These are of reasonable magnitude compared with the observed maximum pH gradients, 4.0 and 3.8, for the cyt c_3 and C_4V^{2+} liposomes, respectively. This fact strongly supports the proposed mechanism for the generation of the pH gradient.

For elucidation of the detailed mechanism of the observed accelerated H^+ influx coupled to the rapid electron flow, further investigations are necessary. However, we just wish to point out the following in order to clarify the situation.

The observed $J_{H^+}^{o\rightarrow i}$ was dependent on the outside reductant concentration at the early stages of the electron transport under the present conditions. It was also mildly affected by the pH gradient initially applied. Therefore, $\bar{J}_{H^+}^{o\rightarrow i}$ may be expressed by eq 15 where S is surface area. According to our preliminary

$$\lim_{t\rightarrow 0} J_{H^+}^{o\rightarrow i}(\text{total}) = (\lim_{t\rightarrow 0} \bar{J}_{H^+}^{o\rightarrow i})S = \bar{P}_{H^+}[e]_o S \quad (15)$$

experiments, the permeability coefficient for the coupled H^+ flow, P_{H^+}, was larger than P_{H^+} by a factor of 10^1-10^2. This enhanced H^+ permeability plays the key role of coupling in the generation of a large pH gradient. A tentative mechanism for coupling, then, is that H^+ flow is associated with electron flow in order to maintain electroneutrality and thus H^+ flow is accelerated remarkably by rapid electron flow.

Registry No. Cytochrome c_3, 9035-44-3; $K_3Fe(CN)_6$, 13746-66-2; cytochrome c, 9007-43-6; C_4V^{2+}, 47082-19-9; hydrogen ion, 12408-02-5; hydroxide, 14280-30-9.

Conformational Effects in the Hydrolyses of Benzo-Ring Diol Epoxides That Have Bay-Region Diol Groups[1]

J. M. Sayer,*[†] D. L. Whalen,[‡] S. L. Friedman,[‡] A. Paik,[‡] H. Yagi,[†] K. P. Vyas,[†] and D. M. Jerina[†]

Contribution from the Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205, and the Laboratory for Chemical Dynamics, Department of Chemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228.
Received March 10, 1983

Abstract: Kinetics of the hydronium ion catalyzed (k_H) and pH-independent (k_o) hydrolyses of several benzo-ring diol epoxides, derived from polycyclic aromatic hydrocarbons, that possess bay-region trans diol groups have been investigated in 1:9 dioxane–water at 25 °C. These epoxides are 1,2,3,4-tetrahydrobenz[*a*]anthracene-1,2-diol 3,4-epoxide, 1,2,3,4-tetrahydrotriphenylene-1,2-diol 3,4-epoxide, and 9,10,11,12-tetrahydrobenzo[*e*]pyrene-9,10-diol 11,12-epoxide. Both possible diastereomers of the diol epoxides in which the two hydroxyl groups are trans to each other were investigated: isomer **1**, in which the benzylic hydroxyl group is cis to the epoxide oxygen, and isomer **2**, in which this hydroxyl group and the epoxide oxygen are trans. The corresponding tetrahydro epoxides that lack a diol group were also investigated. For comparison, isomers **1** and **2** of 1,2,3,4-tetrahydrobenz[*a*]anthracene-3,4-diol 1,2-epoxide, a bay-region epoxide with a non-bay-region trans diol group, and the corresponding benzanthracene tetrahydro epoxide were also studied. The most striking feature of the reactions of the diol epoxides that possess a bay-region diol group is a reversal of the relative reactivity of isomers **1** and **2** in the k_o reaction, when compared with diol epoxides whose diol group is not in a bay region. This reversal of reactivity, which causes k_o to be larger for isomer **2** than for isomer **1** when the diol is in a bay region, is explained by changes in conformational equilibria involving the cyclohexene ring due to steric crowding of the bay-region diol. Preference of this diol group for a pseudodiaxial conformation favors a conformation of isomer **2** in which the benzylic C–O bond of the epoxide is more or less aligned with the π orbitals of the aromatic system and strongly disfavors this aligned conformation of isomer **1**. Reaction via the k_o process is faster for the aligned than for the nonaligned conformer; thus for epoxides with bay-region diol groups, k_o for isomer **2** is faster than k_o for isomer **1**. The pH-independent reaction of isomer **2** of 1,2,3,4-tetrahydrobenz[*a*]anthracene-1,2-diol 3,4-epoxide via the aligned conformer gives, in addition to cis and trans tetraol products, substantial quantitites of a keto diol, whereas no keto diol was detected from the corresponding isomer **1**. This also represents a reversal of the pattern of product formation generally observed with diol epoxides that lack a bay region in the vicinity of the diol group. Rate constants for hydronium ion catalyzed hydrolysis (k_H) are much less sensitive to conformational factors than k_o. The distribution of cis and trans tetraol products from hydronium ion catalyzed hydrolysis of the 1,2,3,4-tetrahydrobenz[*a*]anthracene-1,2-diol 3,4-epoxides and the 1,2,3,4-tetrahydrotriphenylene-1,2-diol 3,4-epoxides can be explained by preferential pseudoaxial attack of water upon the benzylic cations formed from these epoxides. On the basis of these observations and previous findings with non-bay-region diol, bay-region epoxides, rules for predicting the effects of conformation on rates and products of diol epoxide hydrolyses are proposed.

Benzo-ring diol epoxides in which the epoxide group forms part of a bay region of the hydrocarbon have been shown to be the most important ultimate carcinogens formed metabolically from a number of carcinogenic polycyclic aromatic hydrocarbons.[2] For benzo-ring diol epoxides in which the hydroxyl groups are trans to each other, two diastereomers are possible: diastereomer **1**, in which the epoxide oxygen is cis to the benzylic hydroxyl group, and diastereomer **2**, in which the epoxide oxygen is trans to this hydroxyl group (cf. Figure 1). Two rapidly interconvertible conformations of each diol epoxide are possible. In the absence of unusual steric or electronic factors, isomer **1** ordinarily has a slight preference for the conformation in which the hydroxyl groups are pseudodiaxial whereas isomer **2** has a relatively strong preference for the conformation in which the hydroxyl groups are pseudodiequatorial.[3-5] In the conformation normally preferred

[†] National Institutes of Health.
[‡] University of Maryland Baltimore County.

(1) Supported in part by Public Health Service Grants Nos. CA-17278 and CA-26086 from the National Cancer Institute (D.L.W.).
(2) For a recent review and leading references see: Nordqvist, M.; Thakker, D. R.; Yagi, H.; Lehr, R. E.; Wood, A. W.; Levin, W.; Conney, A. H.; Jerina, D. M. In "Molecular Basis of Environmental Toxicity"; Bhatnagar, R. S., Ed.; Ann Arbor Science Publishers: Ann Arbor, MI, 1980; pp 329–357.
(3) (a) Yagi, H.; Hernandez, O.; Jerina, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 6881–6883. (b) Yagi, H.; Thakker, D. R. Hernandez, O.; Koreeda, M.; Jerina, D. M. *Ibid.* 1977, *99*, 1604–1611.

0002-7863/84/1506-0226$01.50/0

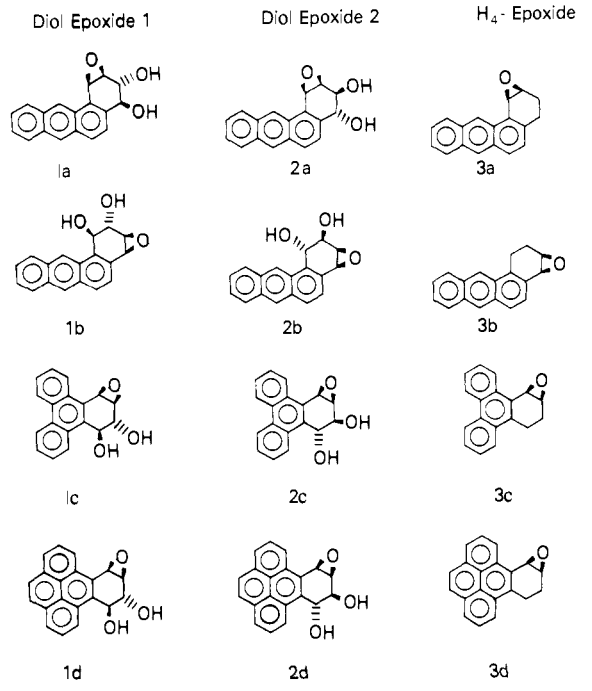


Figure 1. Structures of the diol epoxides and tetrahydro epoxides. Only relative stereochemistry is implied.

