409 (3.1%, M + 1), 408 (0.5%, M^+).

Anal. Calcd for $C_{21}H_{32}N_2O_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.59; H, 7.88; N, 7.00.

(D,L)-**10b** prepared in the same manner from (D,L)-*N*-Boc-ε-bromonorleucine methyl ester had identical spectral, TLC, and HPLC properties.

(D,L)-N^6-Acetyl-N^6-hydroxylysine (2). (D,L)-N^2-Cbz-N^6-Acetyl-N^6-benzyloxylysine benzyl ester (**10a**) (0.240 g, 0.463 mmol) was dissolved in MeOH (10 mL), treated with 5% Pd on carbon (50 mg), and stirred under 1 atm of H_2 at room temperature for 4 h. After the second hour, H_2O (3 mL) was added to redissolve the precipitated product. The reaction mixture was filtered and evaporated, and the residue was crystallized from EtOH (aq) to give flaky white crystals: 84 mg (89%); mp 236–237 °C dec; $FeCl_3$ test for hydroxamic acids +++; ^1H NMR (D_2O) δ 1.1–2.1 (br m, 6 H), 2.0 (s, 3 H), 3.5 (m, 3 H); IR (KBr) 1580, 2950 cm^{-1}.

Anal. Calcd for $C_8H_{16}N_2O_4$: C, 47.04; H, 7.90; N, 13.72. Found: C, 47.42; H, 8.16; N, 14.00.

Hydrogenolysis of 14. When **14** was subjected to the hydrogenolysis conditions above, lysine (**16**) was obtained as shown by amino acid analysis of the reaction mixture.

N^2,N^2-(Anhydromethylene-1,5-citryl)bis[N^6-acetyl-N^6-(benzyloxy)-(L)-lysine methyl ester] (19). (L)-N^2-Boc-N^6-Acetyl-N^6-(benzyloxy)lysine methyl ester (**10b**) (0.678 g, 1.66 mmol) was stirred with CF_3CO_2H (2.5 mL) for 5 min at room temperature. Excess CF_3CO_2H was removed by rotary evaporation. The residue was partitioned between $CHCl_3$ (25 mL) and 1 M Na_2CO_3. The basic chloroform layer containing the free amine, **17**, was briefly dried (K_2CO_3) and chilled to 0 °C. Et_3N (0.231 mL, 1.66 mmol) was added, followed by the slow dropwise addition of anhydromethylenecitryl chloride[55] (**18**) (0.200 g, 0.83 mmol) in $CHCl_3$ (5 mL). After the solution was stirred 5 min at 0 °C, it was warmed to room temperature and stirred an additional 40 min. Next the solution was washed with 0.5 M citric acid (aq), H_2O, 0.5 M $NaHCO_3$, and finally with brine. Finally it was dried ($MgSO_4$) and evaporated to leave a colorless glass: 0.540 g (83%); $[\alpha]^{27}_D$ −13.62 ± 1 (*c* 2.4, MeOH); ^1H NMR ($CDCl_3$) δ 1.1–1.9 (m, 12 H), 2.10 (s, 6 H), 2.83 (s, 4 H), 3.63 (t, 4 H), 3.71 (s, 6 H), 4.49 (m, 2 H), 4.82 (s, 4 H), 5.51 (s, 2 H), 6.92 (br d, 2 H), 7.38 (s, 10 H); mass spectrum, *m/e* 91 (100%, tropylium ion), 785 (10.7%, M + 1), 784 (1.5%, M^+).

N^2,N^2-(1,5-Citryl)bis[N^6-acetyl-N^6-(benzyloxy)-(L)-lysine] (20). Compound **19** (0.354 g, 0.451 mmol) was dissolved in THF–H_2O (1:1, 25 mL), treated with 1 N NaOH (1.36 mL), and stirred at room temperature for 2 h. The reaction mixture was passed through a column of Dowex-50 X-8 (H^+), 25-mL bed, and washed through with a further 60 mL of the same solvent. The sodium-free solution was evaporated at reduced pressure to yield a brittle glass: 0.331 g (98.5%); $[\alpha]^{23}_D$ +12.43 ± 2 (*c* 2, MeOH); ^1H NMR ($CDCl_3$) δ 1.0–2.0 (m, 12 H), 2.08 (s, 6 H), 2.82 (s, 4 H), 3.61 (t, 4 H), 4.5 (m, 2 H), 4.80 (s, 4 H), 7.36 (s, 10 H), 7.92 (d, 2 H); mass spectrum, *m/e* 745 (100%, M + 1), 91 (2%, tropylium ion).

