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General Acid Catalysis in the Hydrolysis of Benzo[*a*]pyrene 7,8-Diol 9,10-Epoxides

Sir:

The most studied of the carcinogenic hydrocarbons is the ubiquitous environmental contaminant benzo[*a*]pyrene (BP). Studies from several laboratories[1] have recently allowed the identification of the metabolite (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro BP ((+)-**2**, Figure 1) as an ultimate carcinogen of BP in newborn mice.[2] Two diastereomeric 7,8-diol 9,10-epoxides of BP are metabolically possible, each capable of existing in enantiomeric forms. Although (±)-**1** is >30-fold more hydrolytically reactive in the physiological pH range[5] and more susceptible to attack by nucleophiles in nonaqueous solution relative to (±)-**2**,[6] only (+)-**2** is tumorigenic in newborn mice. Both **1** and **2** alkylate the phosphate backbone of nucleic acid and effect the strand scission of DNA.[7] Because alkylation at phosphate may play an important role in the mutagenic and carcinogenic activities of **1** and **2**, the mechanisms of reaction of **1** and **2** with hydrogen phosphate species (and other general acids) in aqueous dioxane solutions were determined and are reported in this study.[8]

The rates of reaction of **1** and **2** in 10% dioxane-water solutions at a given pH exhibit a marked first-order dependence on concentration of phosphate buffer (Figure 2).[9,10] The rate data for series of serially diluted buffer solutions of constant pH were fit to

$$k_{obsd} = k_{HA}[HA] + k_{H^+}a_{H^+} + k_0 \qquad (1)$$

where $k_{H^+}a_{H^+}$ and k_0 represent contributions by the acid catalyzed and spontaneous hydrolysis mechanisms, respectively,[5] and HA refers to the dihydrogen phosphate ion ($H_2PO_4^-$). Values of $k_{H_2PO_4^-}$ for hydrolysis of **1** and **2**, obtained from plots of k_{obsd} vs. [$H_2PO_4^-$] for solutions at a given pH, were found to be constant within experimental error over the pH range studied (6.3–7.6, Table I). These data indicate that $H_2PO_4^-$ is the only catalytic or reactive phosphate species in the hydrolysis of **1** and **2** under these conditions.

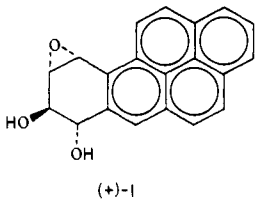

Figure 1. Diastereomeric BP 7,8-diol 9,10-epoxides.[3] The enantiomers of each diastereomer shown are those found bound to nucleic acid when BP is applied to mouse skin.[4]

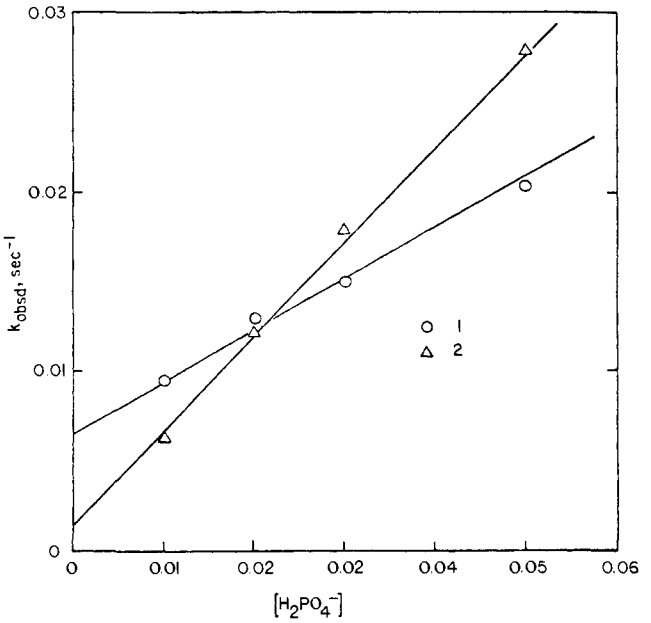

Figure 2. Plots of k_{obsd} vs. [$H_2PO_4^-$] for the hydrolysis of **1** and **2** in 10% dioxane-water solutions at 25 °C, ionic strength 0.2 ($NaClO_4$), pH 7.02.

