Comparative Solvolytic Reactivity of Bay-Region Diol Epoxides Derived from Dibenz[a,j]anthracene and Dibenzacridines

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Abstract: Rates and products have been measured in 1:9 dioxane–water, ionic strength 0.1 M, at 25 °C for the solvolyses of the two diastereomeric bay-region 3,4-diol 1,2-epoxides derived from *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*c,h*]acridine (diol epoxide-1, in which the epoxide and the benzylic hydroxyl groups are cis to each other, and diol epoxide-2, in which these groups are trans). Comparative study of these diol epoxides and their carbocyclic analogues, the dibenz[*a,j*]anthracene 3,4-diol 1,2-epoxides, showed that the nitrogen atom at position 14 in the ring had only a small influence (2–5-fold deceleration) on the rate constants for either acid-catalyzed (k_H) or pH-independent (k_0) solvolysis. In these diol epoxides, the nitrogen is not in direct conjugation with the presumed carbocationic intermediate formed at C-1. These aza- and carbocyclic diol epoxides also closely resembled each other in their product distributions upon acid-catalyzed and pH-independent solvolysis. The use of a rapid-mixing technique permitted measurement of solvolysis rates for the dibenz[*c,h*]acridine diol epoxides at pH values down to ca. 1.2 ($t_{1/2}$ 200–500 ms). At these low pH values, a slight curvature in the plots of log k_{obsd} vs pH was observed, which was consistent with only partial protonation of the nitrogen at the lowest pH values used. From the kinetic data, pK_a values of 0.8–1.2 were estimated for the protonated N-14 in the dibenz[*c,h*]acridine diol epoxides. In contrast, pK_a values of 2.5–3.5 were measured for tetrahydro derivatives of dibenz[*c,h*]acridine that have hydroxyl substituents or are unsubstituted on the tetrahydro benzo ring in the bay region. Dibenz[*a,j*]acridine 3,4-diol 1,2-epoxide-2, which has a nitrogen at position 7, undergoes hydronium ion catalyzed solvolysis (k_H) about 23 times more slowly than does the corresponding carbocyclic dibenz[*a,j*]anthracene diol epoxide. For the dibenz[*a,j*]acridine 3,4-diol 1,2-epoxide, unlike the diol epoxides derived from dibenz[*c,h*]acridine, one resonance contributing form of the benzylic C-1 carbocation associated with solvolysis places positive charge on nitrogen. Thus, stability of the transition state for solvolysis of the dibenz[*a,j*]acridine diol epoxide is decreased. At pH values <2, dibenz[*a,j*]acridine diol epoxide-2 exhibits reactivity consistent with a mechanism for solvolysis that involves protonation on oxygen of the *already N-protonated* substrate. This observation constitutes, to our knowledge, the first example of such a dicationic mechanism for solvolysis of a heterocyclic epoxide derivative of this type. The pK_a for this diol epoxide protonated at N-7 is 4.42 as compared with 5.00 for the corresponding tetrahydro 3,4-diol; thus, the effect of a bay-region epoxide group on the pK_a of this nitrogen is much less than the analogous effect on the bay-region N-14 in the dibenz[*c,h*]acridine derivatives.

Numerous investigations over the past 15 years have indicated that the most important ultimate carcinogens formed upon metabolism of carcinogenic alternant polycyclic hydrocarbons (PAH) are benzo-ring diol epoxides in which the epoxide group forms part of a bay region.[1,2] Analogous metabolites are possible from aza-PAHs.[3] For example, metabolism of the highly carcinogenic aza-PAH dibenz[*c,h*]acridine by rat liver microsomes produces (10–17%) the *trans*-3,4-dihydrodiol,[4] which is further metabolized[5] (to an extent of 15–23%) to the bay-region diol epoxides **1a** and **2a** (Figure 1). We are interested in the effect of the acridine ring nitrogen on both the chemical reactivity and the biological activity of these diol epoxides relative to their carbocyclic analogues **1b** and **2b**. Reactivity of a diol epoxide toward ring opening by solvent depends in large measure upon the ability of the aromatic system to stabilize a positive charge at the benzylic position. Theoretical calculations[6] have suggested that nitrogen should exert only a very slight effect on the ease of cation formation at C-1 when substituted for carbon at position 14. This presumably results from the fact that in the benzylic cations derived from **1a** and **2a**, nitrogen is not in direct conjugation with C-1; if such conjugation were possible a much larger destabilization of the cation by nitrogen would be expected. Although extensive data are available regarding the effects of structure on solvolytic reactivity of diol epoxides in carbocyclic systems, analogous information is lacking for any aza-PAH. Thus the hypothesis that the aza and carbocyclic diol epoxides (**1a** and **2a** vs **1b** and **2b**)

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

parent hydrocarbon[a]	compd	k_H, M^{-1} s^{-1}	10^5k_0, s^{-1}
DB[*c,h*]Acr	**1a**	35	2.7
DB[*a,j*]A	**1b**	76	13
DB[*c,h*]Acr	**2a**	116	0.69
DB[*a,j*]A	**2b**	234	1.5
DB[*a,j*]Acr	**2c**	10.2	

[a] Abbreviations are DB[*c,h*]Acr, dibenz[*c,h*]acridine; DB[*a,j*]Acr, dibenz[*a,j*]acridine; DB[*a,j*]A, dibenz[*a,j*]anthracene.

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(1) Jerina, D. M.; Lehr, R. E.; Yagi, H.; Hernandez, O.; Dansette, P. M.; Wislocki, P. G.; Wood, A. W.; Chang, R. L.; Levin, W.; Conney, A. H. In *In Vitro Metabolic Activation in Mutagenesis Testing*; de Serres, F. J., Fouts, J. R., Bend, J. R., Philpot, R. M., Eds.; Elsevier-North Holland Biomedical Press: Amsterdam, 1976; pp 159–177. 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, England, 1977; pp 709–720.

(2) Thakker, D. R.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Jerina, D. M. In *Bioactivation of Foreign Compounds*; Anders, M. W., Ed.; Academic Press: New York, 1985; pp 177–242.

(3) Lehr, R. E.; Wood, A. W.; Levin, W.; Conney, A. H.; Jerina, D. M. In *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure–Activity Relationships*; Yang, S. K., Silverman, B. D., Eds.; CRC Press: Boca Raton, FL, 1988; Vol. 1, pp 31–58.

(4) Thakker, D. R.; Shirai, N.; Levin, W.; Ryan, D. E.; Thomas, P. E.; Lehr, R. E.; Conney, A. H.; Jerina, D. M. *Proc. Am. Assn. Cancer Res.* **1985**, *26*, 114. Full paper in preparation.

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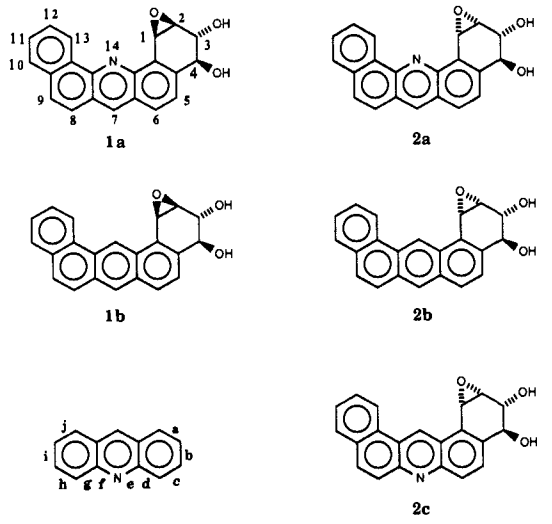


Figure 1. Structures of the diol epoxides. Absolute configuration is not implied. Diastereomers designated as diol epoxide-1 (**1a** and **1b**) have the epoxide oxygen and the benzylic hydroxyl group cis to each other, whereas diol epoxide-2 isomers (**2a–c**) have these group trans. The associated parent hydrocarbons are dibenz[*c,h*]acridine (**a**, DB[*c,h*]Acr), dibenz[*a,j*]anthracene (**b**, DB[*a,j*]A), and dibenz[*a,j*]acridine (**c**, DB-[*a,j*]Acr). Bond lettering of acridine is illustrated.

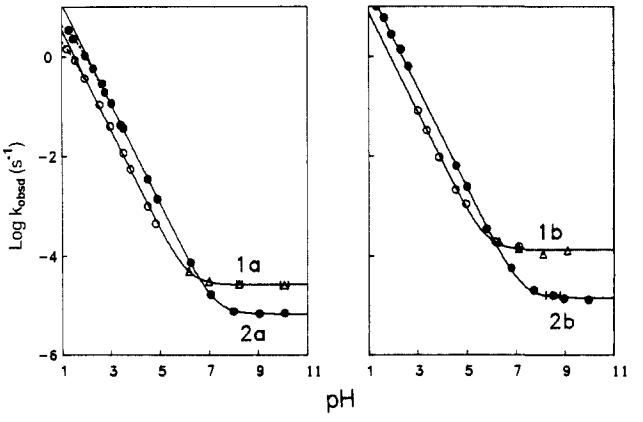


Figure 2. Dependence on pH of the logarithms of the observed pseudo-first-order rate constants for solvolysis of the dibenz[*c,h*]acridine diol epoxides (left panel) and their dibenz[*a,j*]anthracene analogues (right panel). Kinetics were measured at 25 °C in 1:9 dioxane–water, ionic strength 0.1 M ($NaClO_4$). Rate constants were determined spectrophotometrically (circles) or by HPLC following trapping of unreacted diol epoxides (triangles); for experimental procedures, see text. The broken lines at low pH for the dibenz[*c,h*]acridine diol epoxides are based on eq 2 and the rate constants of Table I as well as estimated pK_a values of 0.85 and 1.2 for diol epoxides **1a** and **2a**, respectively.

should exhibit similar reactivities has been purely conjectural. This study represents the first report of the solvolytic kinetics of an aza-substituted PAH diol epoxide, and confirms the expectation that nitrogen in a nonconjugated position has relatively little effect on solvolytic reactivity, whereas the rate for acid-catalyzed solvolysis of a diol epoxide in which *a positive charge at the bay-region benzylic position is in direct conjugation with nitrogen* is substantially retarded.