(−)-Aerobactin (1). Compound **20** (183 mg, 0.246 mmol) was dissolved in MeOH (6 mL) and treated with 5% Pd on carbon (60 mg). The mixture was stirred at room temperature under 1 atm of H_2 for 4 h and then filtered and evaporated. The residue was taken into H_2O (5 mL), Millipore filtered, and lyophilyzed to yield **1** as a hygroscopic, slightly off-white powder, 125 mg (90%). The 100-MHz ^1H (D_2O) and IR spectra (KBr) were identical with those depicted in the literature for natural aerobactin.[31] Paper chromatography (concentrated NH_3–EtOH–H_2O (1:16:3) and *n*-BuOH–HOAc–H_2O (60:15:25)) gave the same R_f values (0.14 and 0.61) as reported for the natural substance (0.13 and 0.63).[31] $[\alpha]^{22}_D$ −10.83 ± 1.4 (*c* 1.7, H_2O); mass spectrum, *m/e* 565 (100%, M + 1); no literature data available.

Acknowledgment. We are grateful for support from the National Institutes of Health (Grant GM 25845) and the Research Corporation, as well as a Lubrizol Fellowship (1981–1982) for P.J.M.

Registry No. 1, 26198-65-2; **2**, 81505-47-7; DL-**5**, 5462-80-6; L-**5**, 6033-32-5; **6a**, 32245-65-1; **6b**, 77611-37-1; **7a**, 81505-48-8; **7b**, 81505-49-9; **8**, 4797-81-3; **9**, 15255-86-4; **10a**, 81505-50-2; **10b**, 81505-51-3; **11a**, 81505-52-4; **11b**, 81505-53-5; **12a**, 81505-54-6; **12b**, 81505-55-7; **13a**, 81505-56-8; **14**, 81505-57-9; **15a**, 81505-58-0; **15b**, 81505-59-1; **16**, 56-87-1; **17**, 81505-60-4; **18**, 81505-61-5; **19**, 81505-62-6; **20**, 81505-63-7; DL-*N*-Boc-ε-hydroxynorleucine, 81505-64-8; *O*-benzylhydroxylamine HCl, 2687-43-6.

Guanosine 5′-Monophosphate Catalyzed Hydrolysis of Diastereomeric Benzo[*a*]pyrene-7,8-diol 9,10-Epoxides

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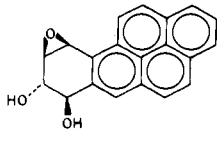
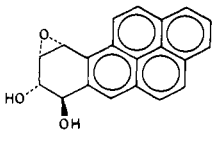
Contribution from the Laboratory for Chemical Dynamics, Deparment of Chemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228, and the Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received August 10, 1981*

Abstract: The rates of reaction of 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in aqueous dioxane solutions in the presence of 5′-guanosine monophosphate (5′-GMP) and guanosine (G) have been determined. 5′-GMP in the monohydrogen form ($GMPH^-$) exhibits pronounced general acid catalysis in the hydrolysis of these epoxides under conditions where the corresponding nucleoside G is unreactive. Although the pK_a values for $GMPH^-$ and inorganic dihydrogen phosphate ion ($H_2PO_4^-$) are very similar, $GMPH^-$ is 60–80 times more efficient than $H_2PO_4^-$ in catalyzing the hydrolysis of the diol epoxides. Significant solvent effects on both $GMPH^-$ and $H_2PO_4^-$ have been observed.