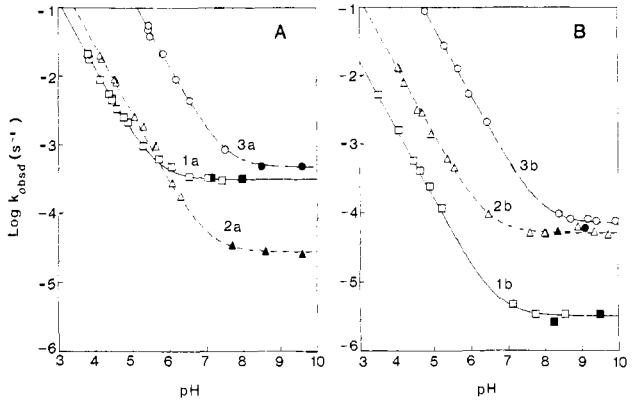


Figure 2. Dependence on pH of the pseudo-first-order rate constants for the hydrolysis of (A) the "normal" epoxides **1a** (□), **2a** (△), and **3a** (○) and (B) the "reverse" epoxides **1b** (□), **2b** (△), and **3b** (○) derived from benz[*a*]anthracene. Rates were measured in 1:9 dioxane–water, μ = 0.1 M ($NaClO_4$), at 25 °C. Open symbols represent rate constants determined spectrophotometrically and closed symbols represent rate constants determined by HPLC. The lines are theoretical curves based on the rate constants given in Table I.

by both isomers, the orientation of the epoxide ring is such that its benzylic C–O bond is not parallel with the π orbitals of the aromatic rings.[5] We have previously referred to this orientation as the "nonaligned" conformation of the epoxide and to the alternative conformation as the "aligned" conformation.[6] Changes in the conformational preference of the diol epoxides can have large effects on their carcinogenic activity: in general, only those diol epoxides with a preferred pseudodiequatorial orientation of the diol group are highly tumorigenic[2,7] We have also shown that conformational effects are important in determining the rates and

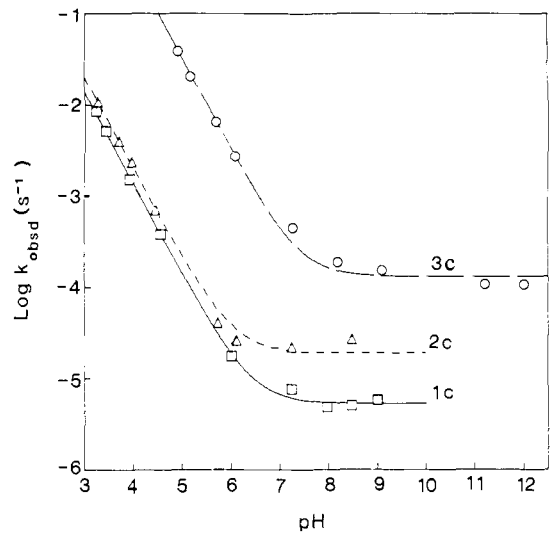


Figure 3. Dependence on pH of the pseudo-first-order rate constants for the hydrolysis of epoxides **1c** (□), **2c** (△), and **3c** (○) derived from triphenylene, in 1:9 dioxane–water, μ = 0.1 M ($NaClO_4$), at 25 °C. Rate constants were determined spectrophotometrically (see text). The lines are theoretical curves based on the rate constants given in Table I.

Table I. Rate Constants for the Hydrolyses of Diol Epoxides and Tetrahydro Epoxides in 1:9 Dioxane–Water, Ionic Strength 0.1 M ($NaClO_4$), 25 °C

compd	k_H, M^{-1} s^{-1}	$10^5 k_o$,[a] s^{-1}
1a	125	31
2a	275	2.8
3a	13500	45[b]
1b	16	0.32
2b	137	5.3
3b	5400	7.2
1c	13.6	0.53
2c	20	1.9 ± 0.5
3c	3200	13 ± 2
1d[c]	9.7	1.3
2d	14	2.0
3d	2800	9.8 ± 1

[a] Estimated errors in k_o were ≤10% unless otherwise indicated. [b] The value of k_o determined spectrophotometrically was ~28 × 10^{-5} s^{-1}. Because the kinetics were not cleanly pseudo first order at pH ≥8, as a result of a pH-dependent secondary reaction, we believe that the value of 45 × 10^{-5} s^{-1} determined from reactant disappearance monitored by HPLC represents a more accurate value of k_o. [c] Biphasic kinetics above pH 7 were observed, presumably due to product instability.

products of hydrolysis of bay-region diol epoxides and related compounds and have proposed mechanisms for these effects.[6] The present study extends our mechanistic investigations to diol epoxides of benz[*a*]anthracene (**1b** and **2b**), triphenylene (**1c** and **2c**), and benzo[*e*]pyrene (**1d** and **2d**) (Figure 1) in which the equilibria between the aligned and nonaligned conformations are altered by the location of the diol group in a bay region of the molecule. For comparison, hydrolyses of the "normal", carcinogenic non-bay-region diol, bay-region epoxides of benz[*a*]anthracene (**1a** and **2a**) have also been investigated.

Results

The pH–rate profiles for the hydrolyses of diol epoxides and tetrahydro epoxides **1a–3a** and **1b–3b** in 1:9 dioxane–water, ionic strength 0.1 M ($NaClO_4$), are shown in Figure 2. These pH–rate profiles are consistent with reaction via both a hydronium ion catalyzed (k_H) and a pH-independent (k_o) pathway, according to the rate law $k_{obsd} = k_H a_{H+} + k_o$. The "normal" diol epoxides **1a** and **2a** show the usual pattern[5,8] of reactivity (Figure 2A) in

(4) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *Tetrahedron Lett.* **1977**, 539–542.
(5) Whalen, D. L.; Ross, A. M.; Yagi, H.; Karle, J. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1978**, *100*, 5218–5221.
(6) Sayer, J. M.; Yagi, H.; Silverton, J. V.; Friedman, S. L.; Whalen, D. L.; Jerina, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 1972–1978.
(7) Jerina, D. M.; Sayer, J. M.; Yagi, H.; Croisy-Delcey, M.; Ittah, Y.; Thakker, D. R.; Wood, A. W.; Chang, R. L.; Levin, W.; Conney, A. H. In "Advances in Experimental Medicine and Biology: Biological Reactive Intermediates IIA"; Snyder, R.; Parke, D. V.; Kocsis, J. J.; Jollow, D. J.; Gibson, C. G.; Witmer, C. M., Eds.; Plenum Press: New York, 1982; pp 501–523.
(8) Whalen, D. L.; Montemarano, J. A.; Thakker, D. R.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 5522–5524.