Results

The pH–rate profiles for the solvolyses of diol epoxides **1a** and **2a** and **1b** and **2b** are shown in Figure 2. As in the case of other diol epoxides,[7,8] the pH dependence of the observed pseudo-first-order rate constants (k_{obsd}) is consistent with a rate law (eq 1) comprising both pH-independent (k_0) and hydronium-ion

$$k_{obsd} = k_H a_{H^+} + k_0 \quad (1)$$

catalyzed (k_H) pathways. Values of the rate constants for the diol epoxides are listed in Table I. We had anticipated that at low pH a leveling off of the rate for the aza compounds would be observed[9] due to protonation of the nitrogen to give an unreactive species (Scheme I), such that the rate law in this region of the pH–rate profile would be given by eq 2, where K_a is the

$$k_{obsd} = K_a k_H a_{H^+}/(a_{H^+} + K_a) \quad (2)$$

Scheme I

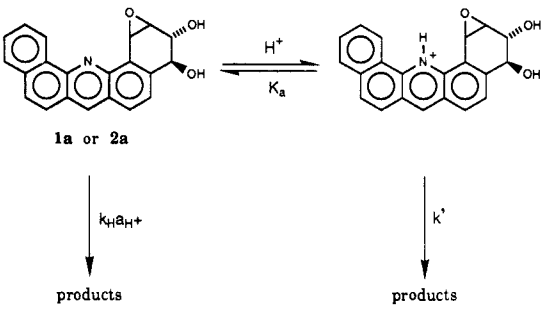


Table II. pK_a Values for Selected Aza Polycyclic Aromatic Hydrocarbon Derivatives in 1:9 Dioxane–Water, Ionic Strength 0.1 M ($NaClO_4$)

compd	pK_a
benz[*c*]acridine[a]	4.14
trans-3,4-dihydroxy-1,2,3,4-tetrahydro-DB[*c,h*]Acr	2.56
trans-**2a** tetraol[b]	2.87
cis-**2a** tetraol[b]	3.45
DB[*c,h*]Acr diol epoxide-1 (**1a**)	≤0.85[c]
DB[*c,h*]Acr diol epoxide-2 (**2a**)	~1.2[c]
benz[*a*]acridine[a]	5.12
trans-3,4-dihydroxy-1,2,3,4-tetrahydro-DB[*a,j*]Acr	5.00
trans-**2c** tetraol[b,d]	4.65
DB[*a,j*]Acr diol epoxide-2 (**2c**)	4.42[c]

[a] See Figure 1 for bond lettering in the nomenclature of acridine derivatives. [b] See Figure 6 for structures of the tetraols. [c] Determined from kinetic data; see text. [d] Major (ca. 95%) product of acid-catalyzed hydrolysis of the corresponding diol epoxide; trans hydrolysis of the epoxide at C-1 is assumed.

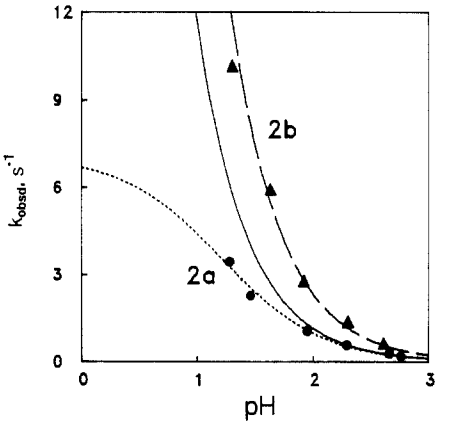


Figure 3. Dependence on pH of the observed rate constants for solvolysis of **2a** (circles) and **2b** (triangles) at low pH. The dotted line for **2a** is calculated from eq 2 on the basis of a pK_a of 1.2 and k_H = 116 M^{-1} s^{-1}. The solid line is a calculated curve for **2a** with the same value for k_H and the assumption that no protonation occurs in the pH range observed (i.e., $pK_a \ll 1$). The corresponding curve for **2b** (k_H = 234 M^{-1} s^{-1}), which has no ionization constant in this pH range, is given by the broken line.

(5) Adams, J. D.; Sayer, J. M.; Chadha, A.; Shirai, N.; Lehr, R. E.; Kumar, S.; Levin, W.; Jerina, D. M. Unpublished work.
(6) Lehr, R. E.; Jerina, D. M. *Tetrahedron Lett.* **1983**, *24*, 27–30. Smith, I. A.; Seybold, P. G. *J. Heterocycl. Chem.* **1979**, *16*, 421–425.
(7) (a) Whalen, D. L.; Montemarano, J. A.; Thakker, D. R.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 5522–5524. (b) Whalen, D. L.; Ross, A. M.; Yagi, H.; Karle, J. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1978**, *100*, 5218–5221.
(8) Sayer, J. M.; Yagi, H.; Croisy-Delcey, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1981**, *103*, 4970–4972.
(9) Bushman, D. R.; Sayer, J. M.; Boyd, D. R.; Jerina, D. M. *J. Am. Chem. Soc.* **1989**, *111*, 2688–2691.

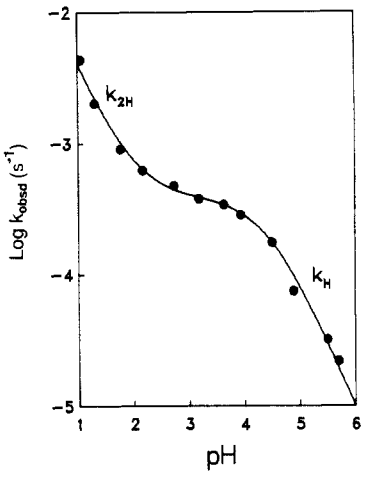
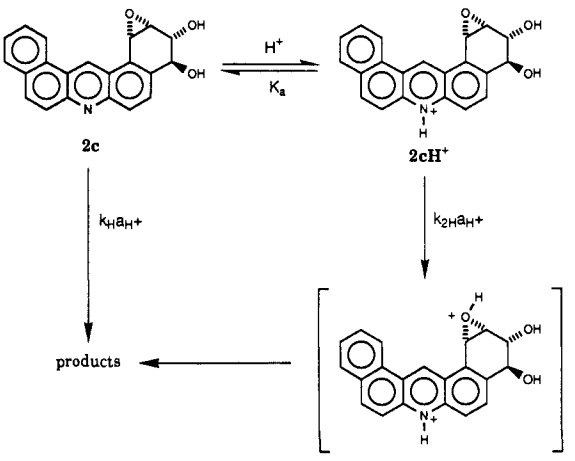


Figure 4. Dependence on pH of the logarithms of the observed pseudo-first-order rate constants for solvolysis of dibenz[*a,j*]acridine 3,4-diol 1,2-epoxide **2c**, at 25 °C in 1:9 dioxane–water, ionic strength 0.1 M ($NaClO_4$). The line was calculated by the use of eq 3c with the values of k_H, k_{2H}, and pK_a that are given in the text.

dissociation constant of the N-protonated epoxide, and at sufficiently low pH ($a_{H^+} \gg K_a$), k_{obsd} would approach a limiting value of $K_a k_H$. Alternatively, if the N-protonated form were to react with water, accounting for the pH dependence at pH values above the pK_a, the observed rate constant at pH values below the pK_a, where nitrogen is fully protonated, would be given by $k_{obsd} = k'$. The break between pH-dependent and pH-independent regions of the profile should occur in either case at the pK_a of the nitrogen. Titration of several model compounds (Table II) suggested that these pK_a values might be in the range of 2–3. In order to detect curvature in the pH–rate profiles that would be diagnostic of protonation at nitrogen in **1a** and **2a**, a rapid-mixing system (as described in the Experimental Section) was required because of the high magnitude of the reaction rates expected at pH 1–3. To verify the accuracy of rate measurements with this system, rates of solvolysis for **2b**, which has no basic nitrogen to protonate in this pH range, were measured (triangles, Figure 3). Rate constants for this compound in the pH range 1.6–3 exhibited no significant deviations from the expected first order dependence on hydronium ion concentration (eq 1) with $k_H = 234$ M^{-1} s^{-1} (as determined at pH 4.5–7), even at half-lives down to 110 ms. Thus, for reactions on this time scale, artifacts due to mixing or to inadequate instrumental response rates were not significant. The fastest rate constant measured for **2b** ($t_{1/2}$ ca. 50–80 ms at pH 1.3) did exhibit substantial scatter among multiple determinations, as well as a small (10–20%) negative deviation of the mean from the theoretical value. Thus, a half-life of 80–100 ms probably represents the lower limit accurately measurable by the method used in these experiments. The observed half-lives of **1a** and **2a** in this pH range are ≥200 ms, and hence systematic experimental errors in these measurements should be negligible. The rate constants for these aza compounds exhibited small to moderate deviations (cf. Figure 3) from the values predicted by eq 1 and k_H determined at higher pH values. These deviations are consistent with incipient curvature in the pH–rate profiles, due to partial protonation of **1a** and **2a**. For example, at pH 1.28, a pseudo-first-order rate constant of 3.45 s^{-1} ($t_{1/2}$ 0.20 s) was observed for **2a**, whereas a rate constant of 6.08 s^{-1} would have been expected on the basis of a first-order dependence on hydronium ion (k_H). Since only partial protonation of nitrogen was indicated even at the lowest pH values studied, the pK_a values for **1a** and **2a** must be substantially lower than expected. Approximate pK_a values of ≤0.85 and 1.2 were estimated by fitting the experimental data at low pH for **1a** and **2a**, respectively, to eq 2 by use of the curve-fitting program MLAB.[10]