The metabolic activation of the environmental carcinogen benzo[a]pyrene leads in part to the two diastereomeric 7,8-diol 9,10-epoxides **1** and **2**.[2a] The mutagenic and carcinogenic



1



2

properties of these highly reactive metabolites have been attributed to their covalent binding to cellular macromolecules.[2b–f] The major products from covalent binding of **1** and **2** to DNA result from

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(2) (a) The complete names of **1** and **2** are (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, respectively. For reviews, see: (b) Miller, E. C. *Cancer Res.* **1978**, *38*, 1479. (c) Levin, W.; Wood, A. W.; Wislocki, P. G.; Chang, R. L.; Kapitulnik, J.; Mah, H. D.; Yagi, H.; Jerina, D. M.; Conney, A. H. In "Polycyclic Hydrocarbons and Cancer"; Gelboin, H. V., T'so, P. O., Eds.; Academic Press: New York, 1978, Vol 1, p 189. (d) Yang, S. K.; Deutsch, J.; Gelboin, H. V. *Ibid.* Vol. 1, p 205. (e) Gelboin, H. V. *Physiol. Rev.* **1980**, *60*, 1107. (f) Harvey, R. G. *Acc. Chem. Res. 1981*, *14*, 218.

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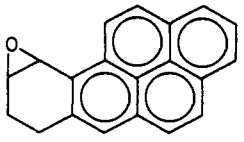
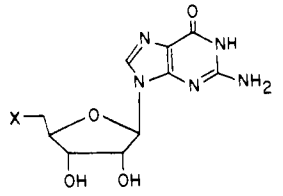
alkylation of the C-2 exocyclic amino group of guanine,[3] but it has been reported that diol epoxide **2** also alkylates at the N-7 position.[4a] Guanine moieties also appear to be the most nucleophilic components of nucleic acids toward other alkylating reagents.[4b] In addition to undergoing covalent binding to the bases of nucleic acids, **1** and **2** also react at the phosphate linkages.[3b,4c] Recent reports[5] show that the rates of reaction of **1** and **2** in aqueous solution are enhanced when DNA is present.

The solution chemistry of **1** and **2** has been extensively studied. Both **1** and **2** undergo acid-catalyzed hydration at low pH[6] and spontaneous reaction with water at higher pH (>~5 for **1** and ~7 for **2**).[6e] Both diol epoxides undergo nucleophilic addition reactions with nucleophiles such as *p*-nitrothiophenolate and aniline.[6c] Marked general acid catalysis by inorganic dihydrogen phosphate ion ($H_2PO_4^-$) and other general acids in the hydrolysis of **1** and **2** has also been reported.[7]

In order to better understand the mechanisms of interaction of epoxides with macromolecules such as RNA and DNA, we have undertaken a kinetic study of the reactions of **1**, **2**, and the tetrahydroepoxide **3** in the presence of nucleosides and nucleotides.



3



4, X = OPO_3H^-

5, X = OPO_3^{-2}

6, X = OH

In this paper we report a dramatic catalysis by 5′-guanosine hydrogen monophosphate (**4**) (5′-GMPH$^-$) in the hydrolysis reaction of **1**–**3** under conditions in which the conjugate base **5** (5′-GMP^{2-}) and the corresponding nucleoside guanosine (**6**) are unreactive. The reactions of **1** and **2** with 5′-guanosine monophosphate at pH 6–7 therefore must not take place by simple displacement mechanisms,[8] since **5** and **6** also contain the guanine base moiety and should be equally or more reactive than **4** in nucleophilic displacement reactions. The distinguishing feature of the reactive species is the presence of the 5′-hydrogen phosphate group. In view of the fact that dihydrogen phosphate ion ($H_2PO_4^-$) acts as an effective general acid in the hydrolysis reactions of **1** and **2**,[7] then it it reasonable to assume that the 5′-hydrogen phosphate group also acts as a general acid in the 5′-GMPH$^-$ catalyzed reactions of **1** and **2**.

