C. S. Menon, J. F. Engel, J. K. Selkirk, and P. P. Roller, "Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis", R. I. Freudenthal and P. W. Jones, Ed., Raven Press, New York, N.Y., 1976, pp 349–411.
(20) R. Criegee, B. Marchand, and M. Wannowius, *Justus Liebigs Ann. Chem.*, **550**, 99–133 (1942).
(21) R. P. Hanzlik, M. Edelman, W. J. Michaely, and G. Scott, *J. Am. Chem. Soc.*, **98**, 1952–1955 (1976).
(22) Mass spectra of all four unlabeled tetrols exhibit characteristic *m/e* 260 ions. This *m/e* 260 ion is absent in the mass spectra of BP (7/8,9)-triol and (7,9/8)-triol, which exhibited characteristic *m/e* 244 ions (ref 18). These results indicate that the *m/e* 260 ion derived from the tetrols and the *m/e* 244 ions derived from the triols are the results of cleavage of C(7)–C(8) and C(9)–C(10) bonds. This is further supported by the mass spectra of ^{14}C(7)-labeled tetrols and triols which exhibit *m/e* 262 and 246 ions, respectively. Since the mass spectra of all ^{18}O-labeled tetrols exhibit *m/e* 262 ions, the above results thus indicate that the ^{18}O is at the C(10) position in the ^{18}O-labeled tetrols.
(23) D. J. McCaustland, W. P. Duncan, and J. F. Engel, *J. Labelled Compd.*, **12**, 443–448 (1976).
(24) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, N.Y., 1967, pp 955–956.
(25) Similar results were observed with the trans and cis isomers of 9,10-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.
(26) M. E. Evans, F. W. Parrish, and L. Long, Jr., *Carbohydr. Res.*, **3**, 453–462 (1967).
(27) H. Muxfeldt, G. Hardtmann, F. Kathawala, E. Vedejs, and J. B. Mooberry, *J. Am. Chem. Soc.*, **90**, 6534–6536 (1968).
(28) The mass spectra of acetonide derivatives of some carbohydrates were reported by D. C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, **86**, 67–74 (1964).
(29) L. Anderson in "The Carbohydrates, Chemistry and Biochemistry", Vol. IA, 2nd ed, W. Pigman and D. Horton, Ed., Academic Press, New York, N.Y., 1972, pp 519–579.
(30) S. J. Angyal and R. M. Hoskinson, *J. Chem. Soc.*, 2985–2991 (1962).
(31) Unpublished results.
(32) H. Brockmann, Jr., H. Budzikiewicz, C. Djerassi, H. Brockmann, and J. Niemeyer, *Chem. Ber.*, **101**, 1341–1348 (1968).
(33) S. K. Yang, D. W. McCourt, and H. V. Gelboin, *J. Am. Chem. Soc.*, following paper in this issue.
(34) H. W. S. King, M. R. Osborne, F. A. Beland, R. G. Harvey, and P. Brookes, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2679–2681 (1976).
(35) P. B. Hulbert, *Nature (London)*, **256**, 146–148 (1975).
(36) Following the submission of this paper, J. W. Keller, C. Heidelberger, F. A. Beland, and R. G. Harvey (*J. Am. Chem. Soc.*, **98**, 8276–8277 (1976)) reported NMR studies of tetraacetates of the tetrol mixtures derived from I and II. Our data agree in general with those of Keller et al. but disagree on the ratios of the tetrols derived from I and II.

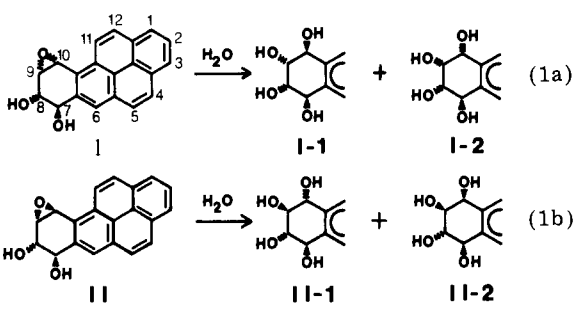
The Mechanism of Hydrolysis of the Non-K-Region Benzo[*a*]pyrene Diol Epoxide *r*-7,*t*-8-Dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene

Shen K. Yang,* David W. McCourt, and Harry V. Gelboin

Contribution from the Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received December 13, 1976*