Table II. Products of the Hydronium Ion Catalyzed (k_H) and pH-Independent (k_o) Hydrolyses of Diol Epoxides and Tetrahydro Epoxides in 1:9 Dioxane-Water, Ionic Strength 0.1 M ($NaClO_4$), 25 °C

compd	pathway	% trans hydration	% cis hydration	% other or unrecovered products[a]
1a	k_H	23	73	⩽4
2a		100	0	
3a		44	56	
1b		77	23	
2b		94	6	
3b		71	29	
1c		85	15	
2c		70	30	
1a	k_o	6	24	70[b]
2a		80	2	18[c]
3a		20	12	68[d]
1b[e]		75-95	0	5-25
2b		44	9	47[f]
3b		29	~1	70[g]
1c[h]		⩾89	1-2	⩽10
2c[i]		44-51	10-13	36-46[b]

[a] Determined for the k_o reaction from the difference in peak areas on HPLC corresponding to tetraols (or diols) observed upon hydrolysis in the k_o region and in 10^{-3} M perchloric acid (see text), 100% recovery of products was assumed under acid conditions. [b] A peak that decreased with time, presumably due to the keto diol, was observed. [c] No product peaks other than those corresponding to the tetraols were detected. [d] A large peak that decreased with time, presumably due to the ketone, was observed. [e] Determined after 20–80% reaction (24–165 h) at pH 8.25 ± 0.15. Calculated recoveries of the tetraol decreased as a function of time, and no chromatographic peaks other than the trans tetraol were detected. Recovery of the tetraol was even lower at a higher pH. We ascribe this low and irreproducible recovery to time-dependent loss of tetraol over the very long periods required for completion of this reaction rather than to formation of unstable primary reaction products other than tetraol. [f] The keto diol formed in this reaction was isolated and characterized; see text. [g] Several unidentified peaks, possibly corresponding to a ketone and/or its degradation products, were detected. [h] Determined after 80–100% reaction at pH 8.2 ± 0.1. [i] Determined after ~67 and 100% reaction at pH 8.30 ± 0.05. Calculated recoveries of the tetraols decreased with time from 64 to 54%.

which diol epoxide **1** reacts much more rapidly than diol epoxide **2** in the k_o region of the pH profile and slightly more slowly than diol epoxide **2** in the k_H region. The non-bay-region epoxides with a bay-region diol group ("reverse diol epoxides") **1b** and **2b** exhibit a striking reversal of reactivity in the k_o region, such that k_o for **1b** is about 17 times slower than k_o for **2b** (Figure 2B). A similar but smaller effect is observed with the diol epoxides **1c** and **2c** (Figure 3) in which both the diol and epoxide groups occupy bay regions. With **1d** and **2d** (rate profiles not shown) k_o for isomer **1** is also larger than for isomer **2**, but the difference between the two values of k_o for these isomers is even smaller. Rate constants for the 12 compounds are listed in Table I.

Products of the reactions of **1a–3a**, **1b–3b**, **1c**, and **2c** (Table II) were determined under kinetic conditions by high performance liquid chromatographic (HPLC) analysis of reaction mixtures. For determination of the product distribution from the k_H reaction, reaction mixtures were analyzed after completion of reaction at pH ~3 (10^{-3} M $HClO_4$). In the k_o region of the pH–rate profile, product formation was followed as a function of time after trapping of the unreacted epoxide as the mercaptoethanol adduct.[9a] Typical HPLC profiles for separation of the products and mercaptoethanol adduct from **1b** and **2b** are shown in Figure 4. For moderately

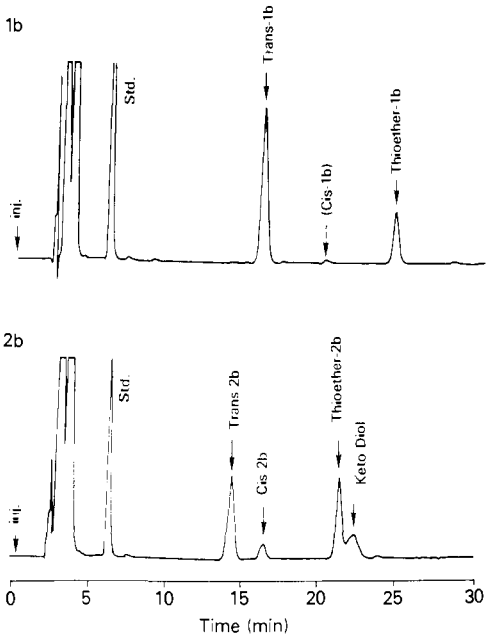


Figure 4. Typical chromatographic traces showing the separation of products from the reaction at pH 8.3 ± 0.2 of **1b** (upper trace) and **2b** (lower trace), after trapping of unreacted diol epoxide as the thioether with mercaptoethanol. Reaction times were 165.3 h for **1b** and 7 h for **2b**. Chromatography was in system A (see text) with an internal standard of *p*-nitrobenzyl alcohol.

rapid reactions, product distribution after completion of the reaction was also measured, but such an end point could not be determined with **1b** because of the slow rate and an apparently time-dependent loss of the tetraol product. Recoveries of diol or tetraol products under conditions of spontaneous (k_o) hydrolysis were determined by comparison of the total peak area corresponding to these products (relative to an internal standard) with the total peak area observed upon hydrolysis of an indentical sample in 10^{-3} M $HClO_4$. The unrecovered material presumably corresponds largely to unstable ketone or keto diol formed in the k_o reaction. In several cases, peaks attributable to these products were observed, but quantitation of the products was not possible, due to their instability and/or poor chromatographic behavior. Because of the unusual finding of keto diol formation from an isomer-**2** diastereomer in the case of **2b**, this product was isolated and characterized by its ultraviolet spectrum (λ_{max} 255 nm in CH_3OH–H_2O) and by the mass spectra of the two isomeric triol triacetates obtained upon sodium borohydride reduction and acetylation. This method for identification of the relatively unstable keto diol is essentially the same as that used previously to demonstrate keto diol formation from isomer **1** of 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8-diol 9,10-epoxide.[3b]

Discussion

Conformational Equilibria of Diol Epoxides. In the absence of unusual steric or electronic features, such as additional steric hindrance in the bay region[9a] or substituents peri to the benzo ring,[9b] *non-bay-region diol, bay-region epoxides* (such as **1a** and **2a**) derived from polycyclic aromatic hydrocarbons generally prefer the conformation in solution in which the benzylic C–O bond of the epoxide ring is not aligned with the π orbitals of the adjacent aromatic system. This corresponds to a pseudoaxial conformation of the two hydroxyl groups in the diastereomer-**1** series of diol epoxides and a pseudoequatorial conformation of these groups in the diastereomer-**2** series.[3-5] The effect of various structural perturbations on this steric preference can be described in terms of the following conformational equilibrium (cf. Table III):

$$\underset{(\mathbf{1}' \text{ or } \mathbf{2}')}{\text{nonaligned conformer}} \overset{K_e}{\rightleftharpoons} \underset{(\mathbf{1}'' \text{ or } \mathbf{2}'')}{\text{aligned conformer}}$$

In the isomer-**1** series, K_e is decreased by the *presence of a bay*

(9) (a) Sayer, J. M.; Yagi, H.; Croisy-Delcey, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1981**, *103*, 4970–4972. (b) For a discussion and references concerning the effect of peri substituents on the conformations of the related dihydrodiols, see: Vyas, K. P.; Shibata, T.; Highet, R. J.; Yeh, H. J.; Thomas, P. E.; Ryan, D. E.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1983**, *258*, 5649–5659.

Table III. Conformational Equilibria of Diol Epoxides

	bay-region epoxide	bay-region diol	J_{diol}, Hz	preferred conformation at equilibrium[a]	K_e
Isomer-1 Series					
			1′ (nonaligned)	1″ (aligned)	
1a	yes	no	7[a]	both present	~1
1b	no	yes	2[b]	1′	very small
1c, 1d	yes	yes	~2[c]	1′	small
Isomer-2 Series					
			2′ (nonaligned)	2″ (aligned)	
2a	yes	no	8.5[a]	2′	small
2b	no	yes	6.8[b]	both present	~1
2c, 2d	yes	yes	3.5[c]	2″	large

[a] In $(CD_3)_2SO$ (ref 4). [b] In $(CD_3)_2CO$ (ref 11). [c] In $(CD_3)_2CO$ (ref 10).

region in the vicinity of the diol group (as in **1b–1d**) since such a bay region strongly favors the nonaligned conformation that has a pseudodiaxial diol.[10] The opposite effect, namely an increase in K_e, is observed for bay-region diols in the isomer-**2** series, again because of the tendency of the diol to assume the pseudodiaxial conformation. In both the isomer-**1** and isomer-**2** series, the absence of a bay region in the vicinity of the epoxide group should decrease K_e, since steric hindrance involving the benzylic hydrogen of the epoxide is decreased when the epoxide is not in a bay region. Thus the tendency of this hydrogen to be forced out of the plane of the aromatic rings (giving the aligned conformation of the epoxide) is decreased. The effects of these factors on the conformation of the diol epoxides to be discussed in this work, as indicated by the coupling constants (J_{diol}) for the C–H protons of the diol group and on predicted relative magnitudes of K_e, are summarized in Table III.