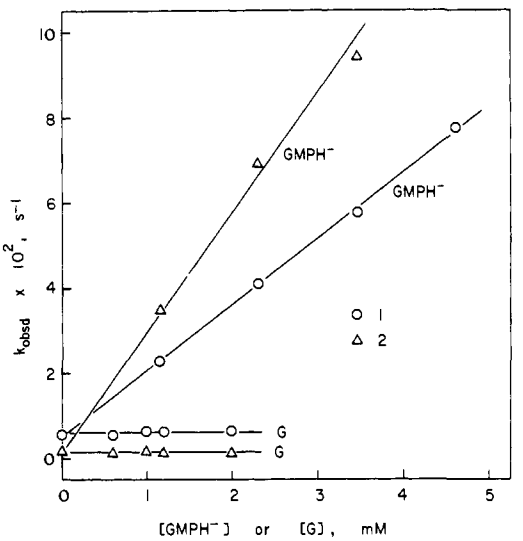


Figure 1. Plots of k_{obsd} vs. [GMPH$^-$] (**4**) or [G] (**6**) for hydrolysis of **1** and **2** in 10% dioxane–water solutions at 25 °C, μ = 0.1 ($NaClO_4$), pH 6.32.[12b] The solutions of G also contained 10^{-3} M cacodylic acid buffer for maintenance of pH.

Table 1. Values of k_{GMPH^-} for Hydrolysis of **1** and **2** in 10% Dioxane–Water (v/v) Solutions at 25 °C[a,b]

pH[b]	k_{GMPH^-}(1), M^{-1} s^{-1}	k_{GMPH^-}(2), M^{-1} s^{-1}
6.02	16.1 ± 0.3	30.1 ± 0.3
6.32	15.8 ± 0.2	28.7 ± 0.9
6.61	17.7 ± 0.5	31.5 ± 2.0

[a] μ = 0.1 ($NaClO_4$). [b] Rates were monitored spectrophotometrically at 348 nm in the thermostated cell compartment (25.0 ± 0.2 °C) of a Gilford 2400 spectrophotometer. Rate constants were determined from weighted least-squares plots of k_{obsd} vs. [GMPH$^-$] for solutions of constant pH. Reference 12b.

Experimental Section

Materials. Diol epoxides **1** and **2** were prepared according to published procedures.[6c] 5′-Guanosine monophosphate disodium salt and guanosine were purchased from Aldrich Chemical Co., Milwaukee, WI. The pK_a and purity of 5′-GMP were determined in 10% dioxane–water solution by titration with a Radiometer automatic titrator assembly. Buffer solutions of 5′-GMP in 10% dioxane–water (v/v) were prepared by addition of the appropriate amount of perchloric acid to a solution of the disodium salt of 5′-GMP. Dioxane was distilled from sodium metal prior to its use, and 10% dioxane–water solutions were prepared by mixing 1 volume of dioxane with 9 volumes of water at 25 °C.

Kinetic Procedures. Stock solutions containing ca. 0.1 mg of diol epoxides **1** or **2** in 0.1 mL of Me_2SO were prepared. For each kinetic run, approximately 5–10 μL of stock solution was added to 2.5 mL of reaction solution in the thermostated cell compartment (25.0 ± 0.2 °C) of a Gilford 2400 spectrophotometer. Reactions were monitored at 348 nm.

The psuedo-first-order rate constants (k_{obsd}) were obtained by nonlinear regression analysis of the data, for each kinetic run, by a Wang 700 desk calculator.

Results and Discussion

Kinetic data for the reactions of **1** and **2** in 10% dioxane–water solutions containing 5′-GMP and guanosine are outlined in Figure 1. The data show striking first-order dependencies of the reaction rates of **1** and **2** upon the concentrations of 5′-GMP. However, the rates of reaction of **1** and **2** in solutions containing up to 0.002 M guanosine (existing as the nonionized form **6** at pH 6.3) are within experimental error the same as the rates of hydrolysis of **1** and **2** in the absence of guanosine. Therefore, nucleophilic