Abstract: The non-K-region benzo[*a*]pyrene diol epoxide *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, a potent mutagen and possibly the ultimate carcinogenic form of benzo[*a*]pyrene, has been found to undergo specific- and general-acid-catalyzed hydrolysis. The kinetics of hydrolysis was studied in 5% (v/v) aqueous tetrahydrofuran solutions at 25 °C with varying concentrations of buffer and ionic strength. The pH of the solutions was controlled by buffers of which the conjugated acid (HB) bears a negative ($H_2PO_4^-$), neutral (CH_3COOH), or positive ($(CH_2OH)_3CNH_3^+$) charge. The observed first-order rate constants (k_{obsd}) are linearly proportional to the buffer concentrations at constant pH. With increasing ionic strength at constant pH, k_{obsd} increases in Tris buffer, remains relatively constant in acetate buffer, and decreases in phosphate buffer. The observed rate constants can be expressed as $k_{obsd} = K_{eq,H}k_H[H^+] + K_{eq,HB}k_{HB}[HB]$. The value for $K_{eq,H}k_H$ is 1000 s^{-1} M^{-1} and for $K_{eq,HB}k_{HB}$ (s^{-1} M^{-1}) are 1.21, 1.03, and 0.07 for $H_2PO_4^-$, CH_3COOH, and $(CH_2OH)_3CNH_3^+$, respectively. These results indicate that the mechanism of hydrolysis involves a prior equilibrium (with equilibrium constant $K_{eq,H}$ and $K_{eq,HB}$) involving hydrogen bonding of the diol epoxide with an acid followed by a rate-determining proton transfer to form a benzylic carbonium ion intermediate at C(10). The planar carbonium ion intermediate undergoes an S_N1 nucleophilic attack by solvent water to form a (7,10/8,9)-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and a (7/8,9,10)-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in an approximately 3:1 ratio. The relative amounts of the two stereoisomeric tetrahydroxytetrahydrobenzo[*a*]pyrenes (tetrols) were analyzed by high-pressure liquid chromatography and the formation of the major tetrol was found to decrease slightly with increasing pH.

The diol epoxide *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (I) is more highly mutagenic in mammalian cells than 15 other benzo[*a*]pyrene (BP) derivatives including the stereoisomeric diol epoxide *r*-7,*t*-8-dihydroxy-*c*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (II).[1] A single enantiomer of I is formed predominantly from BP via the (−)-*r*-7,*t*-8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP (−)-*trans*-7,8-diol) by the mammalian microsomal mixed-function oxidases[2,3] and it is the major form bound to mammalian cellular DNA and RNA. I and II are each hydrolyzed in aqueous medium to a pair of stereoisomeric tetrahydroxytetrahydrobenzo[*a*]pyrenes (tetrols I-1, I-2, II-1, and II-2) and the stereochemistry of the tetrols has been elucidated.[3,7,8] The finding that I is formed predominantly in the biological system prompted us to carry out a detailed kinetic study of the hydrolysis of I. The results indicate that the hydrolysis is a specific- and general-acid-catalyzed S_N1 reaction and the intermediate is a benzylic carbonium ion at C(10). A carbonium ion intermediate indicates that diol epoxide I can react with cellular macromolecules as an alkylating agent.



(1a)

(1b)

Experimental Section

Materials. Synthetic diol epoxides I and [7-^{14}C]diol epoxide I were obtained through National Cancer Institute Contract NO1-CP-

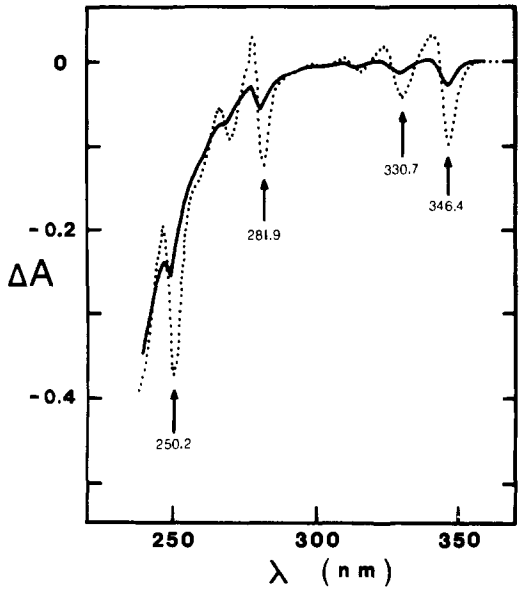


Figure 1. Ultraviolet absorption difference spectra between benzo[*a*]-pyrene diol epoxides I (dotted curve) and II (solid curve) and their hydrolysis products. Arrows and wavelengths indicated are those used in monitoring the absorbance change in the hydrolysis of I.

33387[9,10] and the diol epoxide II by synthesis on NCI Contract NO1-CP-33385.[11] The stereochemistry of the diol epoxides has been elucidated.[11,12] Information on the availability of these compounds can be obtained from the Manager, Information and Resources Segment, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. 20014.

Difference Spectra. Benzo[*a*]pyrene diol epoxides I and II are stable in dry tetrahydrofuran (THF) solution, but are hydrolyzed to tetrols in aqueous THF solutions. Both the ultraviolet absorption spectra of I (or II) and tetrols have characteristics of a pyrene ring and their absorption maxima differ only by approximately 1 nm. However, these minor differences were used to monitor the changes in absorbance during the hydrolysis of either I or II. The monitoring wavelengths were determined with two cuvettes each containing equal amounts (ca. 0.5 μg) of I (or II); the sample cuvette contains I (or II) in THF and the reference cuvette contains completely hydrolyzed I (or II) in 1% aqueous THF. The difference spectra obtained for I and II are shown in Figure 1. The four wavelengths (nm) for absorbance monitoring of the hydrolysis of I are 250.2, 281.9, 330.7, and 346.1, and of II are 248.8, 280.5, 230.0 and 346.1, respectively.