In order to investigate protonation at a nitrogen farther removed from the epoxide function, rates of solvolysis of the 3,4-diol 1,2-epoxide-2 diastereomer (**2c**) derived from dibenz[*a,j*]acridine were measured at pH values <6. The rate vs pH profile for this

Scheme II



compound (Figure 4) is remarkably different in shape from those observed in the same pH range for **1a** and **2a** (Figure 2). In particular, there is a distinct break in the profile at pH ca. 4.4, which we ascribe to protonation of **2c** at nitrogen; the protonated species is less reactive toward acid-catalyzed hydrolysis than is the neutral species. A similar observation has been made in the case of the 5,6- and 7,8-arene oxides of quinoline.[9] Unlike the quinoline oxides, **2c** exhibits a second acid-catalyzed process, significant at pH values <2, that corresponds to the reaction of hydronium ion at oxygen of the *N-protonated* ionic form of **2c**. A mechanism that accounts for the observed pH–rate profile (Scheme II) corresponds to the rate law derived in eqs 3a–c.

$$\text{rate} = k_H[\mathbf{2c}]a_{H^+} + k_{2H}[\mathbf{2cH^+}]a_{H^+} \quad (3a)$$

$$k_{obsd} = k_H a_{H^+} \frac{K_a}{(a_{H^+} + K_a)} + k_{2H} a_{H^+} \frac{a_{H^+}}{(a_{H^+} + K_a)} \quad (3b)$$

$$= (k_H K_a + k_{2H} a_{H^+}) \frac{a_{H^+}}{(a_{H^+} + K_a)} \quad (3c)$$

Values of k_H, k_{2H}, and pK_a determined by fitting[10] eq 3c to the measured kinetic data were 10.2 M^{-1} s^{-1}, 3.76 × 10^{-2} M^{-1} s^{-1}, and 4.42, respectively. For comparison, the pK_a values for *trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*a,j*]acridine and for the tetraol hydrolysis product of **2c** were determined by titration to be 5.00 and 4.65, respectively.

Products (Table III) of the reactions of **1a,b** and **2a,b** formed under kinetic conditions were analyzed by high-performance liquid chromatography (HPLC) of reaction mixtures. In the k_0 region, the recovery of tetraols from diol epoxide-1 isomers (both **1a** and **1b**) is low (ca. 25%) relative to the tetraol yield under acidic conditions. This is a result of formation of keto diols, as previously reported for a number of related diol epoxide-1 diastereomers,[7,8,11,12] presumably via a 1,2-hydride shift. Since these keto diols derived from other hydrocarbons are known to be unstable, and furthermore, since they may have extinction coefficients that differ from those for the tetraols, direct quantitation of these products was not attempted. By the use of previously described methods,[8,11] keto diol formation was estimated from the difference between tetraol recovery under k_0 reaction conditions and at pH 3, where 100% recovery of tetraols was assumed. In the case of **1a**, formation of a keto diol was verified by an experiment in which this product was trapped by reduction with sodium borohydride after ca. 4 half-lives of reaction at pH 7. The two products formed upon borohydride reduction had molecular weights corresponding to the expected triols (m/z = 331) by EI–mass spectrometry. The

(10) Knott, G. D. *Comput. Prog. Biomed.* **1979**, *10*, 271–280.
(11) Sayer, J. M.; Whalen, D. L.; Friedman, S. L.; Paik, A.; Yagi, H.; Vyas, K. P.; Jerina, D. M. *J. Am. Chem. Soc.* **1984**, *106*, 226–233.
(12) Yagi, H.; Thakker, D. R.; Hernandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604–1611.

Table III. Products of the Hydronium Ion Catalyzed (k_H) and pH-Independent (k_0) Hydrolysis Reactions of Diol Epoxides under Kinetic Conditions[a]

k_H Pathway[b]			
parent hydrocarbon	compd	% cis hydration	% trans hydration
DB[c,h]Acr	**1a**	48	52
DB[a,j]A	**1b**	64	36
DB[c,h]Acr	**2a**		100
DB[a,j]A	**2b**		>99

k_0 Pathway[c]				
parent hydrocarbon	compd	% cis hydration	% trans hydration	% other or unrecovered products[d]
DB[c,h]Acr	**1a**	11	14	75
DB[a,j]A	**1b**	16	7	77
DB[c,h]Acr	**2a**	≤2	≥96	
DB[a,j]A	**2b**	8	92	

[a] At 25 °C in 10–11% dioxane in water, ionic strength 0.1 M ($NaClO_4$). For abbreviations, see notes for Table I. [b] In 1 mM perchloric acid. [c] In 1 mM CHES buffer, pH 8.8–9.1. Results for diol epoxide-1 agreed well with the product distribution observed in kinetic experiments upon quenching of reaction mixtures with mercaptoethanol (see Experimental Section). [d] Determined from the difference between total tetraol recovery under k_0 conditions and in 1 mM perchloric acid.

major triol formed was also characterized by the ^1H NMR spectrum of its triacetate.

Discussion

The most significant findings of this study are as follows: (i) The substitution of nitrogen for carbon in a poycyclic aromatic hydrocarbon, at a position that is not in direct conjugation with the potential benzylic carbocation, has little effect on the solvolytic behavior (rates or products) of the derived bay-region diol epoxides. (ii) By the same token, substitution of nitrogen for carbon at a position (N-7 of dibenz[a,j]acridine) that is in conjugation with the benzylic cation substantially retards the rate of hydronium-ion catalyzed solvolysis (k_H) of the diol epoxide. (iii) In the case of dibenz[a,j]acridine 3,4-diol 1,2-epoxide-2, a novel dicationic pathway (k_{2H}) for acid-catalyzed solvolysis involving reaction of a hydronium ion with the *N-protonated* species is observed under mildly acidic conditions. This process is experimentally observable because of the relatively high pK_a and low value of k_H for this diol epoxide, which together result in the leveling-off (at pH 3–4) of the overall rate for the k_H process at a plateau value that is exceeded by $k_{2H}a_{H^+}$ as the pH is decreased. (iv) The pK_a value for a protonated nitrogen in the bay region of an aza-PAH is substantially lowered when a bay-region epoxide (as opposed to hydroxyl groups or no substituent) is present.

N-Protonation and the Shape of pH–Rate Profiles. Previous studies of the 5,6- and 7,8-arene oxides derived from quinoline[9] have shown that the pH–rate profiles for solvolysis of these compounds in acid, unlike the pH–rate profiles for naphthalene 1,2-oxide,[13] exhibit a break at pH ca. 3–4, the pK_a of the ring nitrogen, such that the rates (which are first order in hydronium ion above the pK_a) become pH-independent below this pH value. Use of the *N*-methyl cation of quinoline 5,6-oxide as a model for the N-protonated species demonstrated[9] that this ionic form is resistant to hydrolysis at acidic pH values (1.9–4). Thus, the pH–rate profile levels off below pH 3, at a plateau value equal to K_ak_H, because the *concentration* of the reactive species (unprotonated at nitrogen) is *inversely* proportional to a_{H^+} below the pK_a, whereas the *rate* constant for hydrolysis of this species is *directly* proportional to a_{H^+}, due to the requirement for protonation at oxygen in the transition state. We had anticipated a similar break in the pH–rate profiles for the dibenz[c,h]acridine diol epoxides to occur at pH 2.5–3.5, on the basis of pK_a values measured for model compounds (Table II). Surprisingly, such a break was not observed within the easily accessible pH range,[14] although incipient curvature in the pH–rate profiles was detected at pH 1.2–1.5 (Figure 2). Extrapolation to lower pH values by fitting eq 2 to the observed data (cf. Figure 3) gave an estimated pK_a of 1.2 for N-protonation of **2a** and an even lower value (≤0.85) for **1a**. Thus, the bay-region epoxide group decreases the basicity of nitrogen in **1a** and **2a** by about 1.5 units, relative to the analogous tetrahydrodiol that has no substituents in the bay region, and also decreases the basicity by 2–2.5 units relative to the tetraols, which have bay-region hydroxyl substituents. We suggest that the unusually low basicity of these epoxides results from unfavorable steric interactions involving the protonated nitrogen in the bay region as well as an electron-withdrawing inductive effect of the epoxide group. Evidence in support of this hypothesis is provided by the differential effects of bay-region substitution on pK_a values when nitrogen is in the bay region and when it is distant from the bay region.