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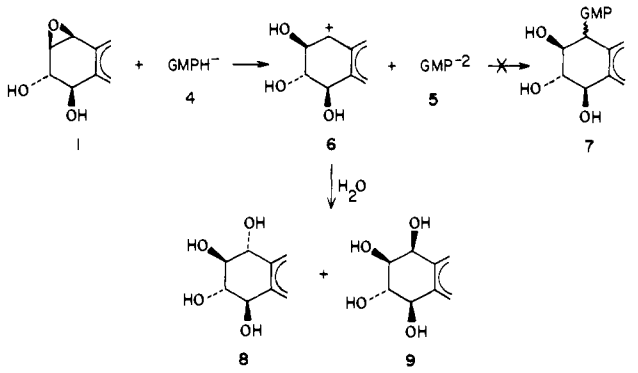
(6) (a) Wood, A. W.; Wislocki, P. G.; Chang, R. L.; Levin, W.; Lu, A. Y. H.; Yagi, H.; Hernandez, O.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1976**, *36*, 3358. (b) Yang, S. K.; McCourt, D. W.; Roller, P. P.; Gelboin, H. V. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2594. (c) Yagi, H.; Thakker, D. R.; Hernandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604. (d) Keller, J. W.; Heidelberger, C.; Beland, F. A.; Harvey, R. G. *Ibid.* **1976**, *98*, 8276. (e) Whalen, D. L.; Montemarano, J. A.; Thakker, D. R.; Yagi, H.; Jerina, D. M. *Ibid.* **1977**, 99, 5522.

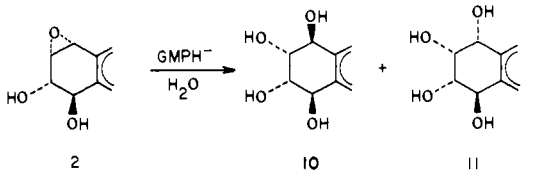
(7) Whalen, D. L.; Ross, A. R.; Montemarano, J. A.; Thakker, D. R., Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1979**, *101*, 5086.

(8) Nucleosides and deoxynucleosides react with simple epoxides and other alkylating agents in aqueous organic or organic solvents, presumably via nucleophilic addition or displacement reactions: (a) Hemminki, K.; Paasivirta, J.; Kurkirinne, T.; Virrki, L. *Chem.-Biol. Interact.* **1980**, *30*, 259. (b) Sugiura, K.; Goto, M. *Ibid.* **1981**, *35*, 71. (c) Lyle, T. A.; Royer, R. E.; Daub, B. H.; Vander Jagt, D. L. *Ibid.* **1980**, *29*, 197. (d) Moschel, R. C.; Hudgens, W. R.; Dipple, A. *Tetrahedron Lett.* **1981**, *22*, 2427.

Scheme I



Scheme II



displacement reactions of guanosine with **1** or **2** are unable to compete successfully with simple hydrolysis reactions under the conditions outlined in Figure 1.[9]

The rates of reaction of **1** and **2** were fit to

$$k_{obsd} = k_{GMPH^-}[GMPH^-] + k_{H^+}a_{H^+} + k_0$$

where $k_{H^+}a_{H^+}$ and k_0 represent contributions by the acid-catalyzed and spontaneous hydrolyses, respectively,[6e] and k_{GMPH^-} is the second-order rate constant for the 5′-GMPH⁻ catalyzed reaction. The slopes of plots such as in Figure 1 are equal to k_{GMPH^-} and are within experimental error of being identical at three different pH values (6.02, 6.32, 6.61).[12] Thus it can be concluded that **4** is the ionization state responsible for the kinetic term in 5′-GMP. Surprisingly, the catalytic effectiveness of 5′-GMPH⁻ was found to be much greater (60–80-fold) than that of $H_2PO_4^-$, although similar reactivities might be expected because 5′-GMPH⁻ and $H_2PO_4^-$ possess similar pK_a values.[10] Values of k_{GMPH^-} for reactions of **1** and **2** in 10% dioxane–water solutions are provided in Table I.