Kinetics. We have recently proposed,[6] on the basis of a study of conformationally locked epoxides that lack diol substituents, that the rate of neutral hydrolysis (k_0) of the aligned conformation is substantially (~40-fold) faster than that of the nonaligned conformation. With this idea as background, we wished to assess the kinetic effects of conformation in conformationally mobile diol epoxides. In particular, we wished to investigate the effect of a bay-region diol group on the reactivity of these compounds. We have studied the kinetics of hydrolysis of diol epoxides and tetrahydro epoxides derived from three such systems, namely, the 1,2-epoxytetrahydrotriphenylene[10] (**1c–3c**), 9,10-epoxytetrahydrobenzo[*e*]pyrene[10] (**1d–3d**), and 3,4-epoxytetrahydrobenz[*a*]anthracene[11] (**1b–3b**) systems. For comparison, we have also investigated the tetrahydro 3,4-diol 1,2-epoxides[4] (**1a** and **2a**) and the tetrahydro 1,2-epoxide (**3a**) derived from benz[*a*]anthracene in which the epoxide group forms part of a bay region but the diol group does not. Our observations are consistent with a mechanism for k_0 in which the reactivity of the aligned conformer of both diastereomers of the diol epoxides is greater than that of the nonaligned conformer; i.e., $k'' > k'$ in eq 1.

$$k_0[\text{total diol epoxide}] = k'[\text{nonaligned conformer}] + k''[\text{aligned conformer}] \quad (1)$$

Examination of the pH–rate profiles in Figure 2 reveals that the most striking qualitative difference between the bay- and non-bay-region diol epoxides of benz[*a*]anthracene is a reversal of the relative reactivities in the k_0 region for isomers **1** and **2** of the diol epoxides, such that isomer **1**, normally the more reactive of the two diastereomers in the k_0 reaction,[5,8] is instead the less reactive isomer when the diol occupies a bay region (**1b** and **2b**).

Analysis of the reasons for the relative values of k_0 for isomers **1b** and **2b** is best accomplished by a quantitative comparison of k_0 values for the non-bay-region epoxides **1b–3b** with expected values based on relative reactivities of bay-region epoxides. On the basis of known k_0 values for a series of non-bay-region diol, bay-region epoxides and previously described Hammett-type linear free energy relationships[12] ($\rho^+ = -6$ for isomer **1**, $\rho^+ = -4.6$ for isomer **2**), use of the σ^+ value[13] of −0.39 for the 2-anthracyl substituent gives calculated values of k_0 for **1b** and **2b** of $\sim 6 \times 10^{-5}$ s^{-1} and $\sim 1 \times 10^{-5}$ s^{-1} respectively. A similar prediction of k_0 for **3b** based on a three-point Hammett plot (naphthalene, phenanthrene, and benz[*a*]anthracene tetrahydro epoxides; $\rho^+ \sim -3.6$)[14] gives k_0(**3b**) = $\sim 1.2 \times 10^{-4}$ s^{-1}. The observed value of k_0 for **3b** of 7.2×10^{-5} s^{-1} is close to this expected value, a finding that suggests that *conformational effects on the rate* arising from the location of the bay region are not large in the case of these tetrahydro epoxides. Similarly, a 4.3-fold difference[15] between values of k_0 for the bay- and non-bay-region tetrahydro epoxides of phenanthrene is in agreement with the 3.5-fold difference predicted from $\Delta\sigma^+ = 0.15$.[13] In contrast k_0 for **1b** (0.32×10^{-5} s^{-1}) is ~19 times slower than expected from electronic effects alone (as measured by σ^+) and the observed k_0 for **2b** (5.2×10^{-5} s^{-1}) is ~5 times faster than predicted from electronic effects. The differences between observed and predicted values of k_0 can be accounted for by conformational differences between the bay- and non-bay-region diol epoxides. Location of the benzylic hydroxyl group of **2b** in a bay region shifts the equilibrium in favor of the aligned conformation with pseudoaxial hydroxyl groups. Thus, although the equilibrium in solution between the aligned and nonaligned conformers of **2a** is almost completely on the side of the nonaligned conformer, both conformers of **2b** exist at equilibrium in approximately equal amounts (cf. J_{diol} values, Table III). Acceleration of k_0 for **2b** can be accounted for if reaction of the aligned conformer proceeds more rapidly than that of the nonaligned conformer ($k'' > k'$). For the diastereomeric compound, **1b**, the observed deceleration of k_0 relative to the predicted value is also consistent with the hypothesis that the reaction of the aligned conformer of isomer **1** (k'') takes place more rapidly than that of the nonaligned conformer (k'), based on the folowing considerations. In solution **1a** (J_{diol} = 7 Hz) exists to an appreciable extent in the more reactive aligned conformation, whereas **1b** (J_{diol} = 2 Hz) exists primarily in the less reactive nonaligned conformation. Therefore, an unfavorable conformational equilibrium must be overcome if **1b** reacts via the aligned conformer. Hence, the reaction of **1b** may proceed predominantly via the less reactive, but more prevalent, nonaligned conformer, whereas the preferred pathway for **1a** will be via the aligned conformer. The observed products (vide infra) are consistent with such a mechanism. In the benzo[*e*]pyrene and triphenylene series (Figure 3 and Table I), the overall pattern of reactivity is qualitatively similar, i.e., k_0 for isomer **1** is smaller than k_0 for isomer **2**, although the difference between the values of k_0 for the two isomers (<4-fold) is less than the corresponding difference of ~17-fold for **1b** and **2b**. In contrast, the "normal" non-bay-region diol, bay-region epoxides **1a** and **2a** exhibit the opposite conformational preferences and relative reactivities (k_0).

These results are consistent with our previous proposals[6] regarding the effect of conformation on rates for diol epoxide

(10) Yagi, H.; Thakker, D. R.; Lehr, R. E.; Jerina, D. M. *J. Org. Chem.* **1979**, *44*, 3439–3442.
(11) Yagi, H.; Jerina, D. M., manuscript in preparation.
(12) Sayer, J. M.; Lehr, R. E.; Whalen, D. L.; Yagi, H.; Jerina, D. M. *Tetrahedron Lett.* **1982**, *23*, 4431–4434.
(13) Streitwieser, A., Jr.; Hammond, H. A.; Jagow, R. H.; Williams, R. M.; Jesaitis, R. G.; Chang, C. J.; Wolf, R. *J. Am. Chem. Soc.* **1970**, *92*, 5141–5150.
(14) Rate constants measured in water for the naphthalene and phenanthrene derivatives were divided by a factor of 4 (ref 8) to correct for the solvent effect of 10% dioxane.
(15) Rogers, D. Z.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 4713–4719.

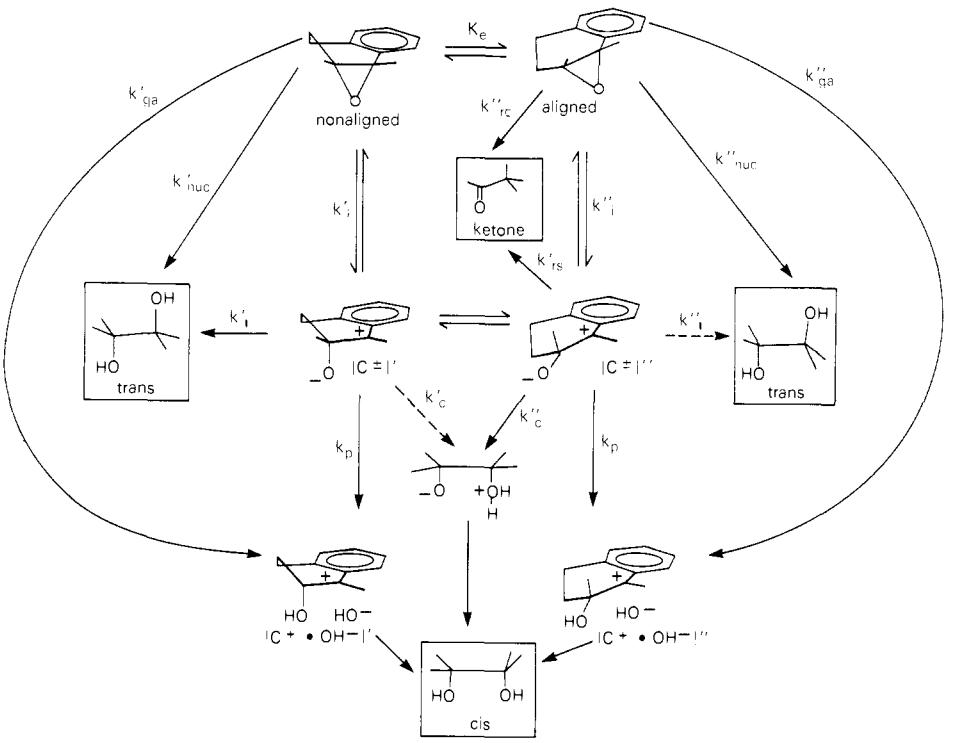


Figure 5. Possible pathways for the k_o reaction. Broken lines indicate pathways that are expected to be unfavorable.