Dibenz[a,j]acridine, which is isosteric with dibenz[c,h]acridine, provides a model for the investigation of the acid–base behavior of tetrahydro benzo-ring derivatives of an aza-PAH in which the nitrogen is *remote* from the bay region. pK_a values for the tetraol obtained upon acid hydrolysis of epoxide **2c** (presumably by trans ring opening of the epoxide) and of the tetrahydro 3,4-diol unsubstituted in the bay region were 4.65 and 5.00, respectively. From pH 6 down to pH 3, the hydrolysis of diol epoxide **2c** exhibits a pH–rate profile (Figure 4) that is similar in shape to the profiles observed for quinoline arene oxides and is consistent with protonation at nitrogen (pK_a = 4.42) to give a species that is less reactive toward acid catalyzed ring opening than is the unprotonated form. At sufficiently low pH, it is reasonable that the N-protonated form can also undergo solvolysis catalyzed by the hydronium ion, via a dicationic transition state (k_{2H}, Scheme II). Such a pathway, which should be manifested by a second acid-catalyzed limb of the pH–rate profile at very low pH, was not detected at pH values ≥1.5 for either quinoline 5,6- or 7,8-oxide.[9] The observation of this pathway for **2c** (Figure 4; points below pH 2) thus constitutes, to our knowledge, *the first observation of such a dicationic mechanism for solvolysis of a heterocyclic epoxide derivative.* The relatively low reactivity of **2c** via the k_H pathway and its relatively high pK_a result in a small value of the observed rate constant (K_ak_H) in the low-pH plateau, such that it becomes easy to observe the alternative pathway (k_{2H}) at conveniently accessible pH values. The fact that nitrogen and oxygen are relatively far apart in space may also increase the magnitude of k_{2H}, thus facilitating the observation of this mechanism for hydrolysis of **2c** under relatively mild acidic conditions. The large difference between the shapes of the pH–rate profiles for hydrolysis of **2c** (Figure 4) and its dibenz[c,h]acridine analogues **1a** and **2a** (Figure 2, left panel) results largely from the much lower pK_a values of **1a** and **2a** relative to **2c**. The pK_a value for the tetrahydro 3,4-diol derived from dibenz[c,h]acridine is ca. 2.4 units lower than that for the corresponding tetrahydrodibenz[a,j]acridine 3,4-diol. These results parallel the relative basicities of the benz[c]- and benz[a]acridines: benz[c]acridine (pK_a 4.14) is ca. 1 order of magnitude less basic than benz[a]acridine (pK_a 5.12).[15] The pK_a of benz[c]acridine is decreased substantially (1.6 units) upon addition of a tetrahydro benzo ring to form tetrahydrodibenz[c,h]acridine 3,4-diol (pK_a 2.56), but a negligible effect (ca. 0.1 unit) on the basicity of benz[a]acridine is observed upon formation of the analogous tetrahydrodibenz[a,j]acridine 3,4-diol (pK_a 5.00). We suggest that the observed decrease in the pK_a of the bay-region nitrogen upon introduction of a neighboring tetrahydro benzo ring may result from increased steric hindrance to solvation of the protonated nitrogen. Interestingly, introduction of hydroxyl groups on this ring in the bay region *increases* the basicity of the tetrahydro-

(13) Kasperek, G. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1972**, *94*, 198–202.

(14) The use of acid concentrations >0.1 M was not possible because of the requirement (chosen for consistency with previous work; cf. ref 7–9, 11) for a constant ionic strength of 0.1 M.

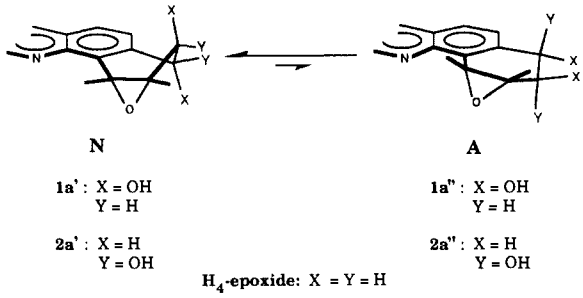
(15) Similar *relative* pK_a values have been measured in 50% aqueous ethanol for benz[c]acridine (pK_a 3.45) and benz[a]acridine (pK_a 4.16): Albert, A.; Goldacre, R.; Phillips, J. *J. Chem. Soc.* **1948**, 2240–2249.

Table IV. ^{13}C and ^{1}H NMR Chemical Shifts (ppm) for Selected Positions in 1,2,3,4-Tetrahydrobenz[*a*]anthracene Derivatives[a]

	unsubstituted	1-hydroxy	1,2-epoxy
H-7	8.37	8.24	8.33
C-7	126.568	126.849	127.036
H-12	8.50	8.66	8.75
C-12	121.147	121.822	120.239

[a] In $CDCl_3$. ^{13}C resonances were assigned by selective decoupling of the proton singlets corresponding to H-7 and H-12.

Scheme III



dibenz[*c*,*h*]acridine tetraols relative to the tetrahydro 3,4-diols, but slightly *decreases* the basicity of the corresponding dibenz[*a*,*j*]acridine tetraol. An inductive effect of oxygen would be expected to decrease the basicity; hence, the opposite effect observed in the dibenz[*c*,*h*]acridine case may result from the ability of a nearby hydroxyl group to solvate the protonated bay-region nitrogen. The observation (Table II) that the pK_a values of the tetraols in the dibenz[*c*,*h*]acridine series are dependent on stereochemistry also supports a possible role of solvation or hydrogen bonding. Thus, the bay-region hydroxyl groups in these tetraols probably do not provide an adequate model for the more sterically restricted epoxide moiety, which may solvate the positive charges on nitrogen less efficiently but whose inductive effect may contribute significantly to the strikingly low basicity of **1a** and **2a**.

The decreased basicity of diol epoxides **1a** and **2a** relative to the corresponding tetrahydro 3,4-diol presumably results in part from an electronic (inductive or field) effect, although several lines of evidence suggest that such an effect of the epoxide group may not be unusually large.

(i) ^{13}C NMR chemical shifts are generally indicative of relative electron densities at carbon[16,17] and, unlike ^{1}H chemical shifts, are relatively insensitive to complicating effects of the magnetic anisotropy of neighboring substituents. Thus, C-7 and C-12 of selected tetrahydrobenz[*a*]anthracene derivatives were used as models for the corresponding nitrogens (N-7 and N-14) in dibenz[*a*,*j*]- and dibenz[*c*,*h*]acridine derivatives, respectively. Table IV summarizes chemical shift data obtained for three model compounds. As expected, C-7 is slightly deshielded in the 1-hydroxy and 1,2-epoxy derivatives, relative to the tetrahydro compound, presumably as a result of electron withdrawal by the oxygen substituent. However, C-12, although deshielded in the presence of the 1-hydroxy group, exhibits an *upfield* shift in the presence of the 1,2-epoxy substituent. Although these effects are too small to permit detailed interpretation, their small magnitude suggests that *a 1,2-epoxy substituent exercises no unusually large electron withdrawing effect* at the bay-region 12-position that is analogous to N-14 in the dibenz[*c*,*h*]acridine derivatives.

(ii) Molecular and quantum mechanics (see Experimental Section) were used to calculate the bonding energies for a series of protonated and unprotonated model compounds containing three fused rings. Structures of these model benzo[*h*]quinoline derivatives and results of the calculations are illustrated in Figure 5.

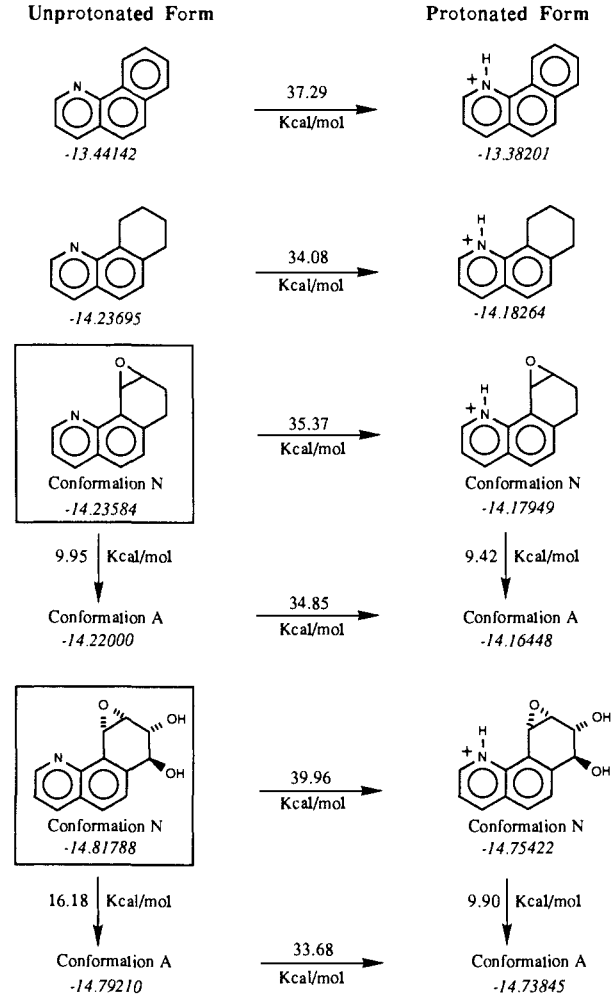


Figure 5. INDO bonding energies and energy differences for protonated and unprotonated benzo[*h*]quinoline derivatives. Bonding energies (in italics) calculated for the individual molecular species are given in atomic units; for a set of related compounds, the most negative value corresponds to the most stable species (as indicated by a box in the case of the epoxides). The numbers accompanying the arrows are energies in kilocalories/mole required for the indicated transformations, and are based on the relationship, 1 au = 627.7 kcal/mol. Larger values of these energies correspond to energetically less favorable processes.