Since **4** is the only ionization state of 5′-GMP effective in bringing about the reactions of **1** and **2** in the pH range studied, it is reasonable to assume that **4** acts as a proton donor (Scheme I) for reactions of **1** and **2**. Protonation of **1** by **4** leads to a benzyl cation intermediate **6** and also produces the conjugate base GMP^{2-} (**5**) as the counterion. Intermediate **6** could therefore potentially undergo collapse with the counterion or other GMP^{2-} ions in solution to yield covalent adduct(s) **7** or react with water to yield tetraols **8** and **9**.[6c]

Product studies were carried out to characterize the reactions of **1** and **2** with GMPH⁻ (**4**). Analysis of the product mixtures from hydrolysis of **1** and **2** in 5 mM 5′-GMPH⁻ at pH 6.3 by HPLC with the aid of an internal standard in the reaction solutions showed that >94% of the products could be accounted for as tetraols (**8** and **9** from **1**; **10** and **11** from **2**) as shown in Schemes I and II.[13] Under these conditions, the kinetic term in GMPH⁻ accounts for 96% of the total rate of **1** and >99% of the total rate for **2**. The absence of significant amounts of detectable covalent adducts between 5′-GMP and either **1** or **2** suggests that **4** acts as a general acid in bringing about hydrolysis of **1** and **2** and that the cation intermediates then react with solvent to yield tetraols as outlined in Schemes I and II. At these rather dilute concentrations, therefore, GMP^{2-} appears not to compete successfully with the solvent for capture of the intermediate cations.[14]

The distributions of tetraols produced from reactions of **1** and **2** with GMPH⁻ in 10% dioxane–water solutions are very similar to the product ratios from hydronium ion catalyzed hydrolysis of **1** and **2**. In 5 mM GMPH⁻ at pH 6.32,[12b] reaction of **1** provided 10% of **10** (trans hydration) and 90% of **11** (cis hydration). Both hydronium ion catalyzed and spontaneous hydrolyses of **1** in 10%

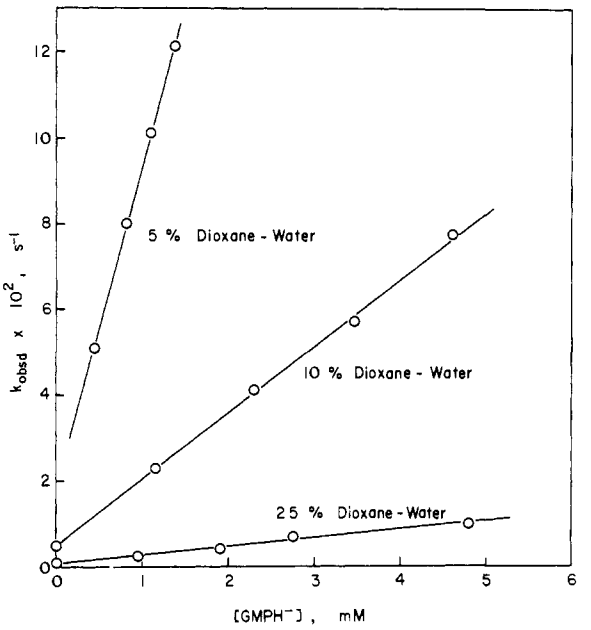


Figure 2. Plots of k_{obsd} vs. [GMPH⁻] (**4**) for hydrolysis of **1** in dioxane–water (v/v) solutions at 25 °C, μ = 0.1 ($NaClO_4$). The apparent pH[12b] values for each set of serially diluted solutions were adjusted to the pH of the most concentrated solution: 5% dioxane–water, pH 6.13; 10% dioxane–water, pH 6.32; 25% dioxane–water, pH 6.72.