cleavage and provide new evidence against the suggestion[8,16] that hydrogen bonding between the epoxide ring and the transannular benzylic hydroxyl group in isomer **1** could account for the higher values of k_o observed for isomer **1** relative to isomer **2** of diol epoxides whose diol groups are not in a bay region. *The present studies demonstrate that the more reactive conformation of isomer* ***1*** *is the one in which internal hydrogen bonding to the epoxide is impossible.* Factors that strongly favor the conformation in which such a hydrogen bond could exist, and that inhibit equilibration to the other conformation, actually retard the rate of spontaneous hydrolysis quite markedly. Thus, for those diol epoxides in which the epoxide group, but not the diol, forms part of a bay region, the larger values of k_o for isomer **1** relative to isomer **2** can be accounted for by the greater ease with which isomer **1** can assume the more reactive aligned conformation in solution, as reflected by NMR spectra[3-5] (cf. **1a** and **2a**). X-ray studies have shown that, in the crystalline state, isomer **1** of 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8-diol 9,10-epoxide prefers the aligned conformation whereas isomer **2** exists in the nonaligned conformation.[17] Acceleration by hydrogen bonding is not required to account for the relative reactivities of these diastereomers in the k_o reaction. The same conclusion regarding the kinetic insignificance of internal hydrogen bonding in the k_o reaction of isomer **1** in aqueous solution was reached from studies of methylated diol epoxides by Becker, Janusz, and Bruice, who proposed that "....the differences in reactivity between [isomers] **1** and **2** are due to conformational differences".[18] The present results have elucidated the nature of these conformational effects.

The absence of a demonstrable effect on k_o (in predominantly aqueous solutions) of a hydrogen bond between the epoxide oxygen and the benzylic hydroxyl group in the isomer-**1** series does not necessarily imply that such a hydrogen bond is not present, but only that it provides little if any added stabilization of the transition state relative to the reactants, when compared with a hydrogen bond to water. The existence of a transannular hydrogen bond in a benzylic hydroxy epoxide analogous to isomer **1** has been demonstrated in the crystalline state and in partly aqueous solutions (~1.2:1.0 (v/v) dioxane–water).[19] However, significant rate accelerations attributable to this hydrogen bond appear to be limited to similar solvent systems containing a large proportion of organic solvent (e.g., *tert*-butyl alcohol or 1:1 dioxane–water), in which substantial accelerations of *nucleophilic* attack on isomer **1** relative to isomer **2** are observed.[3a,9a,18]

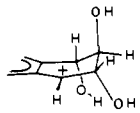
In contrast to the k_o reaction, conformational effects on k_H appear to be relatively small,[20,21] in accordance with our observation that such effects are also quite small in conformationally locked model compounds.[6]

Products—The k_H Reaction. We previously proposed that products of the k_H reaction of diol epoxides arise by preferential pseudoaxial attack[22] upon a cation derived from the epoxide. For

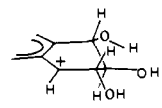
(16) Hulbert, P. B. *Nature (London)* **1975**, *256*, 146–148.
(17) Neidle, S.; Subbiah, A.; Cooper, C. S.; Riberio, O. *Carcinogenesis (N.Y.)* **1980**, *1*, 249–254; Neidle, S.; Cutbush, S. D. *Ibid.* **1983**, *4*, 415–418.
(18) Becker, A. R.; Janusz, J. M.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 5679–5687.

(19) Glusker, J. P.; Zacharias, D. E.; Whalen, D. L.; Friedman, S.; Pohl, T. M. *Science (Washington, D.C.)* **1982**, *215*, 695–696.
(20) (a) Compounds **1b**, **1c**, and **1d**, which are more strongly constrained in the conformation with pseudodiaxial hydroxyl groups than are the "normal" diol epoxides, react ~8–12 times more slowly by the k_H pathway than **1a**. Although part of this difference may be electronic in origin (cf. **3b–3d** relative to **3a**), the cis axial benzylic hydroxyl group present in **1b–1d** may also slightly retard their rate of hydronium-ion-catalyzed hydrolysis. This retardation could result from an effect on the ease of proton transfer to the epoxide oxygen, as suggested by Keller et al. (Keller, J. W.; Heidelberger, C.; Beland, F. A.; Harvey, R. G. *J. Am. Chem. Soc.* **1976**, *98*, 8276–8277). We do not ascribe this difference to the conformation (aligned vs. nonaligned) of the epoxide per se, because the results with locked epoxides lacking hydroxyl substituents suggests that this stereoelectronic effect on k_H is very small (ref 6). (b) In the isomer-**2** series, **2c** and **2d** are most rigidly constrained in the aligned conformation, and react ~7 times more slowly by the k_H pathway than **2b**. Ring opening of this conformation requires the eclipsing of the epoxide oxygen and an adjacent hydroxyl group. Thus, there may be a small kinetic disadvantage in the k_H reaction for isomer-**2** compounds that are strongly constrained in this conformation.
(21) A recent molecular orbital study (Adams, S. M.; Kaminsky, L. S. *Mol. Pharmacol.* **1982**, *22*, 459–464) has suggested that differences in enthalpy between the aligned and nonaligned conformations of *protonated* diol epoxides are small (1–2 kcal/mol), as are the analogous enthalpy differences between the conformations of the corresponding carbocations. Transition-state enthalpies were not calculated. For several diol epoxides, the nonaligned conformation of protonated isomer **2** and the carbocation derived from it were calculated to have slightly higher enthalpies than the corresponding aligned conformations, a result that was unexpected in view of our experimental observations of conformational equilibria for the unprotonated diol epoxides. Because of the small magnitude of these calculated differences, their significance is unclear.

example, if a diol epoxide reacts predominantly via the nonaligned conformer, the initially formed cation (illustrated for isomer **1** as C^+-1′) should give predominantly trans addition of water. For



C^+-1′



C^+-1″

diol epoxides with a bay-region epoxide and a non-bay-region diol, the ratio of cis to trans tetraols formed from isomer **1** depends on the stability of the cation, since highly stabilized cations can undergo conformational inversion (to cations, e.g., C^+-1″, that give predominantly cis hydration) prior to water attack.[6] The stabilities of the cations derived from **1b** and **1c** are roughly comparable to those of the cations derived from the non-bay-region diol, bay-region epoxides of chrysene or phenanthrene, as shown by comparable values of $\Delta E_{deloc}/\beta$[23] or σ^+.[13] Thus, if all other factors were equal, we would expect **1b** and **1c** to give approximately the same amount of trans hydration (~40–50%)[5] as these epoxides. Instead 77–85% trans hydration occurs (Table II). In the ground state, isomers **1** of the non-bay-region diol, bay-region epoxides derived from chrysene and phenanthrene consist of mixtures of conformers, whereas **1b** and **1c** are almost entirely in the nonaligned conformation, **1b**′ or **1c**′. This conformation gives a cation, C^+-1′, that should directly undergo preferential trans hydration, and also should not readily convert to the conformation, C^+-1″, that gives mainly cis hydration. Our hypothesis that the products of the k_H reactions are determined by the conformation of the cation that is attacked by water and that this attack occurs preferentially from a *pseudoaxial* direction thus accounts well for the observed products from **1b** and **1c**. The suggestion of Yang and co-workers[24] that cis addition of water to isomer **1** of 7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8-diol 9,10-epoxide (benzo[*a*]pyrene diol epoxide **1**) involves predominantly pseudoequatorial attack on conformation C^+-1′ is not consistent with our present observations of **1b** and **1c**, for which this conformation is strongly favored, yet which give relatively little cis hydration. Attack on C^+-1′ is not required to explain the product distribution from benzo[*a*]pyrene diol epoxide **1**.[25]

For the compounds whose products have been determined, little cis hydration of isomer **2** in the k_H reaction has been observed,[5,8,9a] with an exception in the case of the triphenylene derivative **2c** in which the aligned conformer is most strongly favored. Although cis hydration would be expected from reaction via the aligned conformer of **2b** and its derived cation, it should be pointed out that this conformer of the reactant is present only to the extent of ~50% and may react by the k_H reaction somewhat more slowly than the nonaligned conformer, because of steric hindrance by the hydroxyl group vicinal to the epoxide.[20b] Thus, the overall reaction of **2b** may occur mainly via the nonaligned confomer, **2b**′, to give a cation that undergoes predominantly trans attack by water.