Kinetic Measurements. The changes in absorbances were recorded on a Cary 15 spectrophotometer. Rates of hydrolysis were measured at 25 ± 1 °C by monitoring the decreases in absorbance (Figure 1). The reaction rates were independent of monitoring wavelengths and concentration of BP diol epoxides within the limit of solubility (ca. 5 μg per mL of 5% aqueous THF). All reactions were initiated by adding 5 μg of the diol epoxides in 0.05 mL of THF to 0.95 mL of aqueous buffer in a cuvette of 1 cm light path length. Absorbance recording was started at the time of adding the THF solution of the diol epoxide into the aqueous buffer. The aqueous THF solution was immediately and thoroughly mixed by inverting the cuvette several times by hand. The mixing process is achieved in about 10 s. Observed rate constants were derived from plots of log $(A_t - A_\infty)$ vs. time. The semilogarithmic analysis of diol epoxides I and II in 1 mL of solution containing 5% (v/v) THF and 95% 0.1 M Tris HCl, pH 7.4 is shown in Figure 2. The observed absorbance vs. time curves for diol epoxide I were strictly first order. THF concentration dependent measurements indicate that the observed rate constants decrease linearly with increasing concentration of tetrahydrofuran from 2.5 to 10% (v/v). Two first-order rate constants were observed in the hydrolysis of diol epoxide II; the fast and the slow kinetic effects constitute 91 and 9% of the absorbance change, respectively.

Quantitation of Tetrols I-1 and I-2. Tetrols I-1 and I-2 were separated by high-pressure liquid chromatography.[1,2,7] The relative amount of tetrols produced by [7-^{14}C]diol epoxide I[10] at the end of hydrolysis was separated by HPLC and determined by liquid scintillation counting.

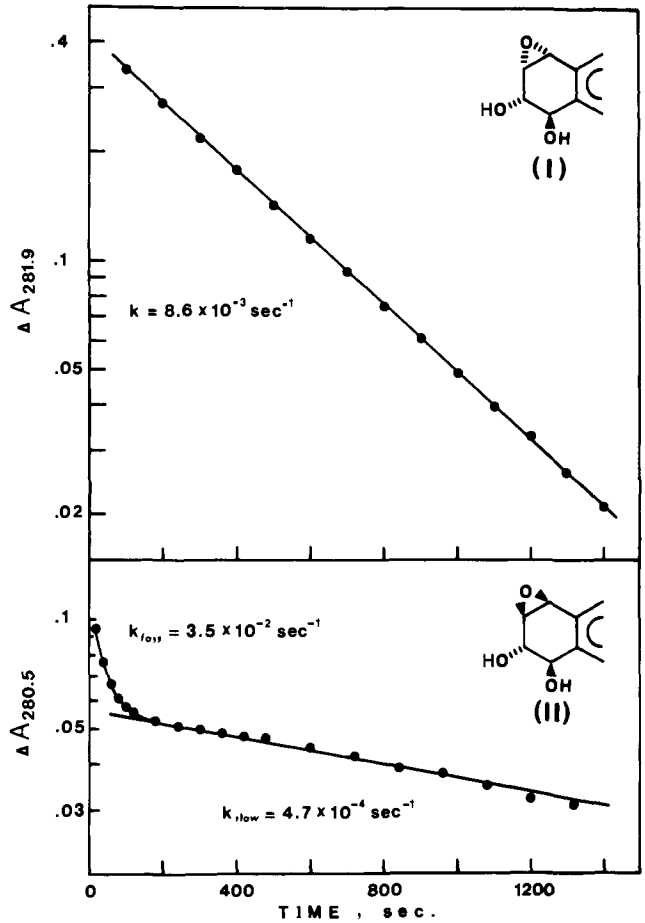


Figure 2. Semilogarithmic analysis of I and II in 5% (v/v) THF and 95% 0.1 M Tris HCl buffer, pH 7.4 at 25.0 ± 0.5 °C.

Table I. Values of k_{obsd} in Phosphate and Tris Buffer for Diol Epoxide I[a]

Buffer	k_{obsd} × 10^3, s^{-1}
0.01 M KH_2PO_4	4.5
0.01 M Tris HCl	1.2
0.01 M KH_2PO_4 + 0.01 M Tris HCl	4.3

[a] At 25.5 ± 0.5 °C, μ = 0.1 with KCl, pH 7.4.

Results

The observed first-order rate constants for hydrolysis of I were determined at constant pH in acetate, Tris, and phosphate buffers at 25 ± 1 °C. The k_{obsd} (Figure 3) increases linearly with buffer concentration. Since the ratio [HB]/[B$^-$] is constant at constant pH, the results in Figure 3 indicate that the hydrolysis is a general-acid-catalyzed reaction. The different values of nonzero intercepts at pH 5.0 and 7.4 (Figure 3) at zero buffer concentration suggest that the reaction is also subjected to specific hydrogen ion catalysis.

At the same buffer concentration, the ratio of k_{obsd} in phosphate buffer to k_{obsd} in Tris buffer is found to be 3.8 (Table I). However, the concentration of $H_2PO_4^-$ to that of $(CH_2OH)_3CNH_3^+$ is 7.9 as calculated by the acid dissociation constants (K_{HB}) at constant pH and buffer concentrations. Thus the k_{obsd} is not directly proportional to the K_{HB} values of the acids and the results indicate that $(CH_2OH)_3CNH_3^+$ is a less effective catalyst than $H_2PO_4^-$.