Table II. Rate Constants for GMPH⁻- and $H_2PO_4^-$-Catalyzed Hydrolysis of 1–3 at 25 °C in Dioxane–Water Solutions[a]

solvent[b]	1	2	3[c]
	k_{GMPH^-}, M^{-1} s^{-1}		
5% dioxane–water	75 ± 1	142 ± 4	
10%[d] dioxane–water	16 ± 1	30 ± 1	
25% dioxane–water	2.0 ± 0.2	3.6 ± 0.1	11.3 ± 0.6
	$k_{H_2PO_4^-}$, M^{-1} s^{-1}		
5% dioxane–water	0.96 ± 0.03	1.8 ± 0.1	
10%[d] dioxane–water	0.27 ± 0.01	0.48 ± 0.02	

[a] μ = 0.1 ($NaClO_4$). [b] v/v. [c] Because of the low solubility of **3** in water, kinetic measurements were made only in 25% dioxane–water solutions. [d] Reference 7.

(9) The low solubility of guanosine in 10% dioxane–water prevented kinetic determinations in solutions with concentrations >0.002 M.

(10) The pK_a for 5′-GMPH⁻ (**4**) is reported to be 6.7 (ref 11b), and the pK_a for $H_2PO_4^-$ was determined to be 6.4 (μ = 1.0, ref 11a). We have determined the apparent pK_a values for 5′-GMPH⁻ (**4**) and $H_2PO_4^-$ in 10% dioxane–water solutions (μ = 0.1, $NaClO_4$) to be 6.50 and 7.09, respectively.

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(12) (a) The intercepts of eq 1 are equal to ($k_{H^+}a_{H^+}$ + k_0), and values of k_{H^+} and k_0 for hydrolysis of **1** and **2** in 10% dioxane–water solutions are given in ref 6e. (b) The pH values listed are apparent values, as provided by pH meter readings.

(13) Products were analyzed on a Waters Radial-Pak C18 reverse-phase HPLC column with 40–60% methanol–water as eluent. Internal standards utilized were either 1-acenaphthenol or 7-methoxy-1-tetralone. Relative retention times were similar to those reported previously: Thakker, D. R.; Yagi, H.; Lu, A. Y. H.; Levin, W., Conney, A. H.; Jerina, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3881.

(14) The formation of labile adducts that either react further under the reaction conditions or decompose under analytical conditions cannot be ruled out. However, phosphate esters from reaction of **1** and **2** with inorganic phosphate have been shown to be relatively stable under the reaction times and analysis conditions but do hydrolyze slowly back to tetraols (ref 3b and 7). It is assumed that phospho diester adducts between **1** and **2** and GMPH⁻ would have comparable stability.

dioxane–water have been found to yield ca. 10–12% of trans hydration and 85–90% cis hydration products.[6e] The reaction of **2** in 5 mM $GMPH^-$ in 10% dioxane–water at pH 6.32 yielded ca. 83% of **12** (trans hydration) and 17% of **13** (cis hydration). This ratio is similar to that reported[6e] for H_3O^+-catalyzed hydrolysis of **2** (81–86% trans hydration in water and 92–93% in 10% dioxane–water) but quite different from the product ratio for spontaneous reaction of **2** (ca. 45:55 trans/cis hydration).

Additional evidence consistent with the proposed mechanisms for reaction of **1** and **2** with $GMPH^-$ is provided by the solvent effect on their rates of reaction (Figure 2 for **1**). For instance, the rate-limiting step in the hydrolysis of **1** catalyzed by $GMPH^-$ is presumably the protonation of **1** by $GMPH^-$ to yield the carbocation **6** and GMP^{2-} (**5**). This reaction involves the generation of charge, and therefore its rate would be expected to decrease as the polarity of the solvent decreases. From Figure 2, it can be seen that the rates for reaction of **1** in $GMPH^-$ solutions fall off markedly (ca. 46-fold) as the polarity of the solvent is decreased in going from 5% dioxane–water to 25% dioxane–water. By comparison, the apparent bimolecular rate constant (k_{H^+}) for **1** is decreased by only about 30% as the solvent is changed from water to 25% dioxane–water.[6e,15] A summary of the rate constants for the $GMPH^-$ reactions of **1** and **2** as a function of solvent is provided in Table II. Benzo[*a*]pyrene tetrahydroepoxide **3** is even more reactive with $GMPH^-$ than either **1** or **2** (Table II). Therefore the relative reactivities of **1–3** toward $GMPH^-$ (**3** > **2** > **1**) parallel their order of reactivities toward hydronium ion catalyzed hydrolysis.[6e]