These models were selected since the INDO program used could not handle molecules as large as the dibenzacridine derivatives. Calculations were done for both conformations of the tetrahydro benzo ring as illustrated in Scheme III: conformation A (aligned) in which the benzylic C–O bond is aligned with the π orbitals of the aromatic system, and conformation N (nonaligned) in which this bond is less well aligned with the π system. Several results of the calculations are worthy of note: (1) In the case of both the tetrahydro epoxide and diol epoxide-2, conformation N is favored (lower energy) for both the protonated and unprotonated forms. This is in accord with experimental ^{1}H NMR data for unprotonated diol epoxide-2 (see subsequent discussion). However, this conformational preference appears to be significantly larger in the case of the unprotonated diol epoxide than in the case of the tetrahydro epoxide. This difference may result from unfavorable interactions between an axial hydroxyl group at C-3 and the epoxide oxygen in conformation A of the diol epoxide.[7b] This conformational preference is less strong for the N-protonated diol epoxide, presumably because protonated conformation N is destabilized by an unfavorable interaction between the proton on nitrogen and the benzylic hydrogen of the epoxide (see below). (2) For both the diol epoxide and the tetrahydro epoxide, it is somewhat easier to protonate conformation A than conformation N. This difference presumably results from the close contacts between the N–H proton and a benzylic hydrogen of the epoxide (cf. Scheme III). Results of the molecular mechanics program

(16) Hehre, W. J.; Taft, R. W.; Topsom, R. D. *Prog. Phys. Org. Chem.* **1976**, *12*, 159–187.
(17) Nelson, G. L.; Williams, E. A. *Prog. Phys. Org. Chem.* **1976**, *12*, 229–342 and references therein.
(18) 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.

indicated a distance of 2.68 Å between these two hydrogens in conformer A and a corresponding distance of 1.97 Å in conformer N of the N-protonated diol epoxide. In the fully aromatic parent compound, benzo[*h*]quinoline, the calculated distance between a proton on nitrogen and the bay-region H-10 is 1.81 Å. (3) The calculations predict that it is somewhat more difficult (by 1.3 kcal/mol) to protonate the nitrogen in the predominant conformer N of the tetrahydro epoxide than to protonate this nitrogen in the unsubstituted tetrahydrobenzo[*h*]quinoline, and significantly more difficult (by 5.9 kcal/mol) to protonate this nitrogen in the predominant conformer N of the diol epoxide. This large difference in protonation energy that is calculated for the diol epoxide in the "nonaligned" conformation N seems to be associated with a decrease in delocalization of electrons but not with changes in conformation or bond order, which are negligible. In the unprotonated species several of the lowest energy molecular orbitals involve the nitrogen atom and all three oxygen atoms, in addition to several carbon atoms. In the protonated form, there are fewer such orbitals. The significant conclusion is that the differences in basicity between tetrahydrobenzo[*h*]quinoline and its epoxide derivatives predicted by calculation are in the same direction as those observed experimentally for the analogous dibenz[*c*,*h*]-acridine derivatives. These calculations suggest a role for both steric and electronic factors in the low basicity of the dibenz-[*c*,*h*]acridine diol epoxides. However, direct quantitative comparison between theory and experiment is not appropriate because the measurements of pK_a are made in solution, whereas the theoretical calculations refer to vacuum.

Structure–Reactivity Correlations. As in the case of other bay-region diol epoxides studied to date[7] that lack unusual conformational influences,[11] the dibenz[*a*,*j*]anthracene diol epoxides-1 and -2 exhibit pH–rate profiles that cross, such that $k_H(2) > k_H(1)$, whereas $k_0(2) < k_0(1)$. The pH–rate profiles (Figure 2) for the diastereomeric bay-region diol epoxides derived from dibenz[*c*,-*h*]acridine are very similar to those for the bay-region dibenz-[*a*,*j*]anthracene diol epoxides in their overall appearance. In the case of the hydrocarbon, dibenz[*a*,*j*]anthracene, the rate constants k_H and k_0 for solvolysis of the two isomeric diol epoxide derivatives are normal in that they exhibit no marked deviations from plots[19] of log k_0 or log k_H for a given isomer vs the reactivity index $\Delta E_{deloc}/\beta$, as obtained from perturbational molecular orbital (PMO)[20] calculations. As predicted by this and other calculated indices of reactivity, the bay-region dibenz[*a*,*j*]anthracene 1,2-diol 3,4-epoxides ($\Delta E_{deloc}/\beta = 0.722$) are slightly less reactive than the corresponding benz[*a*]anthracene derivatives[11] ($\Delta E_{deloc}/\beta = 0.766$) in both hydronium ion catalyzed (1.2–1.6-fold) and pH-independent reactions (1.9–2.4-fold).

Both PMO and Hückel molecular orbital (HMO) calculations[6] suggest that nitrogen at a position that is not formally conjugated with a benzylic carbocation should have little or no effect on the ease of formation of such a cation, and thus that solvolytic rate constants for the aza- and carbocyclic PAH diol epoxides should be similar. PMO calculations (ΔE_{deloc}) predict that such a non-conjugated nitrogen atom will have no effect, whereas HMO calculations (ΔE_π) predict that it will produce only a very small decrease in stability of the cation derived from the aza, as compared with the unsubstituted, diol epoxide; thus, for **1a** or **2a** $\Delta E_\pi/\beta$ is 0.829, whereas for **1b** or **2b** this value is 0.833. On the basis of the reported[19] dependence on $\Delta E_\pi/\beta$ of log k_H and log k_0 for solvolyses of diol epoxides-1 and -2, k_H values for **1a** and **2a** are predicted to be ca. 0.9 times the corresponding rate constants for **1b** and **2b**. For pH-independent solvolysis, k_0 for the aza compounds was predicted to be ca. $0.75k_0$ for the corresponding carbocyclic diol epoxides. With one exception, the observed rate constants for the aza compounds (Table I) are about one-half as large as the corresponding constants for their carbocyclic analogues. Thus, these aza compounds are only very slightly (about 1.5–2-fold) less reactive than predicted by calculation.

In the case of dibenz[*a*,*j*]acridine diol epoxide **2c**, one resonance contributor for the carbocation places positive charge on nitrogen, and thus, as expected,[9] the rate constant for its hydronium ion catalyzed hydrolysis is more significantly retarded than that of **2a**, relative to its carbocyclic analogue, **2b**. Simple Hückel molecular orbital calculations[21] indicate that $\Delta E_\pi/\beta$ for **2c** is 0.799 as compared to 0.829 for **2a**, indicative of the anticipated decrease in resonance stabilization of the cation derived from **2c**. Although these calculations qualitatively predict the decreased solvolytic reactivity of **2c** relative to **2b** and **2a**, the measured value of k_H for **2c** is about 10-fold less than expected on the basis of a linear free energy relationship between log k_H and $\Delta E_\pi/\beta$ derived for the carbocyclic diol epoxides.[19] Thus, quantitative predictions of the reactivity of aza relative to carbocyclic diol epoxides based on simple Hückel calculations appear to be unreliable, and more sophisticated approaches may be required.

A notable exception to the 2-fold rate retardation observed upon substitution of nitrogen for carbon in the dibenz[*c*,*h*]acridine diol epoxide series involves k_0 for diol epoxide-1, which is decreased 5-fold upon nitrogen substitution. The somewhat larger deceleration by a bay-region nitrogen in this isomer may result from conformational differences that arise upon substitution of a nitrogen atom for a C–H group at position 14 in the bay region. ^{1}H NMR spectra of **1a**[22] and **1b**[23] in dimethyl sulfoxide exhibit values of 3.7 and 5.8 Hz, respectively, for the coupling constant ($J_{3,4}$) between the methine hydrogens at C-3 and C-4 of the tetrahydro benzo ring. This observation suggests that the hydroxyl groups at C-3 and C-4 of **1a** have a stronger preference for the diaxial orientation than the corresponding hydroxyls of **1b**; i.e., the conformational equilibrium of Scheme III lies somewhat farther to the left in the case where nitrogen, rather than carbon, occupies a position in the bay region. Similarly, diol epoxide-1 derived from benz[*c*]acridine exhibits a small coupling constant, $J_{3,4}$, of ca. 2.5 Hz.[24] These conformational effects presumably result from the decreased steric requirement, and diminished interaction with H-1, of the nitrogen lone-pair electrons relative to a hydrogen atom. A related observation is that the bay-region hydroxyl groups in the dihydro-1,2-diols derived from dibenz-[*c*,*h*]acridine[22,25] and benz[*c*]acridine[24] are equatorial when in chloroform solution ($J_{1,2}$ = 11.8 and 11.5 Hz, respectively), although the diaxial conformation is preferred by the benz[*c*]acridine dihydrodiol in dimethyl sulfoxide.[24] This solvent effect indicates that hydrogen bonding of a hydroxyl to the heterocyclic nitrogen also contributes to the observed conformational preferences.[24] We have previously proposed[11,18] that diol epoxides in the isomer-1 series that strongly prefer conformation N, corresponding to **1a′** in Scheme III, will exhibit diminished reactivity in neutral solvolysis (k_0) reactions, relative to diol epoxides whose conformational equilibrium lies further in the direction of conformation A (**1a″**). This effect may result from stabilization of the developing carbocation derived from **1a″** (relative to **1a′**) by a more favorable resonance interaction with the π electrons of the aromatic system[7b] when the C–O bond that is cleaved is "aligned" with these π orbitals (as in conformation **1a″**). This stereoelectronic effect appears to be significant only in pH-independent (k_0) and not in acid-catalyzed (k_H) solvolysis reactions. Thus, the 5-fold decrease in rate observed upon substitution of nitrogen for C–H in the bay region, which is peculiar to k_0 for isomer 1, can probably be ascribed to the increased preference of **1a** (relative to **1b**) for the less reactive conformation **1a′**. If, prior to ring opening, this conformer must convert to **1a″** via an equilibrium that is less

(19) Sayer, J. M.; Lehr, R. E.; Whalen, D. L.; Yagi, H.; Jerina, D. M. *Tetrahedron Lett.* **1982**, *23*, 4431–4434.