The k_{obsd} for hydrolysis of diol epoxide I was determined over the pH range 4.0–9.0 in acetate, phosphate, and Tris

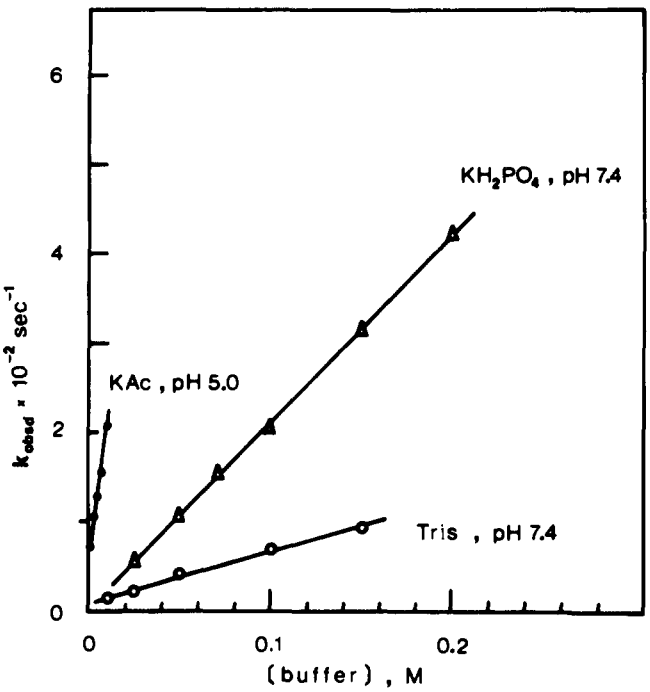


Figure 3. Dependence of k_{obsd} on the buffer concentration at 25 ± 1 °C. All data were obtained with 5% THF-95% buffer (v/v). Acetate buffer (●), pH 5.0, μ = 0.1 with KCl; phosphate buffer (△), pH 7.4, no KCl added; Tris HCl buffer (○), pH 7.4, no KCl added.

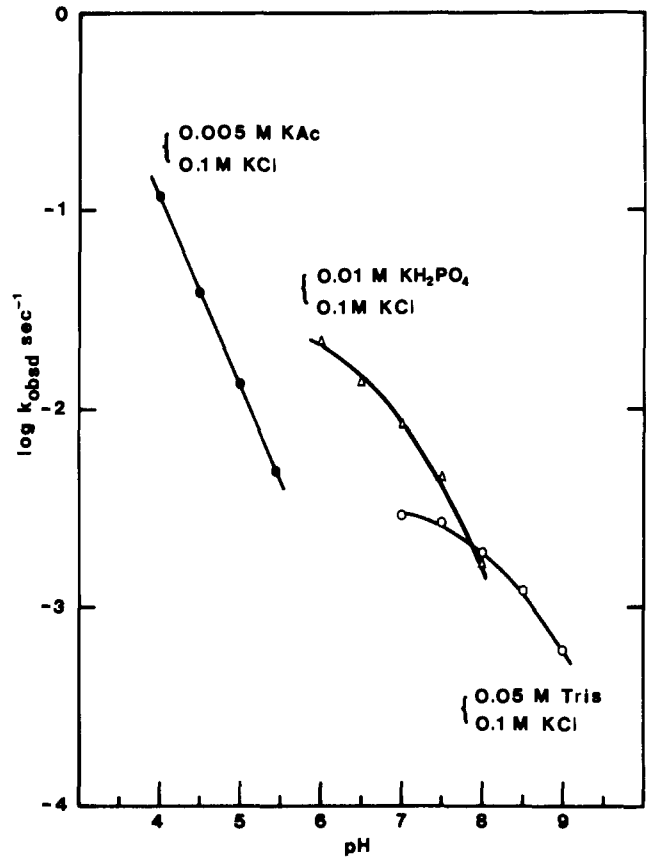


Figure 4. Plots of log k_{obsd} vs. pH for the hydrolysis of I at 25 ± 1 °C. The samples contains 5% THF (v/v) and 95% 0.005 M acetate buffer (●), 0.01 M phosphate buffer (△), and 0.05 M Tris HCl buffer (○), respectively. μ = 0.1 with KCl.

buffers at 25 ± 1 °C and at μ = 0.1 (with KCl). The slope of the log k_{obsd} vs. pH plot in acetate buffer (Figure 4) is −0.95, which indicates that the hydrolysis of I is subjected to both specific and general acid catalysis. This conclusion is further supported by the log k_{obsd} vs. pH plots determined in phosphate and Tris buffers (Figure 4).

A specific- and general-acid-catalyzed reaction can be expressed by either eq 2a or eq 2b with the rate-determining step indicated by rds. The observed first-order rate constants consistent with eq 2a and 2b are expressed by eq 3a and 3b, respectively[13a]

$$\mathrm{I} + \mathrm{HB} \overset{K_{eq,HB}}{\rightleftharpoons} \mathrm{I \cdot HB} \xrightarrow[-\mathrm{B^-},\ \mathrm{rds}]{\mathrm{H_2O},\ -\mathrm{H^+}} \mathrm{I\text{-}1} + \mathrm{I\text{-}2} \qquad (2a)$$

$$\mathrm{I} + \mathrm{HB} \overset{K_{eq,HB}}{\rightleftharpoons} \mathrm{I \cdot HB} \xrightarrow[\mathrm{rds}]{-\mathrm{B^-}} \mathrm{IH^+} \xrightarrow[-\mathrm{H^+}]{\mathrm{H_2O}} \mathrm{I\text{-}1} + \mathrm{I\text{-}2} \qquad (2b)$$

