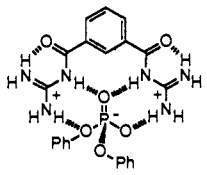
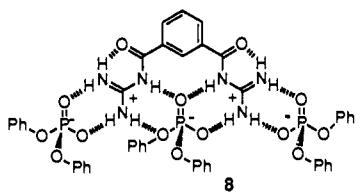
ensuring that the only flexibility is in the phenyl–carbonyl bonds.

Addition of 1 equiv of tetrabutylammonium diphenylphosphate (TDPP) to a $CD_3CN$ solution of **6**-$TPB_2$ leads to both a sharpening and a downfield shifting of the b protons (0.22 ppm) and c protons (1.27 ppm) on the guanidinium. There is little shift, however, in the position of the outwardly-directed a protons. These results are consistent with the formation of a complex of type **7**,[11] in which both guanidiniums converge on the central cavity to form four hydrogen bonds to a single phosphate substrate.[12] The a protons do not participate in this hydrogen bonding and so hardly shift. Similar behavior, with up to 1 equiv of substrate, was seen with bis-imidazoline **5**.



**7**



**8**

With **6**, however, a second series of $^1H$ NMR changes occurs on further addition of TDPP (from 1 to approximately 3 equiv) to the $CD_3CN$ solution. Now the guanidinium a protons shift dramatically downfield (2.48 ppm), the b protons move slightly upfield (0.22 ppm), and the c protons do not move. The outwardly-directed a protons represent two additional binding sites for the phosphodiester anions, and these NMR changes indicate the formation of a 3:1 complex, as in **8**.[14] These binding stoichiometries are supported by Job's plots,[15] generated by following the different H-bonding protons. The b and c protons give a maximum in the curve at a mole ratio of 0.5 (corresponding to a 1:1 complex), whereas the a proton reaches the maximum complex concentration at a mole ratio of receptor to substrate of 0.25 (corresponding to a 1:3 complex). Distinct spectroscopic changes of this type indicate strong 1:1 binding followed by weaker association of the second and third substrates. The absence of any significant higher order complexes until after 1 equiv of substrate has been added simplifies the analysis of the 1:1 complex. Dilution of a 1:1 mixture of **6**-$TPB_2$ and TDPP in $CH_3CN$ gave a binding isotherm (following UV absorption at 266 nm) that was analyzed[16] by nonlinear regression methods to give an association constant for **7** of $(4.6 \pm 1.7) \times 10^4$ $M^{-1}$. In contrast, simple benzoylguanidinium tetraphenylborate has a $K_a$ with TDPP of $(2.7 \pm 1.2) \times 10^3$ $M^{-1}$.

Receptors **5** and **6** are well-suited for the catalysis of phosphodiester cleavage. They possess a dicationic trigonal binding cavity that should be complementary both in terms of shape and electrostatics to the dianionic trigonal bipyramidal intermediate for nucleophilic attack on a phosphodiester.[5] Most importantly, the reduced basicity of the acylguanidines means that they can function not only as a binding site but also as an acid for protonating the alcohol leaving group. These studies will be the subject of a future publication.

**Acknowledgment.** We thank the National Institutes of Health (GM 35208) for financial support of this work and Ken Zweig for his assistance with the syntheses.

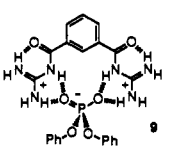
**Registry No.** **3**·DPP, 137695-76-2; **4**, 36145-66-1; **5**·$(chloride)_2$, 137718-31-1; **5**·$(picrate)_2$, 137718-30-0; **5**·$(TBP)_2$, 137718-32-2; **5**·DPP, 137718-33-3; **6**, 137695-71-7; **6**·$(chloride)_2$, 137695-72-8; **6**·$(picrate)_2$, 137695-70-6; **6**·$(TBP)_2$, 137695-73-9; **7**, 137695-74-0; **8**, 137695-75-1; TDPP, 429-42-5; DPP, 48168-03-2; dimethyl isophthalate, 1459-93-4; 2-aminoimidazolinium *p*-toluenesulfonate, 64103-00-0; *N,N'*-bis(3,4-dihydro-1*H*-imidazol-2-yl)-1,3-benzenedicarboxamide, 137718-29-7; guanidinium hydrochloride, 50-01-1.

