The ratio of trans:cis tetrols from the spontaneous reaction of **1** at pH 8.1 in the absence of sodium azide was determined by HPLC to be 55:45.[12]

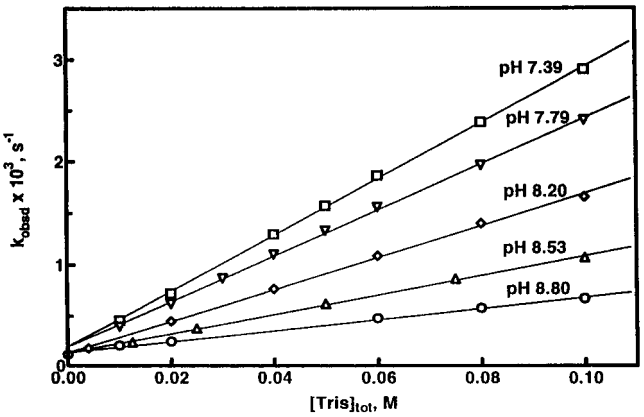

Figure 1. Plots of k_{obsd} reaction of **1** versus total concentration of Tris buffer in 1:9 dioxane−water (v/v) solutions, μ = 0.2 ($NaClO_4$), 25.0 ± 0.1 °C.

Results and Discussion

Reaction of 1 with Amines. We have determined the rates of reaction of **1** in 1:9 dioxane−water solutions of primary amines whose pK_a values span the range of 5.4−10.7. The first-order rate constants (k_{obsd}) for reactions of **1** increase significantly with increase of buffer concentration at constant pH. For those amines with pK_a < ca. 8, rate data for a given buffer plot were fit to eq 1, where k_H, k_o, and k_{buff} are the specific rate constants for the hydronium ion-catalyzed, spontaneous, and buffer-catalyzed reactions, respectively. Figure 1 shows kinetic data for the reaction of 1 in Tris (tris-hydroxymethylaminomethane) buffer solutions at several pH values.

$$k_{obsd} = (k_H[H^+] + k_o) + k_{buff}[Buffer]_{tot} \qquad (1)$$

The slope (k_{buff}) of any given buffer plot at constant pH that complies with eq 1 is a weighted sum of the kinetic terms due to acid and base forms of the buffer (slope = $k_{buff} = f_A k_A + f_B k_B$), where f_A is the mole fraction of acid form and f_B is the mole fraction of base form. A plot of k_{buff} versus the mole fraction of the base form of the buffer (f_B) will be linear. Extrapolation of the plot to a mole fraction of unity for the acid form of the buffer (f_B = 0) yields k_A, and extrapolation of the plot to a mole fraction of unity for the base form of the buffer (f_B = 1) yields k_B. The plot of k_{buff} for reaction of **1** versus the mole fraction of base form of Tris buffer (f_B) is provided in Figure 2. This plot is linear, with a *Y* intercept at f_B = 0 equal to k_A and a *Y* intercept at f_B = 1 equal to zero within experimental uncertainty. Thus, for the reaction of **1** in Tris buffer solutions, only the acid form of Tris is found to contribute a significant kinetic term to the rate expression. For the other

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(12) Rate and product studies of the reaction of **1** by acid-catalyzed and spontaneous pathways in 1:9 dioxane−water solutions, μ = 0.1 ($NaClO_4$) have been reported.[6]

(13) The *cis* and *trans* prefixes for tetrols **3** and azidohydrins **6** refer to the relative stereochemistry of the C-9 and C-10 groups.

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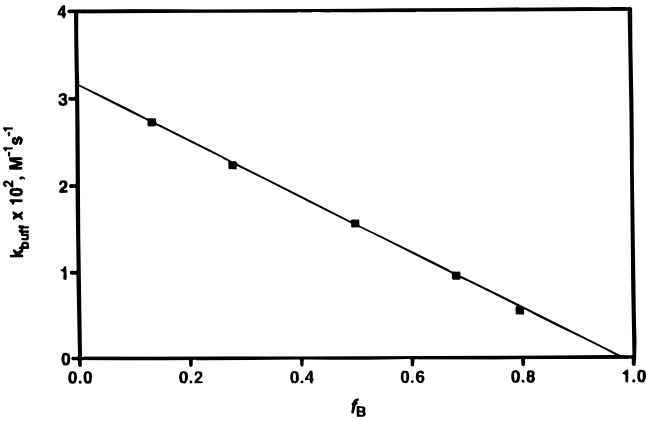


Figure 2. Plots of k_{buff} for reaction of **1** in Tris buffer solutions as functions of the mole fraction of the base form of the amine (f_B), 1:9 dioxane−water (v/v) solutions, μ = 0.2 ($NaClO_4$), 25.0 ± 0.1 °C.