$$k_{obsd} = K_{eq,H}k_H[H^+][H_2O] + K_{eq,HB}k_{HB}[HB][H_2O] \qquad (3a)$$

$$k_{obsd} = K_{eq,H}k_H[H^+] + K_{eq,HB}k_{HB}[HB] \qquad (3b)$$

where k_H and k_{HB} are the rate constants for the rate-determining step catalyzed by hydrogen ion and acid (HB), respectively. $K_{eq,H} = [I{\cdot}H^+]/[I][H^+]$, $K_{eq,HB} = [I{\cdot}HB]/[I][HB]$, and HB = $H_2PO_4^-$, CH_3COOH, or $(CH_2OH)_3CNH_3^+$. $K_{eq,H}$ and $K_{eq,HB}$ are the equilibrium constants. Equation 3a or 3b can be rearranged to eq 4a or 4b by dividing through with $[H^+]$ $[H_2O]$ or with $[H^+]$ and substituting the [HB] by $C[H^+]/(K_{HB} + [H^+])$

$$k'_{obsd}/[H^+] = K_{eq,H}k_H + K_{eq,HB}k_{HB}C/(K_{HB} + [H^+]) \qquad (4a)$$

$$k_{obsd}/[H^+] = K_{eq,H}k_H + K_{eq,HB}k_{HB}C/(K_{HB} + [H^+]) \qquad (4b)$$

where $k'_{obsd} = k_{obsd}/[H_2O]$, $C = [HB] + [B^-]$, and K_{HB} is the acid dissociation constants of the conjugated acid HB. Equation 4a or 4b predict that the data in Figure 4 are straight lines by plotting $k_{obsd}/[H^+]$ (or $k'_{obsd}/[H^+]$) vs. $C/(K_{HB} + [H^+])$ with intercept = $K_{eq,H}k_H$ and slope = $K_{eq,HB}k_{HB}$.

The product analysis of the hydrolysis of I indicated that a benzylic carbonium ion at C(10) was the intermediate.[7] In order to elucidate the rate-determining step in the specific- and general-acid-catalyzed hydrolysis reaction, an ionic strength dependent study was carried out in phosphate, acetate, and Tris buffers whose conjugated acid (HB) bears negative, neutral, and positive charge, respectively.

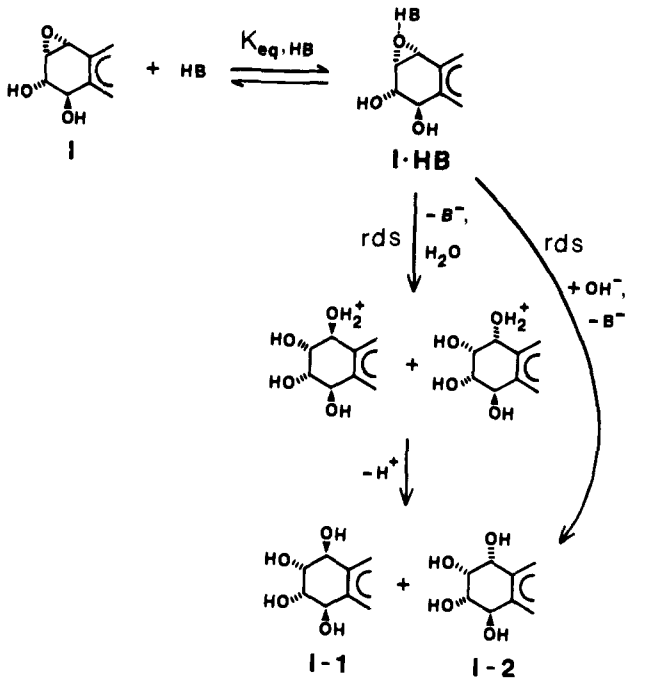
The log k_{obsd} vs. $\sqrt{\mu}$ profiles (Figure 5) indicate that with increasing $\sqrt{\mu}$, log k_{obsd} increases linearly in Tris buffer, decreases linearly in phosphate buffer, and remains relatively constant in acetate buffer. The ionic strength dependence of k_{obsd}[14] thus suggests that the reactant in the rate-determining step is an ionic species.

A reaction expressed by eq 2a implies that the rate-determining step is bimolecular,[13] i.e., the reactants are I·HB and H_2O. According to activated complex theory,[14] this bimolecular reaction is independent of ionic strengths regardless of the charge of I·HB. If I·HB and OH^- are the reactants, with increasing ionic strength the k_{obsd} should increase if I·HB is negatively charged, decrease if I·HB is positively charged, and remain constant if I·HB is uncharged. The ionic strength dependence of k_{obsd} (Figure 5) clearly eliminates the possibility expressed by eq 2a.