**Supplementary Material Available:** Crystallographic details for 2,6-dibutyramidopyridinium diphenylphosphate and 2-(benzoylamino)imidazoline, including tables of atomic coordinates, thermal parameters, bond angles, and bond lengths (18 pages). Ordering information is given on any current masthead page.

(10) The two $NH_2$ groups of each guanidinium interconvert rapidly at room temperature; hence, only three signals are seen. Assignments were based on comparison to **5** and related derivatives.

(11) $^1H$ NMR of 1:1 complex **7** ($CD_3CN$): $\delta$ 12.30 (br s, 2 H, CONH), 8.67 (s, 4 H, picrate), 8.50 (br s, 4 H, endo NH), 8.41 (s, 1 H, 2-isophth), 8.22 (dd, $J$ = 8, 2 Hz, 2 H, 4,6-isophth), 7.76 (t, $J$ = 8 Hz, 1 H, 5-isophth), 7.51 (br s, 4 H, exo NH), 7.26 (t, $J$ = 8.5 Hz, 2 H, 4-phenyl), 7.09 (m, 8 H, 2,3,5,6-phenyl), 3.06 (m, 8 H, $NCH_2$), 1.58 (m, 8 H, $NCH_2CH_2$), 1.35 (m, 8 H, $CH_2CH_3$), 0.95 (t, $J$ = 7 Hz, 12 H, $CH_3$).

(12) At this point we cannot discount a complex structure as in **9**. Similar six-membered-ring H-bonding arrangements have been seen in urea–triphenylphosphine oxide cocrystals.[13]



**9**

(13) Etter, M. C.; Urbañczyk-Lipkowska, Z.; Zia-Ebrahimi, M.; Panunto, T. W. *J. Am. Chem. Soc.* **1990**, *112*, 8415.

(14) The upfield shift of the b protons is presumably due to a decrease in their acidity on phosphate binding to the geminal proton.

(15) Connors, K. A. *Binding Constants*; John Wiley & Sons: New York, 1987; p 24.

(16) Wilcox, C. S. *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H. J., Durr, H., Eds.; VCH: Weinheim, Germany, 1990.

## Rate Constants for the Decomposition of a Simple Alkanediazoate at Physiological pH†

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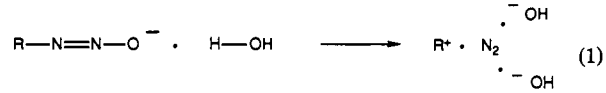
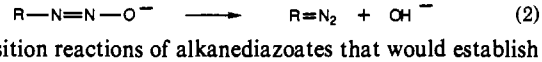
Alkanediazoates (**1**) are postulated to be reactive intermediates in the DNA-alkylating activity of a large number of compounds that contain an *N*-nitroso-*N*-alkyl functionality and are mutagenic, carcinogenic, or cancer-chemotherapeutic agents.[1] Simple *syn*- and *anti*-alkanediazoates were synthesized separately nearly 100 years ago.[2] Alkanediazoates are generally unstable in aqueous solutions, decomposing with the evolution of nitrogen gas, though some of the *anti* forms are reportedly stable in cold water.[3] The elegant isotope labeling studies of Moss and co-workers on the decomposition of secondary *syn*-alkanediazoates in heterogeneous aqueous media have been interpreted as supporting a mechanism involving rate-limiting fragmentation to yield an ion triplet (eq 1, R = secondary alkyl).[4a] In the case of some other *syn*-al-



(1)

**1**

kanediazoates, the products of decomposition in heterogeneous partly aqueous and homogeneous nonaqueous protic media include diazoalkanes to varying extents (eq 2, R = benzyl,[2b] allyl,[4a,b] cinnamyl,[4b] primary alkyl,[4a,c,d] and methyl[2b]), but the mechanism for the reaction of eq 2 is uncertain. Kinetic studies on the



(2)

decomposition reactions of alkanediazoates that would establish experimental criteria for possible mechanisms have not been carried out. We report in the present work the first rate constants for the decomposition of an *anti*-alkanediazoate (**2**) in aqueous

†Alkanediazoates have appeared in the literature as alkanediazotates.