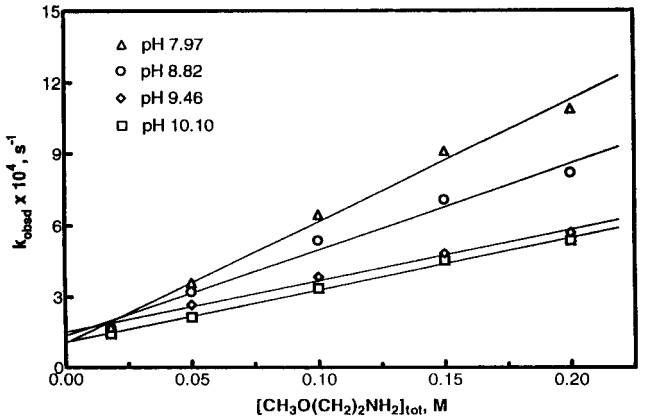


Figure 3. Plots of k_{obsd} for reaction of **1** versus total concentration of 2-methoxyethylamine buffer in 1:9 dioxane−water (v/v) solutions, μ = 0.2 ($NaClO_4$), 25.0 ± 0.1 °C.

amines with pK_a < 8 studied, the acid form of the buffer, but not the base form, reacts with **1**.

For amines with pK_a values greater than that of Tris, some of the buffer plots of k_{obsd} for reaction of **1** versus $[buffer]_{tot}$ appear to be slightly curved. Figure 3 shows kinetic data for the reaction of **1** in methoxyethylamine solutions at several pH values. The amount of curvature that is present in several of the plots is very slight, however, and might easily be attributed to nonrandom experimental error. Linear least-squares correlation lines are arbitrarily drawn through data for each buffer plot. What is clear from these plots and similar ones for reaction of **1** with other amines with pK_a values greater than that of Tris is that the kinetic term in the base form of the amine becomes significant. For example, the rate constants for reaction of the base forms of 4-hydroxybutylamine and propylamine with **1** are larger than the rate constants for reaction of the corresponding acid forms with **1**.

In an effort to obtain rough estimates of the rate constants for reaction of **1** with the acid and base forms of the more basic amines, we analyzed the plots of k_{buff}, the slopes of plots such as those in Figure 3, versus the mole fraction of the base form of the buffer. Plots of k_{buff} for reaction of methoxyethylamine, methoxypropylamine, and hydroxybutylamine with **1**, which are provided in Figure 4, deviate substantially from linearity. This observation and the apparent nonlinearity of buffer plots in Figure 3 indicate that the rate expression given in eq 1 for reaction of **1** in solutions containing amines with pK_a > ca. 9 does not hold.

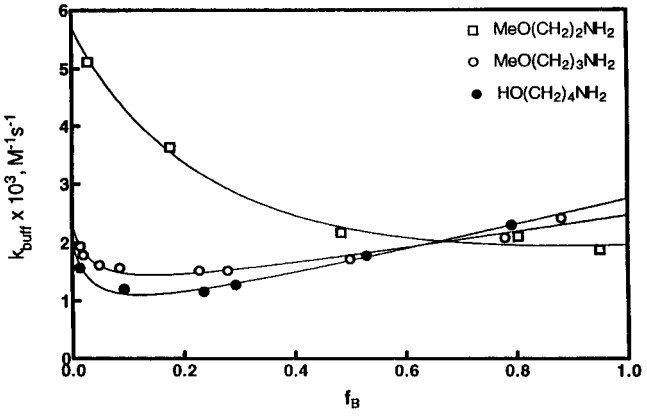


Figure 4. Plots of k_{buff} for reaction of **1** in amine buffer solutions as functions of the mole fraction of the base form of the amine (f_B), 1:9 dioxane−water (v/v) solutions, μ = 0.2 ($NaClO_4$), 25.0 ± 0.1 °C.

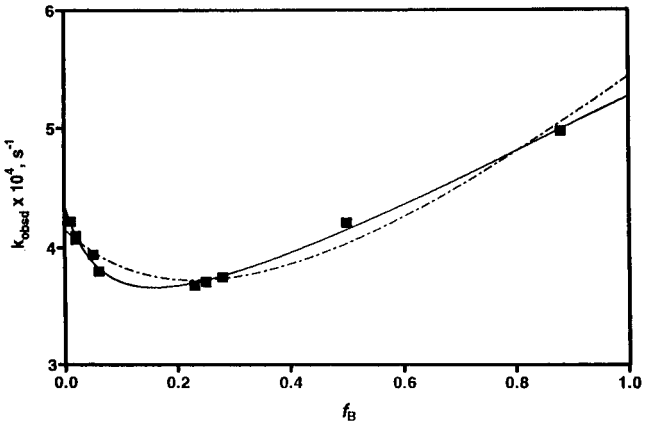
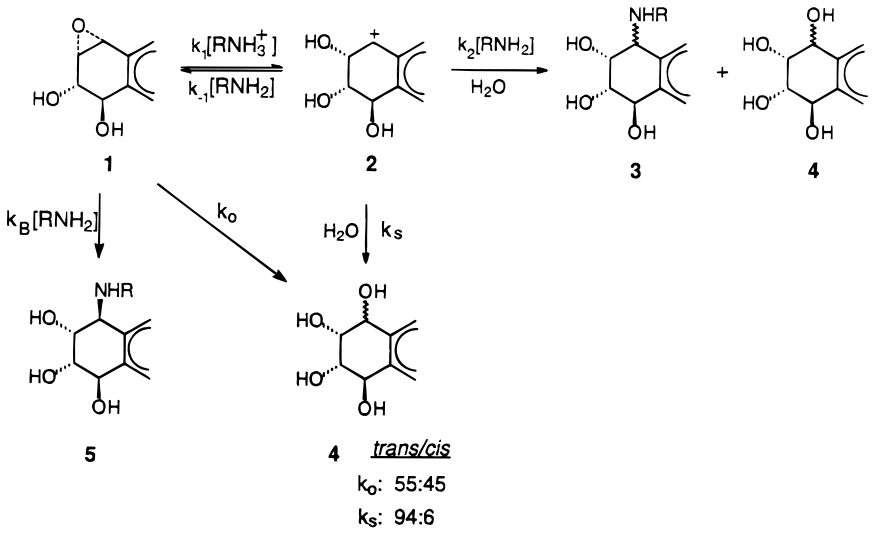