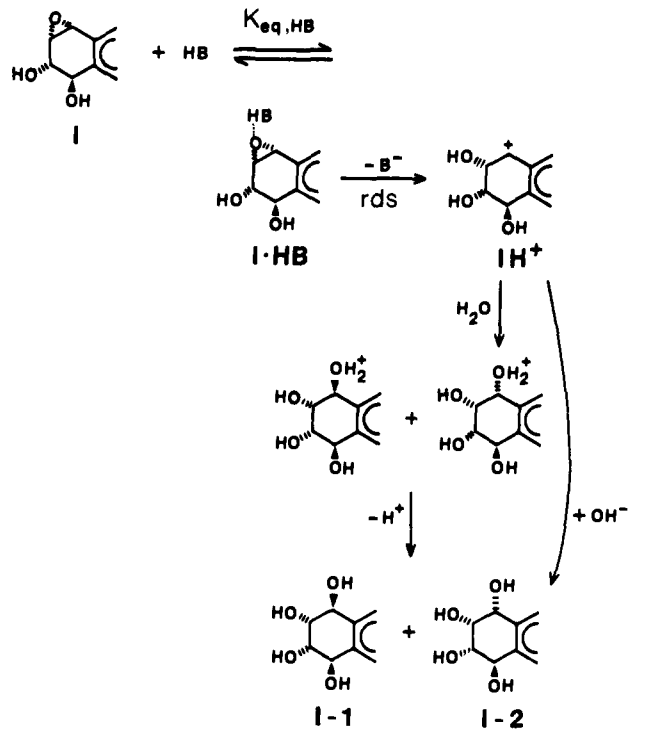
The data in Figure 4 were plotted according to eq 4b (Figure 6) which confirms the rate equation (3b) and produced straight lines with intercept $K_{eq,H}k_H$ = 1000 s^{-1} M^{-1} and slope $K_{eq,HB}k_{HB}$ = 1.21, 1.03, and 0.07 s^{-1} M^{-1} for $H_2PO_4^-$, CH_3COOH, and $(CH_2OH)_3CNH_3^+$, respectively.

The relative amounts of tetrols I-1 and I-2 produced over the pH range 4.0–9.0 (Figure 7) indicate that, within the same buffer system, the trans (relative to the C(9)OH) nucleophilic attack by H_2O is slightly more favored at lower pH.

Scheme I



Scheme II



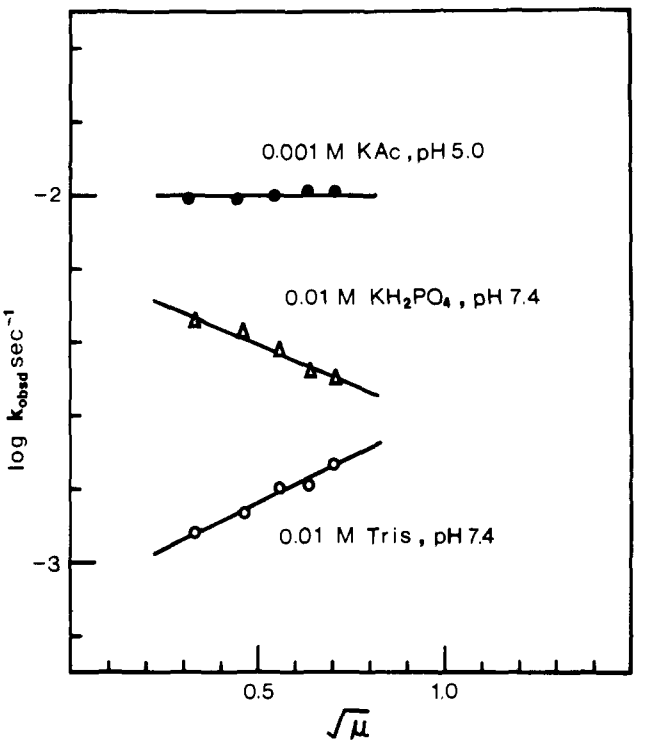


Figure 5. Plots of log k_{obsd} vs. $\sqrt{\mu}$ for the hydrolysis of I in 0.001 M acetate buffer, pH 5.0 (●); 0.01 M phosphate buffer, pH 7.4 (△), and 0.01 M Tris HCl buffer, pH 7.4, (○), Ionic strengths were varied by KCl.

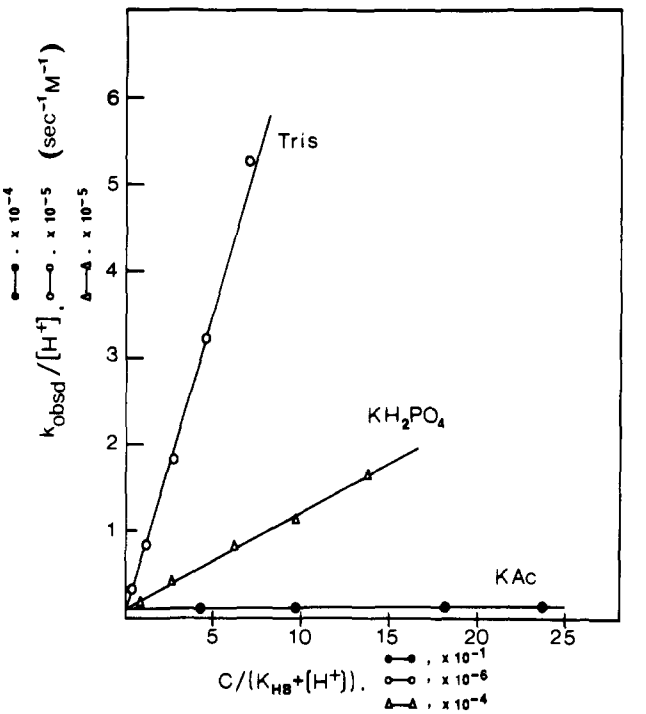


Figure 6. Plots of $k_{obsd}/[H^+]$ vs. $C/(K_{HB} + [H^+])$ for the hydrolysis of I according to eq 4b. The kinetic data are identical with those shown in Figure 4.