(20) Dewar, M. J. S. *The Molecular Orbital Theory of Organic Chemistry*; McGraw-Hill: New York, 1969; pp 214–217, 304–306.

(21) The program *Hückel Molecular Orbitals*, by J. J. Farrell and H. H. Haddon, was used, with values of $h = 0.5$ and $k = 1.0$ for nitrogen.

(22) Lehr, R. E.; Kumar, S.; Shirai, N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 98–107. The NMR data reported for diol epoxides **1a** and **2a** (compounds **12** and **13** in Table I of this reference) were obtained in Me_2SO-d_6 rather than in $CDCl_3$ as had been inadvertently indicated in the table.

(23) Yagi, H.; Jerina, D. M.; Lehr, R. E.; Kumar, S. Manuscript in preparation.

(24) Lehr, R. E.; Kumar, S. *J. Org. Chem.* **1981**, *46*, 3675–3681.

(25) Kitahara, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1980**, *28*, 1958–1961.

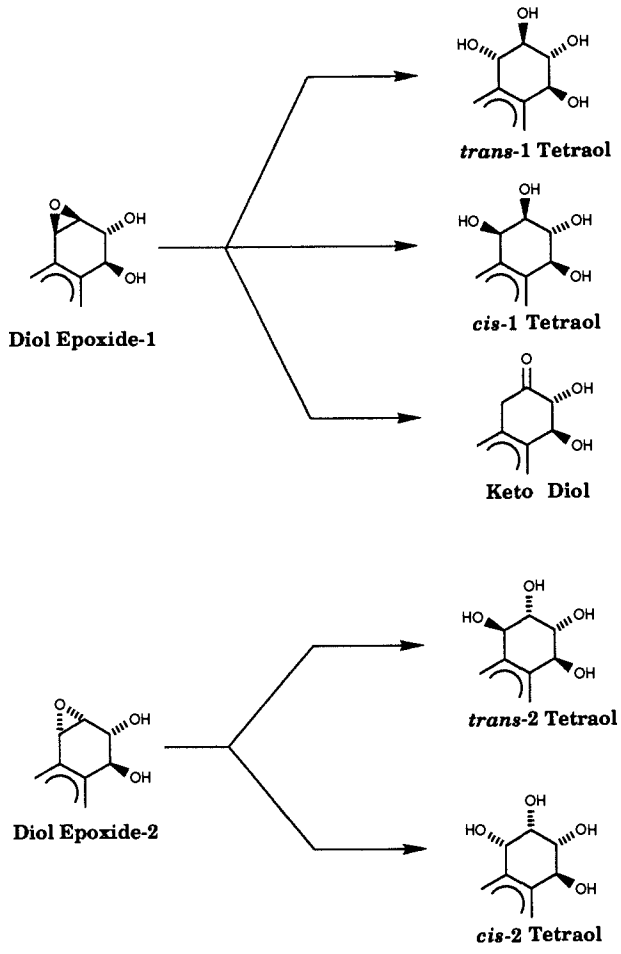
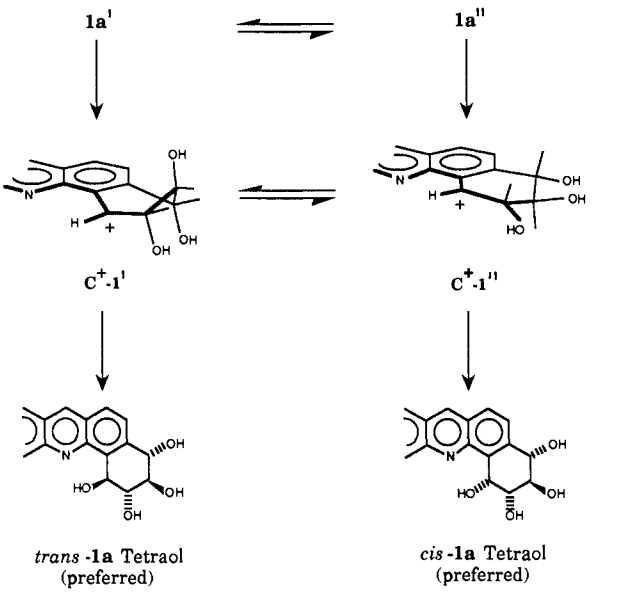


Figure 6. Structures of the hydrolysis products of the diol epoxides.

favorable for **1a** than for **1b**, a retardation of the observed rate for **1a** would result. For the bay-region diol epoxides described herein, there is no apparent difference between the conformations preferred by the diol epoxide-2 isomers in the aza- and carbocyclic cases: values of $J_{3,4}$ for the methine hydrogens at C-3 and C-4 in **2a**, **2b**, and **2c** are 8.9, 8.6, and 8.1 Hz, respectively. Similarly, coupling constants, $J_{3,4}$, close to 8 Hz have been observed for the bay-region 3,4-diol 1,2-epoxides-2 from both benz[*c*]- and benz[*a*]acridine.[24,26] The values of k_0 for **2a** and **2b** differ only by a factor of 2. As in the case of k_H for both diastereomers, this difference is probably ascribable to a small electronic effect upon nitrogen substitution.

Products. Structures of the products formed upon solvolysis of the diol epoxides are shown in Figure 6. The product distributions from **1b** and **2b** are similar to those observed for the corresponding benz[*a*]anthracene derivatives.[11] Bay-region diol epoxide-2 isomers in both series give exclusively or almost exclusively the tetraols derived from trans opening of the epoxide ring, under both acidic (k_H) and neutral (k_0) conditions. In acid, **1b** yields tetraols from both cis (64%) and trans (36%) attack of water at C-1, whereas benz[*a*]anthracene diol epoxide-1 gives 73–75% cis and 25–27% trans hydration. Diol epoxide-1 derived from chrysene, which yields a less stable carbocation ($\Delta E_{deloc}/\beta$ = 0.639) than either the benz[*a*]anthracene ($\Delta E_{deloc}/\beta$ = 0.766) or dibenz[*a,j*]anthracene analogues, undergoes cis hydration to the extent of only 57% under conditions where k_H is the dominant reaction pathway.[7b] Thus, **1b**, whose cation ($\Delta E_{deloc}/\beta$ = 0.722) is intermediate in stability between those derived from the corresponding chrysene and benz[*a*]anthracene diol epoxides, gives an intermediate yield of the cis hydration product, in accordance with expectation. Previous analysis of product data for the k_H reaction of a series of bay-region diol epoxides in the isomer-1 series had led to the proposal that increasing the stability and lifetime of the bay-region benzylic carbocation (increasing $\Delta E_{deloc}/\beta$) should lead to an increase in the extent of cis vs trans hydration.[18] This occurs because increasing the lifetime of the cation permits its equilibration from the conformation that is initially formed (analogous to **C⁺–1′**) to the alternative conformation (analogous to **C⁺–1″**) that undergoes cis hydration via pseudoaxial attack of water (Scheme IV). The aza compound, **1a**, gives somewhat less cis hydration than **1b**, in accordance with its slightly lower reactivity (less stable, shorter lived cation), as well as the greater preference of the diol epoxide for the conformation (**1a′**, "nonaligned") that opens initially to give **C⁺–1′**, the conformation of the cation for which trans attack of solvent is preferred. There appears to be no need to invoke any special directive effect of nitrogen upon the incoming solvent molecule in order to explain the observed product distribution.

Scheme IV



Under conditions of pH-independent solvolysis, both **1a** and **1b**, like other diol epoxide-1 isomers of similar reactivity, afford low yields of tetraols relative to those observed under acid conditions. The material that is not recovered as tetraols presumably corresponds to a keto diol arrangement product (which was partially recovered and identified as its sodium borohydride reduction products from a reaction mixture at pH 7). Similar results have previously been observed with the diol epoxide-1 isomers from benz[*a*]anthracene (ca. 70% keto diol)[11] and chrysene (ca. 65% keto diol).[27]

Experimental Section

Dioxane was distilled from sodium and stored frozen. Syntheses of diol epoxides **1a**, **2a**[22] and **1b**, **2b**[23] are described in detail elsewhere. Diol epoxide **2c** was prepared by reaction of 6.2 mg (0.02 mmol) of *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]acridine[28] with *m*-chloroperoxybenzoic acid (0.02 mmol) in tetrahydrofuran[29] at room temperature in the dark. Progress of the reaction was monitored by the change in UV spectrum from a broad peak centered at ca. 292 nm to a pair of peaks at 280 and 290 nm; an impurity with absorbance at ca. 320 nm also appeared with time. After 2 h, additional peroxy acid (0.02 mmol) was added, and the mixture was stirred for another hour. The reaction mixture was then diluted with ethyl acetate, washed twice with 2% triethylamine in water and then with saturated sodium chloride, and dried over sodium carbonate. The product was purified by HPLC on a Du Pont Golden Series SIL column, 6.2 × 80 mm, eluted with 10% methanol and 1% triethylamine in hexane at a flow rate of 5 mL/min: t_R (min), dihydrodiol, 4.1; diol epoxide **2c**, 5.9. ^{1}H NMR spectrum of **2c** (300 MHz, Me_2SO-d_6): δ (ppm) 3.86 (apparent d, H-2, $J_{2,3} \leq 1$ Hz; $J_{1,2}$ 4.5

(26) Engelhardt, U.; Schaefer-Ridder, M. *Tetrahedron Lett.* **1981**, *22*, 4687–4690.

(27) Sayer, J. M.; Jerina, D. M. Unpublished observation.

(28) Rosario, C. A.; Holder, G. M.; Duke, C. C. *J. Org. Chem.* **1987**, *52*, 1064–1072.