Figure 5. Plot of k_{obsd} for reaction of **1** in solutions of methoxypropylamine at constant 0.15 M concentration as a function of the mole fraction of the base form of the amine (f_B), 1:9 dioxane−water (v/v) solutions, μ = 0.2 ($NaClO_4$), 25.0 ± 0.1 °C. The solid line is theoretical, based on eq 5 and best-fit parameters $k_1 = 2.2 \pm 0.04 \times 10^{-3}$ M^{-1} s^{-1}, $k_B = 2.9 \pm 0.04 \times 10^{-3}$ M^{-1} s^{-1}, $k_2/k_s = 40 \pm 12$ M^{-1}, $k_{-1}/k_s = 34 \pm 7$ M^{-1}. The dotted line is also theoretical, based on eq 5 with the assumption that $k_2/k_s = 0$. In this case, computer fit of the data yielded values of $k_1 = 2.1 \pm 0.04 \times 10^{-3}$ M^{-1} s^{-1}, $k_B = 3.0 \pm 0.08 \times 10^{-3}$ M^{-1} s^{-1} and $k_{-1}/k_s = 12 \pm 2$ M^{-1}.

To facilitate the quantitative analysis of rate data for reaction of **1**, we have determined the rates of reaction of **1** in solutions of methoxypropylamine in which the total concentration of amine is constant, but the mole fraction of the amine in its base form is varied. A plot of k_{obsd} for reaction of **1** versus f_B for methoxypropylamine should also be linear if the rate expression for reaction of **1** conforms to eq 1. However, this plot, shown in Figure 5, is also clearly nonlinear. There is a substantial negative deviation of k_{obsd} from a straight line connecting the intercepts $f_B = 0$ and $f_B = 1$.

The nonlinearity of the plots shown in Figures 3−5 are not consistent with a mechanism in which the epoxide ring opening reactions of **1** induced by both acid and base forms of the buffer are irreversible. For this mechanism, the rate expression of eq 1 should hold. However, the nonlinearity of these plots is consistent with the mechanism outlined in Scheme 1, in which the rate-limiting step for the general acid-catalyzed reaction of **1** changes from epoxide ring opening to form carbocation **2** in solutions containing mostly the acid form of the buffer (RNH_3^+) to partially rate-limiting reaction of **2** with the base form of the buffer (RNH_2) in solutions containing higher concentrations of unprotonated amine. The reaction of RNH_2 with α-hydroxy-

Scheme 1



carbocation **2** to form ring-opened products can occur with direct attack of amine at the electron-deficient C-10 carbon to yield amine product **3** or via general base-catalyzed addition of water to C-10 to yield tetrol product 4.[15] The kinetic term for the reaction of **1** with the base form of the amine (k_B) is presumably nucleophilic attack of the amine on the epoxide group, since this term does not exist for Tris and other amines with pK_a < 8, but becomes significant for the more basic amines and increases with their base strength.

The rate expression for reaction of **1** by the mechanism outlined in Scheme 1, with the assumption that α-hydroxycarbocation **2** is a steady-state intermediate, is given by eq 2.[16] This rate expression holds for reaction of **1** at pH > ca. 8, where the kinetic term due to the hydronium ion-catalyzed reaction is much smaller than that due to the spontaneous reaction. In this

$$k_{obsd} = k_o + k_B f_B[\text{buffer}]_{tot} + \frac{k_1(1 - f_B)[\text{buffer}]_{tot}(k_s + k_2 f_B[\text{buffer}]_{tot})}{k_s + (k_{-1} + k_2)f_B[\text{buffer}]_{tot}} \quad (2)$$

expression, k_o is the first-order rate constant for the spontaneous reaction, k_B is the second-order rate constant for the reaction of the base form of the amine with **1**, f_B is the mole fraction of the amine in its base form, k_1 is the second-order rate constant for reaction of the acid form of the amine with **1** to yield α-hydroxycarbocation intermediate **2**, k_{-1} is the second-order rate constant for the reversal of this step, k_s is the first-order rate constant for reaction of **2** with solvent, and k_2 is the second-order rate constant for reaction of **2** with the base form of the amine, in which the amine acts as a general base and/or as a nucleophile. This equation predicts a nonlinear dependence of k_{obsd} on $[\text{buffer}]_{tot}$ and would account for the nonlinear plots of Figures 3−5.