Discussion

The rate dependence of acid concentration and pH in the hydrolysis of BP diol epoxide I indicates that the hydrolysis is a specific- and general-acid-catalyzed reaction. The mechanism of this hydrolysis will be discussed in light of the kinetic data. In contrast to the extensively studied arene oxides,[15] it is clear from product analysis that I and II do not undergo rearrangement.

For a specific- and general-acid-catalyzed hydrolysis, the mechanism can be depicted either according to eq 2a as in Scheme I or according to eq 2b as in Scheme II.

In Scheme I the rate-determining step is a bimolecular reaction. The product of the ionic charges of the two reactants (I·HB, and H_2O or OH^-) can be positive, zero, or negative. The activation complex theory[14] predicts that the rate constants of this bimolecular reaction in ionic solution depend on the product of the charge of the reactants. If H_2O is one of the reactants, changes in ionic strength should have little effect on the reaction rate. For a system such as $H_2PO_4^-$ where I·HB is negative, the rate of reaction with a negative hydroxide ion would be increased by increasing ionic strength, whereas the

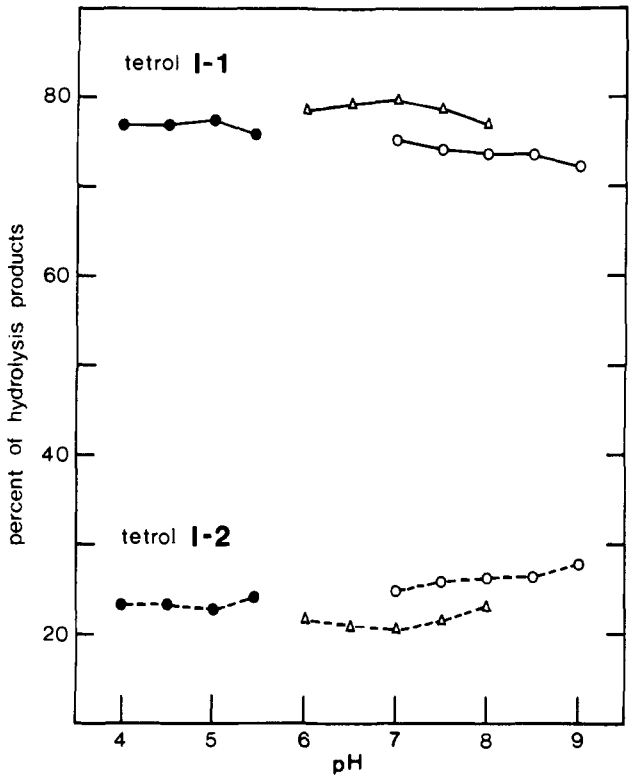


Figure 7. pH dependence of tetrol formation in the hydrolysis of I. The products of [7-^{14}C]diol epoxide I (specific activity 53.9 mCi/mmol)[10] were analyzed on HPLC. Samples were directly injected into HPLC without organic solvent extraction at the end of hydrolysis in the following 95% (v/v) buffers and 5% THF: 0.005 M acetate buffer (pH 4.0–5.5), ●; 0.01 M phosphate buffer (pH 6.0–8.0), △; and 0.05 M Tris HCl buffer (pH 7.0–9.0), ○.

rate would decrease if I·HB were positive and remain unchanged for a neutral I·HB. However, the rate constants were found (Figure 5) to increase with increasing ionic strength when I·HB was positively charged and to decrease with increasing ionic strength when I·HB was negatively charged. The results above are inconsistent with a bimolecular reaction and the mechanism depicted in Scheme I can therefore be ruled out. Similar arguments also rule out the direct proton transfer (i.e., $I + HB \rightarrow IH^+ + B^-$) as the rate-determining step.

A single reactant in the rate-determining step (Scheme II) satisfies the requirements of the ionic strength dependence for the observed first-order rate constants. With increasing μ at constant pH, in addition to specific acid catalysis by H_3O^+, the k_{obsd} in the general-acid-catalyzed reaction increases linearly with the positively charged $(CH_2OH)_3CNH_3^+$, remains relatively constant with uncharged CH_3COOH, and decreases with negatively charged $H_2PO_4^-$. Thus the results are consistent with the mechanism (Scheme II) that the ring opening of I·HB to the C(10) benzylic carbonium ion intermediate IH^+ is the rate-limiting step. Subsequent S_N1 nucleophilic attack by H_2O at both sides of the planar C(10) benzylic carbonium ion yields I-1 and I-2. The tetrol (I-1) resulting from the trans (relative to the C(9)OH) is expected to be the major product due to the steric hindrance of C(9)OH.[7] However, within the same buffer system, the amount of tetrol I-1 relative to that of tetrol I-2 decreases slightly with increasing pH (Figure 7).