The rates of inorganic phosphate ($H_2PO_4^-$) catalyzed hydrolysis of **1** and **2** were also determined in 5% and 10% dioxane–water solutions, and the data are summarized in Table II. It has been postulated that $H_2PO_4^-$ acts as a general acid in the hydrolysis of **1** and **2** in a mechanism similar to that outlined in Scheme I for reaction of **1** with $GMPH^-$.[7] The values of $k_{H_2PO_4^-}$ are also found to be lower in 10% dioxane–water, consistent with the fact that $H_2PO_4^-$ is of the same charge type as $GMPH^-$. Noteworthy, however, is the observation that $GMPH^-$ is ca. 60–80 times more effective than H_2PO_4 in catalyzing the hydrolysis of **1** and **2**, even though the pK_a of $GMPH^-$ is very similar to that of $H_2PO_4^-$.[10,16]

In summary, 5′-GMP acts as a general acid in the hydrolysis of **1** and **2** at pH ~7 and not as a nucleophilic reagent. Ionization state **4** ($GMPH^-$) is the reactive species, and it is much more effective as a general acid than one would predict, based on its pK_a value. The enhanced effectiveness of $GMPH^-$ as a general acid compared to $H_2PO_4^-$ might possibly be due to an association complex between $GMPH^-$ and **1** or **2**. If such association complexes exist, then it would be feasible to design reagents capable of forming more favorable complexes with **1** and **2** and acting as even more efficient catalysts in the hydrolyses of **1** and **2**. Such reagents might block the mutagenic and carcinogenic actions of **1** and **2** by acting as highly efficient scavengers that detoxify **1** and **2** by promoting their hydrolyses to inactivate tetraols.

The foregoing results also suggest that site-selective alkylation of nucleic acids and proteins by epoxide metabolites may not be determined strictly by the relative basicities of the nucleic acid or protein bases but may also depend on the nearness of groups capable of acting as general acid catalysts.

Acknowledgment. This investigation was supported by Public Health Service Grant No. CA-26086 from the National Cancer Institute (D.L.W.).

Registry No. 1, 58917-91-2; **2**, 58917-67-2; **3**, 64608-56-6; **8**, 62697-17-0; **9**, 62697-13-6; **10**, 62697-19-2; **11**, 62697-16-9; 5′-GMP, 85-32-5; guanosine, 118-60-3.

(15) The relatively small solvent effect on k_{H^+} is reasonable since the rate-limiting step for this reaction does not involve the generation of charge but rather a dispersal of charge.

(16) From titration curves, the apparent pK_a values of 5′-$GMPH^-$ (**4**) in 5%, 10%, and 25% dioxane–water solutions (v/v, μ = 0.1 ($NaClO_4$)) were determined to be 6.42, 6.50, and 6.86, respectively.

Tin-Assisted Sulfuration: A Highly Potent New Method for the Conversion of Carbonyl Units into Their Corresponding Thiocarbonyl Analogues[1a,b]

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Abstract: Treating bis(tricyclohexyltin) sulfide with boron trichloride in the presence of a carbonyl-containing compound converts the carbonyl unit into its corresponding thiocarbonyl analogue in high yield. Using this technique, several examples of thioaldehydes, thioketones, thiolactones, and thiolactams were prepared. This potent new sulfurating method is efficient even with highly hindered ketones such as di-*tert*-butyl ketone (65% isolated yield di-*tert*-butyl thioketone) hitherto inert to direct sulfuration. Other group 4 organometallic sulfides such as bis(trimethylsilyl) sulfide, bis(tri-*n*-butyltin) sulfide, and bis(triphenyltin) sulfide can be used in this new sulfurating process with equal effect.

Thiocarbonyl-containing compounds have gained prominence because of their rich photochemistry.[2] However, their recent application to the synthesis of complex natural products[3] as key intermediates, which allow for difficult synthetic transformations[3b,4] under mild reaction conditions,[3c,5] has accentuated interest in this

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