(29) Gill, J. H.; Duke, C. C.; Rosario, C. A.; Ryan, A. J.; Holder, G. M. *Carcinogenesis (London)* **1986**, *7*, 1371–1378.

Hz), 3.93 (dd, H-3, $J_{3,4}$ 8.2 Hz), 4.56 (dd, H-4), 5.54 (d, H-1), 5.72 (d, OH-3, $J_{3,OH-3}$ 4.9 Hz), 5.93 (d, OH-4, $J_{4,OH-4}$ 6.6 Hz), 7.8–8.2 (7 aromatic protons), 9.35 (d, H-13, $J_{12,13}$ 7.8 Hz), 10.33 (s, H-14). *trans*-3,4-Dihydroxy-1,2,3,4-tetrahydrodibenz[*a,j*]acridine was prepared by hydrogenation of the corresponding trans dihydrodiol[28] in methanol for 1 h at atmospheric pressure with 10% palladium on charcoal as catalyst. After purification by HPLC on silica, the identity and purity of the tetrahydrodiol were confirmed by its NMR spectrum. The material obtained upon hydrolysis of **2c** for 1 h at pH 1.3, ionic strength 0.1 M ($NaClO_4$), in 1:9 dioxane–water was shown by HPLC (Du Pont Zorbax ODS column, 4.6 × 250 mm, eluted with methanol (40%) and acetonitrile (10%) in 50 mM Tris–acetate buffer, pH 7, at 1.2 mL/min) to give almost exclusively (ca. 95%) one peak, t_R 7.6 min, detected at 290 nm. This product, presumably *trans*-**2c** tetraol, was used without further purification. pK_a values in 1:9 dioxane–water, ionic strength 0.1 M ($NaClO_4$), were determined by spectrophotometric titration (pH adjusted with perchloric acid) at 296 (*trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*c,h*]acridine) or 295 nm (*cis*-**2a** and *trans*-**2a** tetraols). pK_a values determined at two wavelengths were averaged in the cases of benz[*a*]acridine (276 and 400 nm), benz[*c*]acridine (274 and 292 nm), *trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*a,j*]acridine (280 and 295 nm), and the tetraol derived upon acid hydrolysis of **2c** (278 and 295 nm). With the latter four compounds, pH was controlled by the use of 2 mM buffers.

Reaction rates were measured at 25 °C in 1:9 dioxane–water adjusted to ionic strength 0.1 M with sodium perchlorate, in the presence of buffers as required for pH control. Buffers used were formate, acetate, MES (2-morpholinoethanesulfonic acid), BES (*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), TAPS [3-[[tris(hydroxymethyl)-methyl]amino]-1-propanesulfonic acid], Tris, CHES (2-(cyclohexylamino)ethanesulfonic acid), and CAPS [3-(cyclohexylamino)-1-propanesulfonic acid]. Reaction rates at each pH value were measured in the presence of 1 and 2 mM buffers, except for those reactions of **1a** and **1b** that were followed by HPLC (see below), which were run in 1 mM buffers only. In the other cases, where two buffer concentrations were used, there was no significant effect of buffer concentration on the observed pseudo-first-order rate constants; i.e. rate constants measured at 1 and 2 mM buffers agreed within better than 10%. Rate constants for **2a** and **2b** above pH 3 and for **2c** were measured with a Cary 219 spetrophotometer; wavelength 290 (**2b**), 291 (**2c**, pH >4.5), 293 (**2a**, pH ≥3.5), or 297 (**2a**, pH <3.5 and **2c**, pH <4.5) nm. For **1a** between pH 3 and 6 and **1b** between pH 3 and 8, reactions were followed spectrophotometrically at 293 (**1a**) or 290 (**1b**) nm. Under conditions where k_0 is the predominant reaction pathway, the reactions of diol epoxide-1 diastereomers generally exhibit unstable endpoints, presumably because of the formation and subsequent decomposition of a keto diol. Thus, the progress of reaction under these conditions was most conveniently followed by HPLC subsequent to trapping of unreacted diol epoxide with mercaptoethanol.[8,11] Typical experimental conditions were as follows. (i) For **1a**, 50–100 μL of a dioxane solution containing 1 mM **1a** and ca. 0.02–0.18 μL/mL of (*p*-nitrophenyl)butanol (internal standard) was added to 4 mL of the appropriate buffer solution to give a final concentration of **1a** of 12–25 μM. At appropriate time intervals, 0.3-mL samples were quenched with 0.1 mL of 1 M mercaptoethanol (50% as the base form). After 3–5 min the reaction mixtures were acidified with 0.05 mL of 1 M perchloric acid, and the samples were subjected to HPLC on a Perkin-Elmer HS-3 C_{18} column, 4.6 × 10 mm, eluted at 1.2 mL/min with 5% methanol and 25% acetonitrile in Tris–acetate buffer (50 mM, pH 7.0). Retention times were as follows: internal standard, 7.6 min; *trans*-**1a** tetraol, 9.3 min; *cis*-**1a** tetraol, 9.7 min; thiol adduct, 12.2 min. Peak areas were quantitated at 290 nm, and rate constants were calculated from semilogarithmic plots of the area of the thiol adduct peak relative to internal standard. (ii) With **1b**, reactions were initiated by adding 10–20 μL of a solution containing 0.9–1.8 mM **1b** and 0.16–0.33 μL/mL of (*p*-nitrophenyl)butanol to 4 mL of buffer solution, to give a final diol epoxide concentration of 2.5–9 μM. Samples (0.3 mL) were treated as described with mercaptoethanol, followed by perchloric acid. The samples were analyzed by HPLC (290 nm), on a Perkin-Elmer HS-3 C_{18} cartridge column, 4.6 × 10 mm, eluted at 1.2 mL/min with methanol–acetonitrile–water, 10:25:65, for 12 min, followed by a linear gradient to 75:25 methanol–acetonitrile over 10 min. Retention times were as follows: internal standard, 6.4 min; *trans*-**1b** tetraol, 10.6 min; *cis*-**1b** tetraol, 14.0 min; thiol adduct, 18.2 min.

Fast Reactions. Reactions of **1a** and **2a** at pH values between 1 and 3 were of particular interest, since we had anticipated curvature in the plot of log k_{obsd} vs pH at low pH, as a result of protonation of these compounds at nitrogen. A rapid-mixing technique was required for reactions in this pH range, since half-lives for the compounds at the lowest pH values were expected to be less than 1 s. For this purpose, a SFA-11 rapid kinetics accessory (Hi-Tech Scientific, Salisbury, England), fitted to the Cary spectrophotometer, was used. Spectrophotometric data from the Cary were collected on a microcomputer (IBM PC/AT) as described in the following section. The two solutions to be mixed consisted of equal volumes of (A) the diol epoxide (10 μg/mL) dissolved in 1:9 dioxane–water containing 0.1 M sodium perchlorate and 1 mM Tris buffer, 50% as the perchlorate, and (B) an appropriate dilution of perchloric acid in 1:9 dioxane–water at an ionic strength of 0.1 M (maintained with sodium perchlorate). The progress of reaction was monitored at 297 nm. Measurements at times earlier than 200 ms after triggering were less reliable than those at later times and generally were not used in calculating rate constants. Total absorbance changes in the usable time range were on the order of 0.1 absorbance unit. At least three, and generally five or more, replicate experiments were done at each pH, and the resultant rate constants were averaged; excellent reproducibility was observed. To verify that artifacts due to mixing or instrument response time were not responsible for the negative deviations (Figure 3) of the rate constants for **1a** and **2a** from the calculated values based on measurements using conventional mixing at higher pH, rate constants were also measured for **2b**. In these experiments, a saturated solution of **2b** in the Tris perchlorate buffer described was mixed with appropriate solutions of perchloric acid/sodium perchlorate in the rapid-mixing device, and the absorbance was monitored at 291 nm. Total usable absorbance changes ranged from 0.01 to 0.08 absorbance unit. Rate constants exhibited good reproducibility and agreement with theory down to half-lives of ca. 100 ms, although the fastest rate measured (ca. 50–80 ms) showed scatter in replicate values as well as a slight (ca. 10–20%) negative deviation from the calculated line.

Interface from the Cary 219 Spectrophotometer to the IBM PC/AT. In order to obtain kinetic data from the spectrophotometer when using the rapid-mixing apparatus for reactions with half-lives ca. 0.1–1 s, it was necessary to capture the data directly rather than to rely on the existing digital output of the spectrophotometer, which is limited to one data point/s. In the Cary 219, a 2.18 MHz clock and a CMOS level pulse whose width is proportional to the absorbance are gated together to give a pulse train that is equal to the absorbance value minus an offset. In our installation, the resultant signal is fed to a CTM-05 counter timer card (Metrabyte Corp., Taunton, MA). The pulses are counted for a time interval that is selected by the user from the computer console, and can vary from $^1/_{30}$ to 256 s. The lower limit of $^1/_{30}$ s is a result of the rotation speed of the mirror in the spectrophotometer. The data collection program allows the user to average several successive signals to reduce noise, displays the (averaged) signal and stores it automatically to an ASCII file. The acquisition of data may be triggered in two ways: The signal indicating closure of the sample compartment lid is gated with either (i) a push button or (ii) a switch closure to ground from the stopped-flow apparatus. The data are not affected by any of the external switches (recorder settings, time constants, etc.) on the control panel of the spectrophotometer. The readability is the same as that of the spectrophotometer; i.e., 10^{-4} absorbance unit. Since in our installation, the Cary and the computer were 60 ft apart, line drivers and receivers were used. The data collection program is written in BASIC and is available on request with a 360K formatted disk. Schematics are also available on request.