Diol epoxide II, the minor diol epoxide formed metabolically from BP (−)-*trans*-7,8-diol by the mammalian microsomal enzyme systems,[1-3] undergoes hydrolysis more complex than that of I. At pH 7.4 in 0.1 M Tris buffer, 91 and 9% of the absorbance changes of II (Figure 2) are associated with the rate constants which are 4 times faster and 18 times slower than that of I, respectively. The fast kinetic effect in the hydrolysis of II which is 4 times faster than that of I is presumably due to the anchimerically assisted ring opening of the 9,10-epoxide by the C(7)OH group.[12] The ratio of the two rate constants of II indicates that the two kinetic effects are separated by a factor of 73. Therefore the elucidation of the hydrolysis mechanism of II requires detailed kinetic studies of both the fast and the slow kinetic effects. In nonaqueous solvent, II was found to be 160 times more reactive than I toward *p*-nitrothiophenolate in *tert*-butyl alcohol.[12] The difference in reactivity of I and II toward H_2O and *p*-nitrothiophenolate is most likely due to their different reaction mechanism toward the nucleophiles.

In contrast to a previous hypothesis,[16] our results demonstrated that I can undergo S_N1 reaction mechanism. A carbonium ion as the intermediate in the hydrolysis of I indicates that I can react with cellular macromolecules as an alkylating agent. Previous reports demonstrated that I is the predominant diol epoxide formed metabolically from benzo[*a*]pyrene[1-3] and its exceptionally high mutagenic activity in mammalian cells[1,17,18] thus supports the conclusion that I is a major carcinogenic form in benzo[*a*]pyrene carcinogenesis.[1,17,19]

Acknowledgment. We thank Drs. Paul D. Henson and Donald M. Crothers for their helpful comments during the preparation of the manuscript.

References and Notes

(1) E. Huberman, L. Sachs, S. K. Yang, and H. V. Gelboin, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 607–611 (1976).
(2) S. K. Yang, D. W. McCourt, P. P. Roller, and H. V. Gelboin, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2594–2598 (1976).
(3) S. K. Yang and H. V. Gelboin, *Biochem. Pharmacol.*, **25**, 2221–2225, 2764i (1976).
(4) (a) I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, C. Harris, H. Autrup, H. Kasai, and K. Nakanishi, *Science*, **193**, 592–595 (1976); (b) A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi, *J. Am. Chem. Soc.*, **98**, 5714–5715 (1976).
(5) H. W. S. King, M. R. Osborne, F. A. Beland, R. G. Harvey, and P. Brookes, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2679–2681 (1976).
(6) Synthesis of benzo[*a*]pyrene 7,8-diol 9,10-epoxide and its reactivity toward DNA in vitro was originally reported by P. Sims, P. L. Grover, A. Swaisland, K. Pal, and A. Hewer, *Nature (London)*, **252**, 326–328 (1974). However, the stereochemistry of the synthesized diol epoxide was not known in that study.
(7) S. K. Yang, P. P. Roller, D. W. McCourt, J. R. Miller, and H. V. Gelboin, *J. Am. Chem. Soc.*, preceding paper in this issue.
(8) Preliminary results on the structural identification of four tetrols were reported by D. R. Thakker, H. Yagi, A. Y. H. Lu, W. Levin, A. H. Conney, and D. M. Jerina, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3381–3385 (1976).
(9) D. J. McCaustland and J. F. Engel, *Tetrahedron Lett.*, 2549–2552 (1975).
(10) D. J. McCaustand, W. P. Duncan, and J. F. Engel, *J. Labelled Compd.*, **12**, 443–448 (1976).
(11) F. A. Beland and R. G. Harvey, *J. Chem. Soc., Chem. Commun.*, **3**, 84–85 (1976).
(12) H. Yagi, O. Hernandez, and D. M. Jerina, *J. Am. Chem. Soc.*, **97**, 6881–6883 (1975).
(13) (a) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism", 2nd ed, Wiley, New York, N.Y., 1961, Chapter 9, pp 213–218; (b) R. P. Bell, "The Proton in Chemistry", Methuen, London, 1959.
(14) W. J. Moore, "Physical Chemistry", 4th ed, Prentice-Hall, Englewood Cliffs, N.J., 1972, Chapter 10, pp 420–471, and references cited therein.
(15) (a) D. M. Jerina and J. W. Daly, *Science*, **185**, 573–582 (1974); (b) G. J. Kasperek and T. C. Bruice, *J. Am. Chem. Soc.*, **94**, 198–202 (1972); (c) P. Y. Bruice and T. C. Bruice, *ibid.*, **98**, 2023–2025 (1976); (d) P. Y. Bruice, T. C. Bruice, P. M. Dansette, H. G. Selander, H. Yagi, and D. M. Jerina, *Ibid.*, **98**, 2965–2973 (1976); (e) J. W. Keller and C. Heidelberger, *Ibid.*, **98**, 2328–2336 (1976).
(16) P. B. Hulbert, *Nature (London)*, **236**, 146–148 (1975).
(17) R. F. Newbold and P. Brookes, *Nature (London)*, **261**, 52–54 (1976).
(18) A. W. Wood, P. G. Wislocki, R. L. Chang, W. Levin, A. Y. H. Lu, H. Yagi, O. Hernandez, D. M. Jerina, and A. H. Conney, *Cancer Res.*, **36**, 3358–3366 (1976).
(19) Following the submission of this paper, J. W. Keller, C. Heidelberger, F. A. Beland, and R. G. Harvey (*J. Am. Chem. Soc.*, **98**, 8276–8277 (1976)) reported preliminary results on the kinetics of hydrolysis of diol epoxides I and II. Discrepancies exist between this paper and that of Keller et al. In the interpretation of experimental results on the mechanism of hydrolysis of diol expoxides and the ratios of the hydrolysis products.