Combining eqs 1 and 2 yields eq 3, in which k_{buff} represents that portion of the observed rate constant (eq 1) that is due to buffer catalysis. When the buffer is predominantly in the conjugate acid form ($f_B \rightarrow 0$), eq 3 reduces to $k_{buff} = k_1$. Under

$$k_{buff} = f_B k_B + (1 - f_B)k_1\left[\frac{(k_s + f_B k_2[\text{buffer}]_{tot})}{k_s + f_B(k_{-1} + k_2)[\text{buffer}]_{tot}}\right] \quad (3)$$

these conditions, the rate-determining step for the buffer-catalyzed process is ring opening, since the carbocation proceeds to products (via k_s) faster than it returns to starting material via the base-catalyzed process (k_{-1}). As the proportion of the conjugate base is increased, both base-catalyzed return of **2** to reactants (k_{-1}) and cation trapping (k_2) become kinetically significant relative to k_s. Thus, k_2 becomes the dominant pathway for conversion of the carbocation to products (e.g., $k_2 f_B[\text{buffer}]_{tot} > k_s$) and eq 3 reduces to eq 4. This equation provides

$$k_{buff} = [k_B - k_1k_2/(k_{-1} + k_2)]f_B + k_1k_2/(k_{-1} + k_2) \quad (4)$$

a linear dependence of k_{buff} on f_B, with *Y* intercepts of k_B when $f_B = 1$ and $k_1k_2/(k_{-1} + k_2)$ when $f_B = 0$. The plots of k_{buff} for reaction of **1** versus f_B for methoxypropylamine and hydroxybutylamine solutions in Figure 4 become linear at $f_B > 0.2-0.4$, consistent with this interpretation. The negative deviations of k_{buff} in Figure 4 and k_{obsd} in Figure 5 from a straight line connecting the intercepts $f_B = 0$ and $f_B = 1$ are therefore attributed to a change in the rate-limiting step of the buffer acid-dependent component from rate-determining epoxide ring opening (k_1) to partially rate-determining carbocation trapping [$k_1k_2/(k_{-1} + k_2)$] as the fraction of conjugate base in increased.

Values of k_1 for reaction of **1** in buffer solutions of the more basic amines were evaluated by several approaches. For propylamine, the slope of k_{obsd} versus [buffer] in solutions containing a very high $[RNH_3^+]/[RNH_2]$ ratio (>100) was determined. For methoxyethylamine, methoxypropylamine, and hydroxybutylamine, values of k_{obsd} were measured in solutions in which the total buffer concentration ($[\text{buffer}]_{tot}$) was held constant, but the mole fraction of the base form of the buffer was varied. Revision of eq 2 by dividing both numerator and denominator of the third term of the rate expression by k_s yields eq 5. Nonlinear fitting of the data of Figure 5 to eq 5 yields values of k_B, k_1, k_{-1}/k_s, and k_2/k_s. Table 1 summarizes kinetic parameters for reaction of **1** with various amines of differing pK_a values.

Our rate date for reaction of **1** in amine buffers provide reasonably well-defined values for k_1 and k_B but do not provide

(15) Facile general base-catalyzed addition of solvent to a number of carbocations has been established: (a) Ritchie, C. D.; Virtanen, P. O. I. *J. Am. Chem. Soc.* **1972**, *94*, 4966. (b) Ritchie, C. D. *Acc. Chem. Res.* **1972**, *5*, 348. (c) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1396.

(16) For this mechanism it is assumed that the spontaneous reaction of **1** does not proceed to a significant extent via the α-hydroxycarbocation intermediate **2**, although this reaction pathway cannot be ruled out as a minor component of k_o.

Table 1. Values of k_1 and k_B for Reaction of **DE-2** with Substituted Ammonium Ions in 1:9 Dioxane−Water Solutions (μ = 0.2 ($NaClO_4$) at 25 °C)

acid	pK_a[a]	k_1 (M^{-1} s^{-1})[b]	k_B (M^{-1} s^{-1})
$NCCH_2NH_3^+$	5.44	3.0×10^{-1}	
$CF_3CH_2NH_3^+$	5.77	2.3×10^{-1}	
$EtO_2CCH_2NH_3^+$	7.82	4.5×10^{-2}	
$(HOCH_2)_3CNH_3^+$	8.20	3.2×10^{-2}	
$CH_3O(CH_2)_2NH_3^+$	9.49	7.4×10^{-3}	2.0×10^{-3}
$CH_3O(CH_2)_3NH_3^+$	10.11	2.5×10^{-3}	2.5×10^{-3}
$HO(CH_2)_4NH_3^+$	10.38	2.2×10^{-3}	2.9×10^{-3}
$CH_3(CH_2)_2NH_3^+$	10.66	1.4×10^{-3}	3.6×10^{-3}

[a] Determined by titration in 1:9 dioxane−water, μ = 0.2 (KCl or $NaClO_4$). [b] Values of k_1 for those acids with pK_a < 9 correspond to values of k_A.