Products—The k_o Reaction. Compared to the k_H reaction, the k_o reaction requires a more complicated mechanistic scheme (Figure 5) for product formation. The mechanism of the k_o reaction may vary as a function of the epoxide structure, and clear distinctions cannot be made at present among these possible pathways for each epoxide. Hence, the following discussion is necessarily speculative. For k_o reactions leading to hydration, the solvent may be involved in the rate-determining transition state by providing solvation of charges, more directly by acting primarily as a general acid or as a nucleophile, or by some combination of these mechanisms. The reaction may occur either by an S_N2-type process or via a cationic or zwitterionic intermediate whose formation or whose subsequent reaction to give products may be rate determining. Rearranged ketone is frequently a product of the k_o reaction and can also be formed by several mechanisms. For related arene oxides,[26] evidence has been presented for the involvement in this rearrangement of a zwitterionic intermediate whose formation is rate determining. However, this mechanism may not be general in the case of the tetrahydro epoxides.[27]

Several features of the kinetic scheme of Figure 5 are notable. (1) As previously noted, for diol epoxides in the isomer-**2** series with a bay-region epoxide group and a non-bay-region diol and for **1b–1d**, the equilibrium, K_e, strongly favors the nonaligned conformer. For diol epoxides in the isomer **1** series with a bay-region epoxide and a non-bay-region diol and for **2b**, no large conformational preference exists in the ground states (cf. Table III). The corresponding equilibria between the hypothetical zwitterionic intermediates probably favor $[C^{\pm}]'$ for non-bay-region diol, bay-region epoxides **2** and $[C^{\pm}]''$ for non-bay-region diol, bay-region epoxides **1**.[6] Because of the bay-region diol group in **1b** and **2b**, these equilibria would be expected to favor conformation $[C^{\pm}]'$ for **1b** and $[C^{\pm}]''$ for **2b**. (2) Rearrangement to ketone is postulated to occur only via the aligned conformer (k''_{rc}) or via the zwitterion, $[C^{\pm}]''$, conformationally related to it (k''_{rs}).[6] (3) Direct nucleophilic attack of water to give a trans product by an S_N2-like reaction (k'_{nuc} and/or k''_{nuc}) is also possible, especially for epoxides that give unstable cations. (4) Ring opening by an S_N1-like process may occur either via a zwitterionic intermediate ($[C^{\pm}]'$, $[C^{\pm}]''$) or via an ion pair[28] ($[C^+ \cdot OH^-]'$, $[C^+ \cdot OH^-]''$) depending on whether or not protonation of the epoxide oxygen by water is "concerted" with C–O bond breaking. In either event this ring opening should be more rapid for the aligned conformer;[6] i.e., $k''_i > k'_i$ and $k''_{ga} > k'_{ga}$. If the predominant ionization pathway involves the zwitterion, this ion may undergo competitive trapping reactions with water: namely, either attack at the benzylic carbon or protonation (k_p) to give an ion pair that may collapse preferentially to cis product. (5) Attack of solvent water on the benzylic carbon of the intermediate zwitterion should occur preferentially from a pseudoaxial[6,22] orientation to give predominantly the trans product from $[C^{\pm}]'$ and the cis product from $[C^{\pm}]''$.

These features can account for the observed product distributions in the pH-independent hydrolyses of diol epoxides. (1) The "reverse" diol epoxide, **2b**, like "normal" diol epoxides (with a bay-region epoxide and a non-bay-region diol group) in the isomer-**1** series, and unlike "normal" diol epoxides in the isomer-**2** series, yields significant quantities of keto diol in the k_o reaction. This represents the first example of a diol epoxide–**2** isomer that has been unequivocally shown to undergo this rearrangement to a significant extent under hydrolytic conditions.[29] In contrast,

(22) Goering, H. L.; Josephson, R. R. *J. Am. Chem. Soc.* **1962**, *84*, 2779–2785. Goering, H. L.; Vlazny, J. C. *Ibid.* **1979**, *101*, 1801–1805.

(23) Jerina, D. M.; Lehr, R. E. In "Microsomes and Drug Oxidations (Proceedings of the Third International Symposium)"; Ullrich, V., Roots, I., Hildebrandt, A. G., Estabrook, R. W., Conney, A. H., Eds.; Pergamon Press, Oxford, Engl., 1977; pp 709–720.

(24) Yang, S. K.; McCourt, D. W.; Gelboin, H. V.; Miller, J. R.; Roller, P. P. *J. Am. Chem. Soc.* **1977**, *99*, 5124–5130.

(25) These workers postulated that water attacks cis to the epoxide oxygen of cation C^+-1′, which has the benzylic hydroxyl group, O(7), pseudoaxial and a hydrogen bond between this group and O(9) derived from the epoxide. Such a pseudoequatorial attack was suggested to result from steric hindrance by O(8) to trans (pseudoaxial) attack on C^+-1′. This explanation cannot be correct in its simplest form, since if it were, the cations derived from **1b** and **1c**, for which conformation C^+-1′ should be more strongly favored because of the location of the benzylic hydroxyl group in a bay region, ought to give at least as much cis hydration (~87–89% in acid) as benzo[*a*]pyrene diol epoxide **1**. Instead only 15–23% cis hydration is observed for **1b** and **1c** under acidic conditions. These results suggest that attack on conformation C^+-1′ occurs *preferentially trans* to the epoxide oxygen from a pseudoaxial direction (ref 22). Thus, extensive cis hydration of benzo[*a*]pyrene diol epoxide **1** and other diol epoxide-**1** isomers with a bay-region epoxide group and a non-bay-region diol must result primarily from pseudoaxial attack on the *other possible conformation* (C^+-1″) of the cation. For **1b** and **1c**, the lack of complete trans stereospecificity may indicate either a minor pathway via C^+-1″ or a small contribution of pseudoequatorial attack on C^+-1′.

(26) Kasperek, G. J.; Bruice, T. C.; Yagi, H.; Jerina, D. M. *J. Chem. Soc., Chem. Commun.* **1972**, 784–785.

(27) Gillilan, R. E.; Pohl, T. M.; Whalen, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 4482–4484.

(28) Whalen, D. L.; Ross, A. M.; Montemarano, J. A.; Thakker, D. R.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1979**, *101*, 5086–5088.

the "reverse" diol epoxide **1b** gives no detectable keto diol,[30] whereas as much as 70% of the "normal" diol epoxide **1a** may be converted to this product (cf. Table II). Keto diol formation from **2b** but not from **1b** is accounted for by the reversal of conformational preferences in **1b** and **2b** relative to "normal" diol epoxides such as **1a** and **2a**. Thus, **2b**, unlike **2a**, can easily assume the aligned conformation, which upon neutral ring opening gives a zwitterion in which the hydrogen that migrates to give keto diol is pseudoaxial. For the isomer-**1** compounds, this aligned conformation is more easily assumed by **1a** and is highly disfavored for **1b**.