Table I. Values of k_{HA} for the General Acid Catalyzed Hydrolysis of **1** and **2** in 10% Dioxane-Water[a] at 25 °C[b]

HA	pH	k_{HA}(**1**), M^{-1} s^{-1}	k_{HA}(**2**), M^{-1} s^{-1}
HOAc	4.81	0.72 ± 0.06	1.32 ± 0.02
	5.11	0.71 ± 0.04	1.33 ± 0.03
$H_2PO_4^-$	6.34	0.31 ± 0.01	0.48 ± 0.03
	6.70	0.28 ± 0.03	0.49 ± 0.01
	7.02	0.27 ± 0.01	0.48 ± 0.02
	7.10	0.28 ± 0.01	0.54 ± 0.02
	7.60	0.25 ± 0.01	0.49 ± 0.02
Tris-H^+	7.94	0.055 ± 0.006	0.026 ± 0.001
	8.54	0.056 ± 0.005	0.020 ± 0.002
$HO(CH_2)_2NH_3^+$	8.68	0.048 ± 0.004	0.0043 ± 0.0003
	8.94	0.048 ± 0.002	0.0044 ± 0.0004
phenol	9.22	0.19 ± 0.02	0.046 ± 0.003
	9.58	0.17 ± 0.01	0.046 ± 0.002

[a] Volume/volume; ionic strength, 0.2 ($NaClO_4$). [b] Rates were monitored by observing the absorbance change of the reaction solutions at 348 nm in the thermostated cell compartment (25.0 ± 0.1 °C) of a Gilford 2400 spectrophotometer. Rate constants were determined from least-squares plots of k_{obsd} vs. [HA] for solutions of constant pH but varied buffer concentrations.

Several kinetically indistinguishable mechanisms might account for the $H_2PO_4^-$ term in eq 1. One possibility is the specific acid-general base mechanism outlined in Scheme I (arbitrarily shown for the reaction of **1**). This mechanism involves attack of hydrogen phosphate ion (HPO_4^{-2}) on the protonated epoxide **3** to yield phosphate ester **4**.

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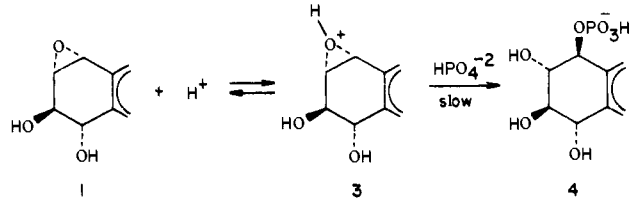
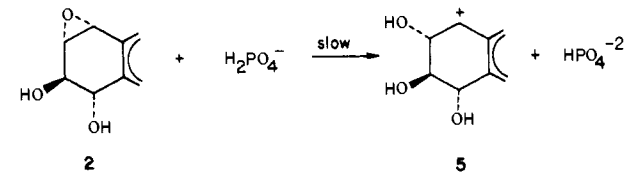
Scheme I



Scheme II



A second possible mechanism is that in which dihydrogen phosphate ion ($H_2PO_4^-$) acts as a general acid and transfers a proton to **1** or **2** in the rate-limiting step to form a benzyl cation (i.e., Scheme II). The intermediate cation can then react either with the solvent to yield tetrols or with HPO_4^{-2} to yield phosphate esters. Whereas the mechanism of Scheme I requires that phosphate ester be the sole product by this pathway, the yield of phosphate ester by the mechanism of Scheme II depends on the ability of HPO_4^{-2} to compete with solvent for the capture of the benzyl cation **5**.