$$k_{obsd} = k_o + k_B f_B[\text{buffer}]_{tot} + \frac{k_1(1 - f_B)\left(1 + \frac{k_2}{k_s} f_B[\text{buffer}]_{tot}\right)[\text{buffer}]_{tot}}{1 + \left(\frac{k_{-1}}{k_s} + \frac{k_2}{k_s}\right) f_B[\text{buffer}]_{tot}} \quad (5)$$

well-defined values for the rate ratios k_{-1}/k_s and k_2/k_s for reaction of **1** in methoxyethylamine, hydroxybutylamine, and propylamine buffers. Values of k_{-1}/k_s and k_2/k_s for reaction of **1** in methoxypropylamine solutions are provided in the legend of Figure 5. Values of k_{-1}/k_2 for reaction of **1** with those amines with pK_a > 9 are estimated to be close to unity, and therefore, the change in rate-limiting step that occurs with increase in the concentration of the more basic amines is not complete. The marked curvatures of the plots given in Figures 4 and 5 require that the base form of the amine reacts with **2** to reform epoxide at a rate comparable to or greater than that at which **2** reacts with solvent, thus establishing the reversibility of the epoxide ring-opening step.[17] Curvature in these plots would also be present if the ring-opening step is reversible and the reaction of **2** with the base form of the amine to form ring-opened products is negligible ($k_2[RNH_2] \ll k_s$); however, a considerably worse fit of the data (dotted line in Figure 5) results if this assumption is made.

Trapping of Carbocation 2 with Azide. The mechanism of Scheme 1 involves the intermediacy of α-hydroxycarbocation **2**, with a sufficient lifetime in water solutions to allow it to be "trapped" by the base form of the amine in a second-order reaction. In order to gain some insight on the relative stability of **2**, we have carried out rate and product studies of reactions of **1** in solutions containing sodium azide. Azide ion is a very effective nucleophile in capturing carbocations[18] and also reacts with **1** by nucleophilic addition to the benzyl C-10 carbon to yield *trans*-azidohydrin **6a**.[14] Scheme 2 provides a mechanism for the reaction of **1** in aqueous solutions containing azide ion.

From plots of k_{obsd} for reaction of **1** versus [N_3^-] at pH 5.5 and at pH 8.2, a value of the bimolecular rate constant k_N was determined to be 9.3×10^{-2} M^{-1} s^{-1}. For reaction of **1** at pH 8.1 in 0.1 M NaN_3, $k_N[N_3^-] \gg (k_H[H^+] + k_o)$ and a single azide product assigned the trans structure **6a** is detected by HPLC. For reaction of **1** at pH 4.7 in 0.04 M total azide concentration, $k_H[H^+] \gg k_N[N_3^-]$ and two azide products are formed in a 1:4 ratio. The major azide product has the same retention time as

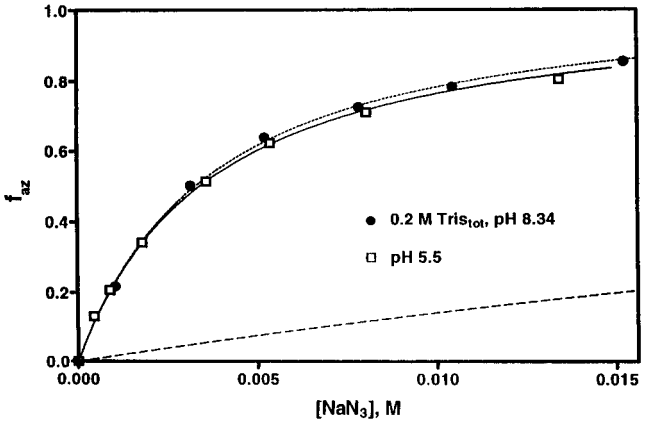
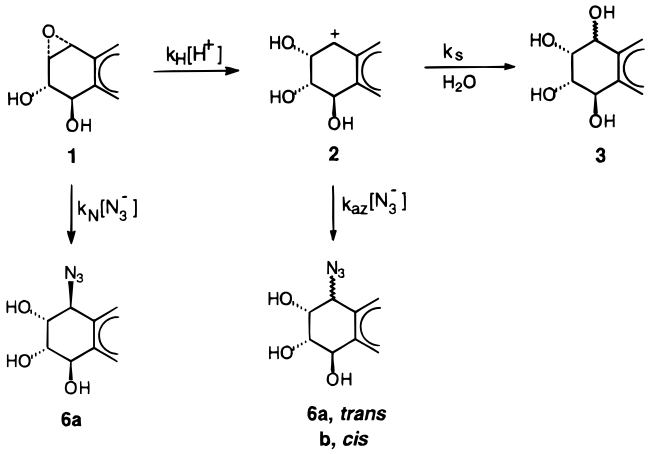


Figure 6. Plots of yield of azide products (f_{az}) from reaction of **1** as a function of sodium azide concentration in 1:9 dioxane−water (v/v) solutions, μ = 0.2 ($NaClO_4$), 25.0 ± 0.1 °C. Fitting of the data for reaction of **1** at pH 5.50 to eq 6 with values of k_H and k_N determined independently by kinetic measurements and fixed at 1.1×10^4 s^{-1} and 9.3×10^{-2} M^{-1} s^{-1} yielded a value for k_{az}/k_s of $2.6 \pm 0.1 \times 10^2$ M^{-1}. Fitting of the data for reaction of **1** at pH 8.34 in 0.2 M Tris$_{tot}$ to eq 6, with $k_H[H^+]$ replaced by k_{Tris}[TrisH$^+$] and fixed at 2.76×10^{-3} s^{-1}, yielded a value for k_{az}/k_s of $2.5 \pm 0.1 \times 10^2$ M^{-1}. The dotted line is a plot of the yield of azide product calculated on the assumption that it is derived only from the second order reaction of N_3^- with **1** at pH 5.50.