(2) Only 10–20% of the products from the k_o reaction of isomers **1** of bay-region epoxides with non-bay-region diol groups consists of the trans tetraol,[5,8,9a] regardless of the parent hydrocarbons from which these diol epoxides are derived. This is consistent with the hypothesis that all of these "normal" isomer-**1** diol epoxides undergo the k_o reaction predominantly via the aligned conformer and/or the related zwitterion $[C^{\pm}]''$. More extensive formation (44–51%) of trans tetraols from the conformationally related "reverse" diol epoxide, **2b**, and from **2c** suggests that, although these compounds react at least in part via the aligned conformer (as shown by relative rates and by keto diol formation), their preference for reaction via this conformer may not be as strong as that of the "normal" isomer-**1** diol epoxides. The k'_i and k'_{nuc} pathways via the nonaligned conformers of **2b** and **2c** avoid an eclipsing interaction between a hydroxyl group and the adjacent epoxide oxygen that would occur in the transition state as the ring opens via the aligned conformer (k''_i). The analogous eclipsing interaction in the non-bay-region diol, bay-region epoxide-**1** series is not so severe, since it involves only a hydrogen.

(3) For the great majority of isomers **2** of bay-region epoxides with non-bay-region diol groups[5,9a] and for the conformationally related "reverse" diol epoxide **1b**, essentially 100% of the product of the k_o reaction corresponds to trans addition of water. All these compounds are postulated to react primarily via the nonaligned conformer (k'_{nuc} or k'_i and k'_i). 7,8,9,10-Tetrahydrobenzo[*a*]-pyrene-7,8-diol 9,10-epoxide **2**, which corresponds to the most highly stabilized benzylic cation in the series studied to date, gives 50–60% cis addition of water[8] and thus constitutes an exception to the generalization that trans hydration is predominant for these compounds in the k_o reaction. We suggest that this exceptional behavior of benzo[*a*]pyrene diol epoxide may result from the unusually high stability of its derived ionic intermediates, according to one or both of the following mechanisms. (i) If zwitterionic intermediates are involved, increasing the stability of the ion should result in a decrease in k'_i but little or no change in k_p; thus, the k_p pathway involving protonation at oxygen may become competitive with k'_i in the reaction of $[C^{\pm}]'$, the favored conformation of the zwitterion from isomer **2**. If this proton-transfer process generates an ion pair $[C^+ \cdot OH^-]'$ that collapses rapidly to the cis tetraol, it provides a new route for cis hydration. (ii) An alternative, or additional, mechanism for increased cis hydration of benzo[*a*]pyrene diol epoxide **2** does not require zwitterionic intermediates although it is not inconsistent with their existence. According to this mechanism, stabilization of the cation will result in a favorable competition of all pathways involving ionic intermediates with the S_N2 processes k'_{nuc} and k''_{nuc} that avoid such intermediates. Since several of these ionic pathways (k'_{ga}, k''_{ga}, k''_c, and k_p) lead to cis product whereas k'_{nuc} and k''_{nuc} give trans product, increasing the importance of any or all of these ionic pathways relative to k'_{nuc} and k''_{nuc} should lead to increased cis tetraol formation.

For the tetrahydro epoxides also, trans hydration in the k_o reaction is favored over cis hydration and/or ketone formation as the stability of the ion derived from ring opening of the epoxide is decreased. For example, in the k_o reaction of the bay-region tetrahydro epoxides of benzo[*a*]pyrene[5] and phenanthrene[5,15] and the 1,2-epoxide of tetrahydronaphthalene,[18] trans hydration occurs to the extent of 14, 60–100, and 100% respectively. The analogous 6-methoxy-1,2-epoxy-1,2,3,4-tetrahydronaphthalene gives only 7% trans hydration,[31] a result similar to that for the benzo[*a*]-pyrene tetrahydro epoxide. Deuterium isotope effects for the *benzylic* hydrogen of the unsubstituted and methoxy-substituted tetrahydronaphthalene epoxides suggest an S_N2-like transition state for the unsubstituted naphthalene derivative and a more ionic transition state for the methoxy-substituted compound,[27] consistent with the idea that the observed products of the k_o reaction could arise from a competition between the k'_{nuc} (or k''_{nuc}) pathway (trans product) and ionic pathways (cis, ketone, and trans product). This interpretation is analogous to that advanced by Battistini et al.[32] to explain similar effects of electron-donating substituents on product distribution in the *acid-catalyzed* hydrolysis of 1-arylcyclohexene oxides, with the added feature that, for the diol epoxide system, conformation as well as cation stability plays a decisive role in determining the mechanistic pathway.

Although trans hydration of non-bay-region diol, bay-region epoxides **2** and tetrahydro epoxides in the k_o reaction is consistent with a possible role for an S_N2-like process for those epoxides that form highly unstable ions upon ring opening, the lack of significant trans hydration in the isomer-**1** series of non-bay-region diol, bay-region epoxides, regardless of the stability of the derived ions, indicates that isomer **1** of these epoxides usually "avoids" a classical S_N2 reaction pathway. Presumably this preference results from the greater ease with which isomer **1** assumes the aligned conformation, in which cation formation and ketonic rearrangement are facilitated relative to the S_N2 reaction.

Summary

We tentatively propose the following "rules" to account for and predict the rates and products of hydrolysis of diol epoxides derived from polycyclic aromatic hydrocarbons. (1) These epoxides can exist in two rapidly interconvertible conformations in which the benzylic C–O bond of the epoxide is either "aligned" with the π orbitals of the aromatic ring (**1''** and **2''**, Table III) or less well aligned with these orbitals (**1'** and **2'**). Ring opening of the epoxide gives a conformationally related pair of carbocations, which may or may not interconvert prior to their capture by water, depending on the stability of the cation. For purposes of this discussion, we define diol expoxides of conformational type A as those in which a significant fraction exists as the *aligned conformer* at equilibrium, and diol epoxides of conformational type N as those in which *all or nearly all* of the epoxide exists as the *nonaligned conformer*.

(2) For those diol epoxides in which an appreciable amount of the reactant is present as the aligned conformer (conformational type A), pH-independent hydrolysis (k_o) occurs more rapidly via this conformer, resulting in a relatively *fast rate* and extensive formation of *cis tetraol* and/or *keto diol products*. Under acid conditions (k_H), epoxides of conformational type A hydrolyze to give cis and trans tetraols. In the k_H reaction, the *cis tetraol* is produced by attack of water upon the cationic intermediate whose conformation is related to the *aligned conformation* of the epoxide. This pathway of attack is favored by the following factors: (a) an increase in the equilibrium concentration of the aligned con-

(29) The failure to detect these products from isomer **2** of the "normal" diol epoxides may be due in part to experimental factors, since k_o usually is small for these epoxides and is consequently predominant only at relatively high pH values. The combination of high pH and relatively low values of k_o could lead to a situation where small amounts of a keto diol product from isomer **2** decompose as rapidly as they are formed, and are not detected. The recovery of only 82% of the expected tetraol products from the "normal" diol epoxide **2a** at pH ~9.1 (Table II) could result from formation of *small* amounts of unstable keto diol from this compound. For comparison, only 53% of the "reverse" diol epoxide **2b** is recovered as tetraols under similar conditions (pH 8.4). Keto diol from **2b** was easily isolatable at pH 7, and was identified after borohydride reduction and conversion to a pair of isomeric triol triacetates. Thus, regardless of whether or not small amounts of keto diol are formed from **2a**, larger amounts of this product are formed from **2b**.

(30) A report (Vyas, K. P.; Yagi, H.; Levin, W.; Conney, A. H.; Jerina, D. M. *Biochem. Biophys. Res. Commun.* **1981**, *98*, 961–969) that a keto diol is formed from 1,2,3,4-tetrahydrochrysene-3,4-diol 1,2-epoxide **1**, a "reverse" diol epoxide analogous to **1b** with a bay-region diol and a non-bay-region epoxide group, was not based on unequivocal identification of the keto diol formed from the diol epoxide under hydrolytic conditions similar to our present ones, and is probably in error.

(31) Gillilan, R. D.; Pohl, T. M.; Whalen, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 4481–4482.

(32) Battistini, C.; Balsamo, A.; Berti, G.; Crotti, P.; Macchia, B.; Macchia, F. *J. Chem. Soc., Chem. Commun.* **1974**, 712–713.

former of the reactant and/or its corresponding cation (e.g., **2c** relative to **2b**), (b) an increase in the lifetime of the initially formed cation (comprising both conformers) that permits equlibration prior to water attack, to give a mixture enriched in the conformer of the cation corresponding to the aligned conformer of the reactant (e.g., in the isomer-**1** series of non-bay-region diol, bay-region epoxides[6]), and (c) the absence of substituents vicinal to the epoxide that may hinder ring opening of the aligned conformer because of an eclipsing interaction in the transition state (e.g., type A epoxides in the isomer-**1** series relative to **2b** and **2c**).