To distinguish between these mechanisms, we have determined the product distributions from the phosphate-induced reactions of **1** and **2**. Hydrolysis of both **1** and **2** in 10% dioxane-water solutions containing 0.08 M NaH_2PO_4 (pH 6.66) yielded ~90% tetrol products and ~10% more polar materials, which were identified as phosphate esters.[11-13] It can be calculated from eq 1 that, in 0.08 M NaH_2PO_4 solution, ~80% of the products from **1** and >90% of the products from **2** arise from the phosphate-promoted reactions. The fact that nearly all of the product from **1** and **2** are tetrols instead of phosphate esters is inconsistent with Scheme I and suggests that $H_2PO_4^-$ acts instead as a general acid (Scheme II).[14] Further evidence in support of this conclusion is that the ratios of cis/trans addition of water to **1** and **2** by the phosphate mechanism are 88:12 and 2:98, respectively.[15] These ratios are very similar to the ratios obtained by hydronium ion catalyzed hydrolysis of **1** and **2** and are consistent with the intermediacy of the same benzyl cations. The small amount of phosphate esters are presumably formed from collapse of the benzyl cations with HPO_4^{-2}, but we cannot rule out the formation of some phosphate esters by minor incursion of the Scheme I mechanism.

We have also observed pronounced general acid catalysis (see Table I) in the hydrolysis of **1** and **2** by acetic acid (pH 4.8-5.1), Tris-H^+ (pH 7.9-8.5), $HOCH_2CH_2NH_3^+$ (pH 8.5-9.0), and phenol (pH 9.2-9.6).[16] These findings are especially significant in that general acid catalysis outside of the pH range 5.5-7.5 had not previously been detected in the hydrolysis of various arene oxides.[17] Dihydrogen phosphate ion ($H_2PO_4^-$) and cacodylic acid have been found to be catalytic in the hydrolysis of cyclohexadiene oxide[18] and cyclopentadiene oxide,[19] but catalysis by other general acids such as $NCCH_2NH_3^+$ (pH 5.6) and Tris-H^+ (pH 7.5-8.5) was not detected.[18]

In Table I are listed the catalytic constants for the general acid catalyzed hydrolysis of **1** and **2**. An interesting observation is that **2** is more reactive than **1** toward hydrolysis catalyzed by those reagents of relatively low pK_a (H_3O^+, HOAc, $H_2PO_4^-$), but **1** is more reactive than **2** toward hydrolysis catalyzed by Tris-H^+ (pK_a = 8.3), $HOCH_2CH_2NH_3^+$ (pK_a = 9.5), and phenol (pK_a = 10.0). The fact that general acids with pK_a values as high as 10 are effective catalysts in the hydrolysis of **1** and **2** is suggestive that water, too, might act as a general acid in their hydrolyses.[20] In agreement with this suggestion is the fact that both the spontaneous (k_0) and hydronium ion catalyzed (k_{H^+}) reactions of **1** yield very similar product distributions (~80% cis addition of water to the oxirane ring). Such rationale would explain the predominant cis addition of solvent to **1** by the k_0 mechanism, since hydrolysis would occur via the same benzyl cation as the hydronium ion catalyzed route.[21] General acid catalysis by water might not be the sole mechanism for the spontaneous hydrolysis of **2**, however, since the product distribution from this reaction is different from that from the H_3O^+ catalyzed reaction.[21]

Perhaps the most important conclusion that can be drawn from our present results is that detoxification of **1** and **2** in the cell might occur by their reactions with noncritical cellular agents by a mechanism that involves general acid catalysis, as opposed to a mechanism in which these agents act as nucleophilic scavengers. However, binding of **1** and **2** to critical cellular reagents such as DNA might also be facilitated by this same type of general acid catalysis. Thus, comparisons of second-order rate constants for the attack of nucleophiles with the solvolytic rate constants for **1** and **2** may be of limited value in predicting their biological activity.[22]

Acknowledgment. This investigation was supported by Public Health Service Grant No. CA-17278 from the National Cancer Institute (D.L.W.). A helpful discussion with Professor Donald Creighton is appreciated.