Scheme 2



that of the *trans*-azidohydrin **6a**. The minor azide product was assigned the cis stereochemistry. The formation of a mixture of azidohydrin products from reaction of **1** at pH 4.7 in solutions containing N_3^- is consistent with a mechanism in which N_3^- reacts with carbocation **2** from both faces of the carbocation via the k_{az} route (Scheme 2), with trans attack of nucleophile favored over cis attack. Collapse of solvent with **2** gives 94% of trans tetrol and only 6% of cis tetrol, and thus trans attack of solvent on **2** is also preferred. A rationale for this favored trans attack of solvent on **2**, involving axial attack of solvent at the electron-deficient carbon of the more stable conformation, has been proposed.[19]

The yield of azide products from reaction of **1** at pH 5.50 as a function of N_3^- concentrations is provided in Figure 6. The calculated yield of azide product formed from the bimolecular reaction of **1** with N_3^- via the k_N pathway under this condition is given by the dotted line. The observed yield of azide products is much greater than that expected solely from the increase in

(17) A change in rate-limiting step with change in pH and buffer concentrations has been observed in the solvolysis of precocene I oxide: Sayer, J. M.; Grossman, S. J.; Adusei-Poku, K. S.; Jerina, D. M. *J. Am. Chem. Soc.* **1988**, *110*, 5068.

(18) (a) Bunton, C. A.; Huang, S. K. *J. Am. Chem. Soc.* **1972**, *94*, 3536. (b) Ritchie, C. D. *J. Am. Chem. Soc.* **1975**, *97*, 1170.

(19) (a) Gillilan, R. E.; Pohl, T. M.; Whalen, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 4481. (b) Sayer, J. M.; Yagi, H.; Silverton, J. V.; Friedman, S. L.; Whalen, D. L.; Jerina, D. M. *Ibid.* **1982**, *104*, 1972.

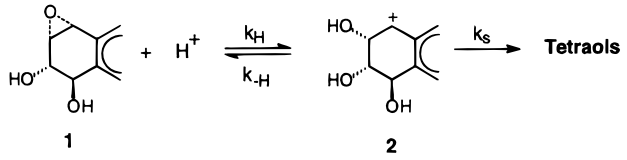
rate of reaction of **1** with increasing $[N_3^-]$, and therefore, N_3^- must be capturing carbocation **2**, subsequent to its rate-limiting formation. Thus, for reaction of **1** at pH 5.5, azide products are formed mostly by capture of **2** by N_3^- (k_{az}) and to a lesser extent by reaction of N_3^- with **1** (k_N). Consistent with this interpretation is the observation that the ratio of azide products is ca. 85:15, and the ratio of the major azide:minor azide products increases slightly with increasing $[N_3^-]$. The mole fraction of azide products formed by both the k_N and k_{az} routes of Scheme 2 is given by eq 6. Nonlinear fitting of the yield of

$$f_{az} = \frac{k_N[N_3^-]}{k_N[N_3^-] + k_H[H^+]} + \left(\frac{k_H[H^+]}{(k_N[N_3^-] + k_H[H^+])}\right)\left(\frac{k_{az}[N_3^-]}{(k_{az}[N_3^-] + k_s)}\right) \quad (6)$$

azide products as a function of $[N_3^-]$ to eq 6, with $k_H = 1.8 \times 10^3$ M^{-1} s^{-1}, $k_N = 9.3 \times 10^{-2}$ M^{-1} s^{-1}, and $[H^+] = 10^{-5.50}$ yields a value of 2.6×10^2 M^{-1} for the ratio k_{az}/k_s. It has been observed that k_{az}/k_s ratios for activation-limited attack of N_3^- on stable carbocations[18] are orders of magnitude greater than that calculated for reaction of **2** with N_3^-. Consequently, it has been argued by Jencks and co-workers that low values of k_{az}/k_s for reaction of certain carbocations indicate that the reaction of N_3^- with the carbocation occurs at the diffusional limit, whereas reaction of the carbocation with solvent (k_s) is activation limited.[20] If it is assumed that azide ion reacts with **2** at the diffusional limit, with a bimolecular rate constant of ca. 5×10^9 M^{-1} s^{-1}, then a value of k_s for reaction of **2** with solvent can be estimated to be ca. 2.0×10^7 s^{-1}.[8,20] These data establish that α-hydroxycarbocation **2** has a sufficient lifetime in water solution to be able to undergo bimolecular reactions with external nucleophiles in competition with its reaction with the solvent.[21]