(3) For those diol epoxides in which the equilibrium *strongly favors* the nonaligned conformer of the reactant (conformational type N), pH-independent hydrolysis (k_o) is relatively *slow* and gives no detectable keto diol product. The predominant or exclusive product of the k_o reaction is the *trans tetraol*, except in the case of the highly reactive benzo[*a*]pyrene diol epoxide **2**, which yields both cis and trans tetraols. Under acidic conditions, all the diol epoxides of type N studied to date give *trans tetraol* as the *major product*. The relative amount of trans tetraol formed in the k_H reaction appears to be somewhat smaller for isomer-**1** than for isomer-**2** compounds of conformational type N.

Experimental Section

Syntheses of diol epoxides **1a** and **2a**,[4] **1b** and **2b**,[11] and **1c**, **2c**, **1d**, and **2d**[10] are described elsewhere. Tetrahydro epoxides were synthesized from the corresponding olefins by oxidation with *m*-chloroperoxybenzoic acid[33] or base-catalyzed cyclization of the bromohydrins.[34] Reaction rates were measured at 25 °C in 1:9 dioxane:water containing 0.1 M sodium perchlorate and 10^{-3} M buffers (sodium formate, sodium acetate, tris(hydroxymethyl)aminomethane, Mes [2-(*N*-morpholino)ethanesulfonic acid] and Caps [3-(cyclohexylamino)propanesulfonic acid]. Pseudo-first-order reactions of **1–3a** and **1–3b** were followed with a Cary 219 spectrophotometer. In some cases where end points were unstable because of product decomposition, end points were estimated from the absorbance change after 4–6 half-lives, with appropriate corrections for incomplete reaction. Wavelengths (nm) for the spectrophotometric kinetics measurements were as follows: 257 or 260 (**1a**); 257 (**2a**); 260 (**3a**, pH 5–8) 264 (**3a**, pH 8.5); 263 (**3a**, pH 9.2); 261 (**1b**, **2b**); 260 (**3b**, pH 4.8–6); 268 (**3b**, pH 8.3–9.5). The wavelengths used for **3a** and **3b** at high pH were chosen to minimize the biphasic kinetics and drifting end points experienced with these compounds as a result of product instability under these conditions. Reactions of **1c–3c** and **1d–3d** were followed on a Gilford spectrophotometer at 259 (**1c** and **2c**), 261 (**3c**), and 277 nm (**1d–3d**). Because of the extremely slow rate of hydrolysis of **1b** under neutral conditions reactions of this compound were followed spectrophotometrically for only ~1.3 half-lives (80–90 h) and pseudo-first-order rate constants were estimated using the Guggenheim method.[35] Rate constants calculated in this way were in acceptable agreement with rate constants determined by chromatographic analysis of reaction mixtures (see below).

High performance liquid chromatography (HPLC) was carried out on a Spectra Physics Model 3500B or a Spectra Physics Model 8000 liquid chromatograph. The following chromatographic systems were used: A, a Waters Associates μBondpak C_{18} column, 3.9 × 300 mm, eluted with 45% methanol in water for 5 min followed by a linear gradient of 1% methanol/min at a flow rate of 1.2 mL/min; B, the same type of column as A, eluted with 50% methanol in water for 5 min followed by a linear gradient of 1% methanol/min at a flow rate of 1.2 mL/min; C, a Du Pont Zorbax ODS column, 6.2 × 250 mm, eluted isocratically with 50% methanol in water at a flow rate of 1.5 mL/min; D, the same type of column as C eluted with 50% methanol in water for 23 min, followed by a linear gradient of 2% methanol/min, at a flow rate of 1.5 mL/min; E, a Du Pont Zorbax ODS column, 4.6 × 250 mm, eluted with 50% methanol in water for 10 min, followed by a linear gradient of 1% methanol/min, at a flow rate of 1 mL/min.

Rate constants (k_o) for **1a–3a** and **1b–3b** were determined chromatographically, and product formation was followed during the course of reactions after trapping of unreacted diol epoxide as the mercaptoethanol adduct.[9a] In a typical experiment, aliquots of a reaction mixture containing $1–12 \times 10^{-6}$ M diol epoxide or $\sim 6 \times 10^{-6}$ M tetrahydro epoxide were quenched with one-tenth volume of 2 M mercaptoethanol, 20% as the sodium salt. After at least 15 min, samples of the quenched solutions were subjected to HPLC with the systems described above, as follows: **1a**, system D; **2a**, system A or C; **3a** and **3b**, system B; **1b** and **2b**, system A; **1c** and **2c**, system E. Trapped epoxide (as the thioether) and products were quantified by integration of peak areas (254–255 nm) relative to an internal standard of *p*-nitrobenzyl alcohol (for the diol epoxides) or benzhydrol (for the tetrahydroepoxides). Typical chromatographic separations of products and thioether from **1b** and **2b** are shown in Figure 4. For **1a–3a**, **3b** and **1c** (not shown), the trans and cis tetraols (or diols from **3a** and **3b**) and the thioether are eluted in the same order as for the compounds illustrated. For **2c**, the order of elution in system E is *trans*-**2c**, thioether, *cis*-**2c**. Rate constants were ordinarily determined from semilogarithmic plots vs. time of the relative peak area corresponding to the thioether, and in most cases were in satisfactory agreement with rate constants calculated from the time-dependent increase in products. In the case of **3b**, measurement of the thioether peak was not possible, because an unstable product gave rise to varying amounts of an unknown compound that co-eluted with the thioether; thus, the rate constant was determined from the time-dependent increase in concentration of the *trans*-tetraol.

Diol and tetraol products were identified by comparison of their chromatographic behavior with that of the known compounds and by co-chromatography with known standards, where necessary.[10,11,36–38] The keto diol product from the hydrolysis (pH 7.0) of **2b** was isolated by HPLC on a Waters Associates μBondapak C_{18} column, 7.8 × 300 mm, eluted with 57% methanol in water at a flow rate of 3.6 mL/min. The chromatographed sample was reduced with sodium borohydride and the resultant mixture of triols was converted to the acetates by treatment with pyridine/acetic anhydride (16 h, room temperature). The acetates were separated by HPLC on a Du Pont Zorbax SIL column, 9.5 × 250 mm, eluted with 1% tetrahydrofuran in methylene chloride at a flow rate of 5.6 mL/min: major isomer, k' 2.7, *m/e* (CI, NO–N_2) 406 (M^+); minor isomer, k' 3.8, *m/e* (CI, NO–N_2) 406 (M^+).

Registry No. 1a, 63493-01-6; **1b**, 76094-81-0; **1c**, 70981-76-9; **1d**, 70981-75-8; **2a**, 63438-26-6; **2b**, 76094-80-9; **2c**, 68151-06-4; **2d**, 68151-05-3; **3a**, 64521-16-0; **3b**, 64521-17-1; **3c**, 74444-59-0; **3d**, 66788-11-2.

(33) Lehr, R. E.; Taylor, C. W.; Kumar, S.; Mah, H. D.; Jerina, D. M. *J. Org. Chem.* **1978**, *43*, 3462–3466.
(34) Yagi, H.; Vyas, K. P.; Tada, M.; Thakker, D. R.; Jerina, D. M. *J. Org. Chem.* **1982**, *47*, 1110–1117.
(35) Frost, A. A.; Pearson, R. G. In "Kinetics and Mechanism", 2nd ed.; Wiley: New York, 1961; pp 49–50.
(36) Thakker, D. R.; Levin, W.; Yagi, H.; Tada, M.; Ryan, D. E.; Thomas, P. E.; Conney, A. H. Jerina, D. M. *J. Biol. Chem.* **1982**, *257*, 5103–5110.
(37) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *J. Org. Chem.* **1977**, *42*, 736–744.
(38) Vyas, K. P.; van Bladeren, P. J.; Thakker, D. R.; Yagi, H.; Sayer, J. M.; Levin, W.; Jerina, D. M. *Mol. Pharmacol.* **1983**, *24*, 115–123.