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(9) Catalysis by phosphate and other buffering reagents in the hydrolysis of both **1** and **2** was previously noted; ref 5, note 8.
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(12) Hydrolysis of **1** and **2** in 10% dioxane-water containing 0.9 M NaH_2PO_4 and 0.45 M Na_2HPO_4 resulted in the formation of ~35 and 25%, respectively, of phosphate esters that could not be extracted into ethyl acetate. Treatment of diluted aqueous solutions of these esters with alkaline phosphatase at pH 8 brought about gradual release (>50% after 18 h, room

temperature) of tetrols that were extracted into ethyl acetate and chromatographed by high pressure liquid chromatography (LC). The ratio of liberated tetrols corresponding to trans and cis addition of water to the diol expoxides at C-10 were 44:56 from **1** and 70:30 from **2**. In a control incubation in the absence of enzyme, the phosphate esters were essentially stable. In contrast, they slowly hydrolyzed ($t_{1/2} > 1$ day) in the concentrated phosphate solvolysis solutions.
(13) Products were analyzed by LC on a Du Pont Zorbax-ODS column (6.2 mm × 25 cm). The material assumed to be phosphate ester was not retained by the column (0–100% linear gradient of methanol–water, 1%/min, 1.2-mL/min flow rate) and displayed a 7,8,9,10-tetrahydrobenzo[*a*]-pyrene fluorophore identical with that of the tetrols that eluted much later.
(14) Kinetic solvent deuterium isotope effects $k_{H_2PO_4^-}/k_{O_2PO_4^-}$ for the phosphate catalyzed reactions of **1** and **2** in 10% dioxane–water were also measured and found to be 2.0 ± 0.2 and 1.9 ± 0.1 for **1** and **2**, respectively.
(15) The ratio of cis:trans addition of water to **2** in 0.02 M NaH_2PO_4 (pH 7.45) was also 2:98. At this same pH in the absence of phosphate, the spontaneous hydrolysis (k_0) of **2** predominates and gives >50% cis addition of solvent.
(16) Reaction of **1** and **2** in 0.4 M phenol (pH 9.23) yielded mainly phenol adducts, with cis addition of phenol favored over trans addition. LC analysis[13] of the products from **1** indicated the formation of 57% material cochromatographic with cis adduct (retention time, 29.5 min),[3] 17% material assumed to be trans adduct (retention time, 29.0 min), ~2% tetrols, and 24% several unidentified materials. Analysis of the products from **2** indicated the formation of 70% material cochromatographic with cis adduct (retention time, 28.0 min),[3] 17% material assumed to be trans adduct (27.5 min), ~2% tetrols, and ~11% several unidentified materials.
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(20) The kinetic solvent deuterium isotope effects (k_{H_2O}/k_{O_2O}) for hydrolysis of **1** and **2** by the spontaneous mechanism in 0.1 M $NaClO_4$ solutions were measured and found to be 2.4 ± 0.1 and 1.7 ± 0.2, respectively.
(21) General acid catalyzed opening of an epoxide by water would generate a carbonium ion–hydroxide ion pair that need not yield the same product distribution as the cation generated in the hydronium ion catalyzed route.
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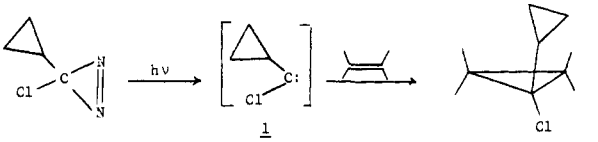
Reactivity of Cyclopropylchlorocarbene: an Interactive Experimental and Theoretical Analysis

Sir:

The geometries, energies, and electronic distributions of isolated singlet and triplet carbenes have been calculated.[1] Related techniques have defined energetically probable reaction pathways for simple carbenic abstraction, addition, or insertion reactions.[1] Rarely, however, have experimental and theoretical approaches been simultaneously focussed on a specific problem of intermolecular carbenic reactivity.[2] In this communication we present an interactive experimental and calculational analysis of the olefinic selectivity of cyclopropylchlorocarbene, illustrating this combined approach's potential for detailed elucidation of carbenic reaction mechanisms.