We have also carried out rate and product studies of the reaction of **1** in Tris buffer solutions at pH 8.2, a pH at which **1** reacts primarily by the spontaneous reaction in the absence of buffer.[6] In 0.1 M Tris-H^+ solution, pH 8.2, the reaction of **1** is >30 times faster than that in the absence of buffer and yields tetrols in a 94:6 (trans:cis) ratio. The trans:cis hydration ratio for reaction of **1** in the absence of buffer under these conditions, where the spontaneous reaction of **1** predominates, is 55:45. The 94:6 trans:cis hydration ratio for reaction of **1** in 0.1 M Tris-H^+ solution is indistinguishable from the hydration ratio of its reaction with hydronium ion[5-7] and suggests that the reaction of **1** with Tris-H^+ yields the same carbocation intermediate (**2**) as that formed from reaction of **1** with H^+. In the reaction of **1** in 0.1 M Tris solutions containing increasing $[NaN_3]$, a decrease in tetrol yields and an increase in the yields of two new products with the same HPLC retention times and relative yields as the azide products from reaction of **1** at pH 5.5 in NaN_3 solutions (Figure 6) is observed. The yield of azide products is much greater than that calculated with the assumption that azide product arises only from the second-order reaction of azide with **1** and is consistent with a mechanism similar to

Scheme 3



1 2

that for reaction of **1** via Scheme 2, with $k_H[H^+]$ replaced by $k_{TrisH}[TrisH^+]$. In this mechanism, k_{-1} and k_2 (reaction of **2** with Tris base, Scheme 1) are considered negligible. Fitting of the yield of azide products from reaction of **1** in Tris solutions to eq 6, with $k_H[H^+]$ replaced by $k_{TrisH}[TrisH^+]$, yields a value for k_{az}/k_s of 2.5×10^2 M^{-1}. This value is within experimental error of that calculated for reaction of **1** at pH 5.5 in azide solutions, and provides support for the proposal that reaction of **1** with $TrisH^+$ and presumably other amine acids yields the same discrete carbocation **2** that is generated from reaction of **1** with H^+.

Reaction of 1 with H^+. Kinetic parameters for the reaction of **1** in amine buffer solutions also provide important information that can be used to estimate the energetics of the reaction of **1** with H^+ to form α-hydroxycarbocation **2**. The magnitude of k_{-1} for reaction of α-hydroxycarbocation **2** with $MeO(CH_2)_3NH_2$ to reform **1** is calculated from the value ca. 34 M^{-1} for k_{-1}/k_s (Figure 3) and 2.0×10^7 s^{-1} for k_s to be ca. 6.8×10^8 M^{-1} s^{-1}. The value of the equilibrium constant (k_1/k_{-1}) for reaction of **1** with $MeO(CH_2)_3NH_3^+$ is therefore estimated to be 3.7×10^{-12}. From this equilibrium constant and K_a for ionization of $MeO(CH_2)_3NH_3^+$, the equilibrium constant $K_H(k_H/k_{-H})$ for reaction of **1** with H^+ to form α-hydroxycarbocation **2** (Scheme 3) is calculated to be 5×10^{-2} M^{-1}. This equilibrium constant cannot be determined directly because of the high reactivity of **2** with solvent.

It is often assumed that the epoxide ring opening step in hydronium ion-catalyzed hydrolysis of epoxides is irreversible. For those epoxides that hydrolyze via carbocation intermediates, this requires that the intermediate reacts with solvent to give ring-opened products faster that it undergoes epoxide ring closure to reform reactant. The acid-catalyzed rate constant k_H for reaction of **1** in 1:9 dioxane−water (μ = 0.2, $NaClO_4$) to yield **2** was measured independently to be 1.8×10^3 M^{-1} s^{-1} (see Experimental Section). From this value of k_H and a value of 5×10^{-2} M^{-1} for K_H, k_{-H} is calculated to be 3.6×10^4 s^{-1}. However, k_s for reaction of **2** with solvent is estimated to be 2.0×10^7 s^{-1}. Therefore, intermediate **2** reacts with solvent via the k_s route approximately 5×10^2 times faster than it reforms epoxide reactant via the k_{-H} pathway.

Brønsted Correlation. In the reactions of **1** with those amine acids possessing pK_a values < ca. 8, epoxide ring opening is always rate-limiting and the kinetic term due to the acid form of the buffer is equal to k_1. For reactions of **1** with ammonium ions having pK_a values > ca. 8, however, epoxide ring opening is reversible under certain conditions, and values of k_1 must be arrived at by fitting of eq 2. A plot of log k_1 versus pK_a for the general acid-catalyzed hydrolysis of **1** by all substituted ammonium ions studied, shown in Figure 7, gives a reasonable correlation with a Brønsted α of 0.45. This value is similar to the Brønsted α of 0.51 observed for the phosphate/phosphonate general acid-catalyzed hydrolysis of **1**, for which a concerted general acid catalysis mechanism was proposed.[8] The epoxide ring-opening reaction of **1** with substituted ammonium ions possessing pK_a values less than that of α-hydroxycarbocation **2**, estimated to be ca. 13,[22] must also be a concerted process.[23]

For reaction of **1** with methoxypropylamine buffer (Figure

(20) Reactions of carbocations with k_{az}/k_s ratios similar to that for **1** have been proposed to occur at the diffusion-controlled limit: (a) Richard, J. P.; Rothenberg, M. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1361. (b) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 4689. (c) Ta-Sha, R.; Rappoport, Z. *Ibid.* **1983**, *105*, 6082.