Products. Identification and characterization of the tetraols obtained upon hydrolysis of **1a** and **2a**[5,22] and of **1b** and **2b**[23] are described in detail elsewhere. For quantitation of products obtained under kinetic conditions (1:9 dioxane–water, ionic strength 0.1 M, maintained with sodium perchlorate), solvolysis of each epoxide at final concentrations of 2.3 and 4.5 μM was carried out in the presence of 1 mM perchloric acid (k_H) or in 1 mM CHES buffer, pH 8.8–9.0 (k_0). The yield of tetraols under k_H conditions was assumed to be quantitative. Recovery of tetraols under k_0 conditions was determined by the difference in tetraol yield in the buffered basic reaction mixtures relative to the yield in 1 mM perchloric acid. To ensure consistency of results, (*p*-nitrophenyl)butanol was added to the stock solutions of epoxides to serve as an internal standard. Analyses of reaction products employed the following chromatographic conditions. For **1a** and **2a**, the Perkin-Elmer C_{18} cartridge column, 4.6 × 100 mm, was eluted at 1.2 mL/min with methanol–acetonitrile–Tris–acetate buffer (50 mM, pH 7), 40:10:50. Detection was at 290 nm. Retention times were as follows: (*p*-nitrophenyl)butanol (internal standard), 3.7 min; *trans*-**2a** tetraol, 7.4 min; *trans*-**1a** tetraol, 8.3 min; *cis*-**1a** tetraol, 9.0 min. The position of the *cis*-**1a** and *cis*-**2a** tetraols as the later-eluting peaks of each pair of tetraols from a given diol epoxide was verified by cochromatography with the tetraol products derived from osmium tetroxide oxidation of the 3,4-dihydrodiol. For analysis of products from **1b**, the Perkin-Elmer cartridge column was eluted at 1.2 mL/min with methanol–acetonitrile–water, 10:25:65, followed by a linear gradient over 10 min to methanol–acetonitrile–water, 10:40:50. Detection was at 294 nm. Retention times were as follows: (*p*-nitrophenyl)-butanol (internal standard), 6.0 min; *trans*-**1b** tetraol, 10.0 min; *cis*-**1b**

tetraol, 13.0 min. The later-eluting tetraol was cochromatographic with the minor tetraol product formed upon osmium tetroxide oxidation of the dihydrodiol. For **2b**, HPLC was run on a Du Pont phenyl column, 4.6 × 250 mm, eluted at 1.2 mL/min with 37% THF in water; detection was at 290 nm. Retention times were as follows: *trans*-**2b** tetraol, 6.5 min; *cis*-**2b** tetraol, 7.8 min; (*p*-nitrophenyl)butanol (internal standard), 8.9 min.

The keto diol product formed from the k_0 reaction of **1a** was identified as follows. Diol epoxide **1a** (ca. 1 mg), in 0.95 mL of dioxane and 0.05 mL of dimethyl sulfoxide, was added to 49 mL of a solution containing 0.1 M sodium perchlorate, 1 mM BES buffer, pH 7, and 8% dioxane. After 24 h, the mixture was acidified to pH ca. 3 with perchloric acid. Excess (ca. 80 mg) solid sodium borohydride was added and the mixture was allowed to stand for 15–20 min. It was then acidified, the products were extracted with ethyl acetate, and the product mixture was subjected to HPLC on a Rainin Microsorb C_{18} column, 10 × 250 mm, equipped with a 50-mm guard column, eluted at 3 mL/min with 40% methanol and 25% acetonitrile in water. Two peaks, t_R 16.7 min (minor) and 23.3 min (major) in a ratio of 1:4.8, which were not present in the mixture prior to borohydride treatment, were collected: *m/z* (EI) 331, for both products. The major triol was acetylated with acetic anhydride in pyridine, and the acetate was purified by HPLC on a Du Pont Zorbax SIL column, 9.4 × 250 mm, eluted at 10 mL/min with 1.25% methanol and 7.5% ethyl acetate in hexane, t_R 5.7 min. The 300-MHz NMR spectrum in $CDCl_3$ exhibited three singlets corresponding to the methyl protons of a triacetate at δ 2.13, 2.18, and 2.21 ppm; the benzo-ring proton resonances were at δ 3.6 (m, 1 H-1), 4.57 (dd, 1 H-1, J_{gem} 17 Hz, $J_{1,2}$ 5.3 Hz), 5.58–5.68 (overlapping multiplets, H-2 and H-3) and 6.47 (d, H-4, $J_{3,4}$ 7 Hz).

Theoretical Calculations. Ionization energies were calculated by using molecular mechanics to obtain a set of Cartesian coordinates (Bruger, W. XICAMM, a computer-aided molecular modeling system, Version 2.0, Xiris Corp., New Monmouth NJ, 1986) and then by using these coordinates as input to the INDO program (Pople, J. A.; Beveridge, D. L.; Dobosh, P. A. CINDO-INDO Program, Program 141, Quantum Chemistry Program Exchange, Indiana University, 1970) to obtain bonding energies. XICAMM has affinities to mainframe molecular mechanics programs but has modifications to allow running on an IBM PC/AT desk computer and also possesses a convenient graphics interface. CINDO is a FORTRAN program and was run on an IBM-3090 computer.

Registry No. 1a, 124508-36-7; **1a**·$HClO_4$, 124578-17-2; **1b**, 124508-37-8; **1b**·$HClO_4$, 124578-22-9; **2a**, 124508-38-9; **2a**·$HClO_4$, 124578-18-3; *trans*-**2a** tetraol, 124400-22-2; *cis*-**2a** tetraol, 124508-40-3; **2b**, 114326-34-0; **2b**·$HClO_4$, 124508-35-6; **2c**, 105500-28-5; *trans*-**2c** tetraol, 124508-34-5; MES, 4432-31-9; BES, 10191-18-1; TAPS, 29915-38-6; Tris, 77-86-1; CHES, 103-47-9; CAPS, 1135-40-6; *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]acridine, 105467-65-0; *trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*a,j*]acridine, 124508-33-4; formic acid, 64-18-6; acetic acid, 64-19-7; benz[*c*]acridine, 225-51-4; *trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*c,h*]acridine, 124508-39-0; benz[*a*]-acridine, 225-11-6.

X-ray Crystallographic Determination of Absolute Configurations and Conformations of Two Conformational Isomers of 1,2,3,4-Dibenzo-*trans*-6,7-dibromo-1,3-cyclooctadiene, a 2,2′-Bridged Biphenyl

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Abstract: The bridged biphenyl 1,2,3,4-dibenzo-*trans*-6,7-dibromo-1,3-cyclooctadiene (**1b**) exists in four stereoisomeric forms as two interconverting enantiomeric pairs, one with axial (a,a) and one with equatorial (e,e) bromine atoms. The stereoisomers can be separated by chromatography on triacetylcellulose. Crystals of the e,e and a,a forms of the pure enantiomers can be separated manually. The structures and absolute configurations of one of the a,a forms and one of the e,e forms were determined by X-ray crystallography, using the anomalous scattering technique. Both stereoisomers were shown to have the eight-membered ring in the twist-boat–chair conformation, confirming predictions by empirical force-field calculation. Two forms were observed by ^1H and ^{13}C NMR in solution and were shown by analysis of the coupling constants to be the same as in the crystals. The free energy barrier to e,e–a,a exchange was found by NMR band-shape analysis to be 23.9 ± 0.1 kcal/mol at 460–470 K, and by following the change in the CD spectrum to be 22.85 ± 0.05 kcal/mol at 298–303 K.

Stereochemical studies of atropisomeric biaryls have played a leading role in the development of organic stereochemistry.[3] In his pioneering work on the absolute configuration of hindered biaryls, Mislow made extensive use of conformationally restricted 2,2′-bridged biaryls, basing his system of correlations by physical and chemical methods on the results of partial asymmetric reduction of dibenzo- and dinaphthosuberones.[4] In their subsequent studies of ORD and CD spectra of bridged biaryls, Mislow and co-workers[5-9] observed regularities, which permitted determination of absolute configurations from the signs of the rotational strengths

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(3) (a) Christie, G. H.; Kenner, J. *J. Chem. Soc.* **1922**, *121*, 614–620. (b) Shriner, R. L.; Adams, R.; Marvel, C. S. In *Organic Chemistry*; Gilman, H., Ed.; Wiley: New York, 1943; Chapter 4. (c) Turner, E. E. In *Steric Effects in Conjugated Systems*; Gray, G. W., Ed.; Butterworths: London, 1958; Chapter 1. (d) Westheimer, F. In *Steric Effects in Organic Chemistry*; Newman, M. S., Ed.; Wiley: New York, 1956; p 523, and references cited therein.
(4) Mislow, K. *Angew. Chem.* **1958**, *70*, 683–689.
(5) Mislow, K.; Glass, M. A. W.; O'Brien, R. E.; Rutkin, P.; Steinberg, D. H.; Weiss, J.; Djerassi, C. *J. Am. Chem. Soc.* **1962**, *84*, 1455–1478.
(6) Bunnenberg, E.; Djerassi, C.; Mislow, K.; Moscowitz, A. *J. Am. Chem. Soc.* **1962**, *84*, 2823–2826, 5003.
(7) Mislow, K.; Bunnenberg, E.; Records, R.; Wellman, K.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 1342–1349.
(8) Mislow, K.; Glass, M. A. W.; Hopps, H. B.; Simon, E.; Wahl, G. H., Jr. *J. Am. Chem. Soc.* **1964**, *86*, 1710–1733.
(9) Joshua, H.; Gans, R.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4884–4892.

0002-7863/90/1512-1185$02.50/0