Cyclopropylchlorocarbene (**1**)[3] was photolytically generated (λ >300 nm, 25 °C) from the diazirine[4] in large excesses of selected binary alkene mixtures (eq 1). Quantitative GC analysis (calibrated tc detector) of the known[3] product chlo-



(1)

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robicyclopropyls, coupled with standard competition reaction analysis,[5] gave the relative reactivities in Table I. Satisfactory cross-check competitions[5] linked the reactivities of the substrate triads $Me_2C{=}CMe_2$, $Me_2C{=}CHMe$, $Me_2C{=}CH_2$ and $Me_2C{=}CMe_2$, $Me_2C{=}CH_2$, *c*-MeCH=CHMe. Least-squares correlation of log (k_i/k_0) for carbene **1** with comparable data for CCl_2[6] afforded the relation shown in Figure 1; the slope of the regression line, m_1^{obsd}, was 0.41.[7]

Carbene selectivity indices (m_{CXY}) can be estimated from the empirically based equation (2),[8] in which the Σ terms represent the sums of the appropriate σ constants[9a] for the substituents of CXY.

$$m_{CXY} = -1.10 \sum_{X,Y} \sigma^+_R + 0.53 \sum_{X,Y} \sigma_I - 0.31 \qquad (2)$$

From the rate constants, and derived values of σ_p^+ and σ_m, for solvolyses of *p*- and *m*-cyclopropyl-*tert*-cumyl chlorides in 90% aqueous acetone,[10] we calculate[11] $\sigma_R^+ = -0.38$ for cyclopropyl. Using this value, and taking $\sigma_I = \sigma_m = -0.04$[10] (eq 2), provides $m_1^{calcd} = 0.73$.[11] The discrepancy between m_1^{obsd} (Figure 1) and m_1^{calcd} (eq 2), $\Delta m = 0.32$, is more than three times the standard deviation in Δm expected from eq 2[8] and must be regarded as significant.

Why is **1** so much *less* selective than predicted by eq 2?[12] A priori, **1** should prefer *bisected* conformation, **1b**, in which

1b 1t

the "bent" cyclopropyl σ bonds can favorably interact with the vacant carbenic p orbital.[3,13] Studies of molecular models, however, indicate that bisected **1** would encounter substantial steric hindrance in electrophilic addition to alkenes: for the vacant p orbital to adequately overlap with the olefinic π orbital, a cyclopropyl carbon and its pair of H atoms must project down and onto the substrate's olefinic carbon atoms or their substituents. *Twisting* the cyclopropyl ring about the σ bond to the carbenic center relieves these adverse steric interactions. Accordingly, **1** should add to alkenes via a twisted conformation (in the limit **1t**), which is largely unstablilized by cyclo-

Table I. Relative Reactivities of Cycloalkylchlorocarbenes[a]

olefin	k_{rel} for *c*-C_3H_5CCl (**1**)	k_{rel} for *c*-C_4H_7CCl (**2**)
$Me_2C{=}CMe_2$	2.41 ± 0.02	1.49 ± 0.02
$Me_2C{=}CHMe$[b]	1.75 ± 0.01	1.75 ± 0.02
$Me_2C{=}CH_2$[c]	1.00	1.00
c-MeCH=CHMe[b]	0.67 ± 0.02	0.96 ± 0.02
t-MeCH=CHMe	0.46[d]	0.46[e]

[a] Errors are average deviations of two experiments. [b] The reactivity is the sum of *syn*-Cl and *anti*-Cl carbenic additions to this alkene.[9b] [c] Standard alkene. [d] This value was obtained indirectly from the competitions: *t*-MeCH=CHMe/*c*-MeCH=CHMe (k_{rel} = 0.68 ± 0.01) and *c*-MeCH=CHMe/$Me_2C{=}CH_2$ (cf. table for k_{rel}). [e] This value was obtained as in *d*, with k_{rel} = 0.48 ± 0.01 for *t*-MeCH=CHMe/*c*-MeCH=CHMe.

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