(21) The rate constant for reaction of α-hydroxycarbocation **2** in 1:9 dioxane−water solvent ($k_s \approx 2.0 \times 10^7$ s^{-1}) is very similar to that calculated for reaction of the α-hydroxycarbocation derived from epoxide ring opening of a "**DE-1**" diol epoxide, isomeric with **1**, in which the C-7 hydroxyl group is cis to the epoxide ($k_s \approx 1.6 \times 10^7$ s^{-1}, ref 8).

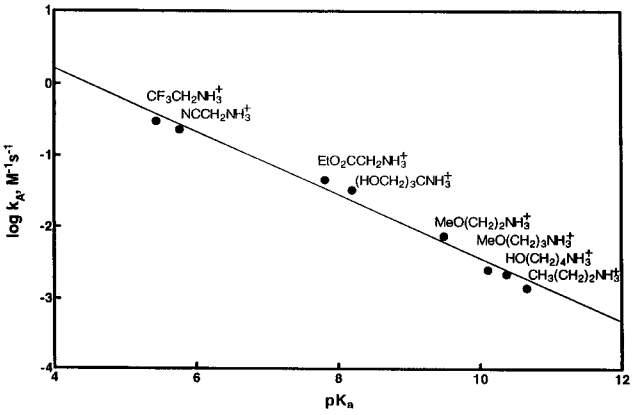


Figure 7. Brønsted plot of log k_1 for general acid-catalyzed hydrolysis of **1** by substituted ammonium ions versus pK_a of the general acid. Data are from values of k_1 listed in Table 1.

5), a value of k_{-1}/k_s is estimated to be ca. 34 M^{-1}. From this value and an estimate of 2×10^7 s^{-1} for k_s, k_{-1} for reaction of **2** with methoxypropylamine to reform **1** is estimated to be ca. 7×10^8 M^{-1} s^{-1}. This rate constant is within approximately an order of magnitude of the diffusional limit for bimolecular reactions in water solution. For reaction of **2** with amines more basic than methoxypropylamine, the bimolecular rate constant should approach the diffusional limit.

Summary

The rate of reaction of benzo[*a*]pyrene diol epoxide **1** in water solutions is enhanced significantly in the presence of amine buffers. In buffer solutions of those amines with pK_a < ca. 8, only the acid form (RNH_3^+) reacts with **1**. The rate-limiting step of this reaction is concerted, general acid-catalyzed epoxide ring opening of **1** to form a discrete α-hydroxycarbocation **2**, which reacts with water in a fast, subsequent step (k_s) to yield tetrol products. For those amines with pK_a values > ca. 8, both acid and base forms of the buffer react with **1**. In the reactions of the less acidic ammonium ions with **1**, the rate-limiting step changes from epoxide ring opening to form carbocation **2** at low amine base concentrations to partially rate-limiting product formation from reaction of **2** at higher base concentrations. *Under this latter condition, the epoxide ring opening step is reversible.* The kinetic data thus far obtained are most consistent with the inclusion of a kinetic term for reaction of amine base with the intermediate α-hydroxycarbocation **2** to form ring-opened products; omitting this term results in a poorer fit of the kinetic data. Thus, the change in rate-limiting step is a consequence of the fact that there are two reaction pathways for **2** to form product, an uncatalyzed pathway involving solvent (k_s) and a second pathway involving amine base (k_2).

The main conclusions that can be drawn from this work are (1) that epoxide ring opening of **1** with acidic reagents yields a discrete α-hydroxycarbocation **2**, with a sufficient lifetime in water solutions (k_s ca. 2×10^7 s^{-1}) to undergo reactions with external nucleophiles, and (2) that the epoxide ring opening reaction of **1** to form α-hydroxycarbocation **2** is reversible in buffer solutions containing strongly basic amines. Thus, sufficiently basic amines react with α-hydroxycarbocation **2** as a base to reform **1** at a rate comparable to or greater than that at which they react with **2** to form ring-opened products. From our product studies, we are not able to conclude whether amines function as general bases to assist in the addition of water (Scheme 2) or as nucleophiles to yield amine products. Product studies necessary for this determination are complicated by the fact that significant yields of amine products are formed from the bimolecular reaction between **1** and the more basic amines.

Acknowledgment. This work was funded in part by a Special Research Initiative Support Award from the UMBC Designated Research Initiative Fund. Helpful discussions with Drs. Ralph Pollack (UMBC), Donald Creighton (UMBC), and Jane Sayer (National Institutes of Health) are greatly appreciated.

Supporting Information Available: Plot of k_{obsd} for reaction of **1** versus [NaN_3] in 0.2 M $Tris_{tot}$ buffer, 1:9 dioxane−water, 25 °C and a derivation of eq 2 (3 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(22) The pK_a for an α-hydroxycarbocation isomeric with **1** is estimated to be ca. 13, ref 8.

(23) For a summary of requirements for concerted general acid catalysis, see: Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 4731.