(1) Lawley, P. D. In *Chemical Carcinogens*; Searle, C. D., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984.

(2) (a) Thiele, J. *Justus Liebigs Ann. Chem.* **1910**, *376*, 239. (b) Hantzsch, A.; Lehmann, M. *Chem. Ber.* **1902**, *35*, 897.

(3) Thiele, J. *Chem. Ber.* **1908**, *41*, 2806.

(4) (a) Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421. (b) Hart, H.; Brewbaker, J. L. *J. Am. Chem. Soc.* **1969**, *91*, 716. (c) Feiring, A. E.; Ciabattoni, J. *J. Org. Chem.* **1972**, *37*, 3784. (d) Kirmse, W.; Wachterhauser, G. *Justus Liebigs Ann. Chem.* **1967**, *707*, 44.

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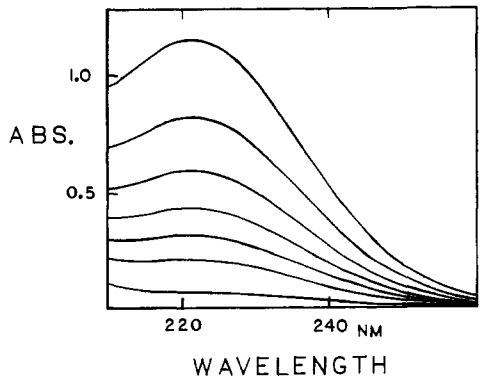



**Figure 1.** Decay of the ultraviolet spectrum of potassium (*E*)-methanediazoate in aqueous solution containing 0.0124 M NaOH at 25 °C, 1 M ionic strength ($NaClO_4$). Spectra were recorded every 300 s except for the last, which was recorded at 2900 s.

solution. A simple rate law for the reaction, encompassing the physiological pH range, is obtained.

Potassium (*E*)-methanediazoate was synthesized with minor modification of the published procedure and recrystallized in methanol/2-propanol.[5] The diazoate, previously reported to be stable in deuteriomethanol,[6] also proved moderately stable in solutions of $D_2O$ containing NaOD. The clean first-order decay of the methyl singlet signal, observed by $^1H$ NMR for three half-lives, gave a rate constant of $k_{obsd} = 4.4 \times 10^{-5}$ s$^{-1}$ at 25 °C in 0.092 M NaOD ([**2**] ~ 0.009 M).

The diazoate **2** was found to have a broad ultraviolet absorption spectrum with a $\lambda_{max}$ of 222 nm (Figure 1).[7] The decay of this absorbance was monitored, in a 1 mm path length cell, under conditions identical to those of the rate experiment monitored by NMR (above); was found to exhibit clean first-order kinetics for 4 half-lives of reaction; and gave a rate constant of $k_{obsd} = 4.8 \times 10^{-5}$ s$^{-1}$ at 25 °C, in agreement with the rate measurement by NMR within 9%.[8]

Decomposition of **2** in aqueous solutions containing sodium hydroxide (0.01–0.1 M) or perchloric acid (0.1 M) gave methanol with an average yield of 92% based on the weight of the starting material (four determinations).[9]

The kinetics of decomposition of the diazoate were subsequently monitored by UV spectrophotometry at 25 °C, 1 M ionic strength ($NaClO_4$), in a number of aqueous buffer systems.[10] At a constant buffer ratio, the value of $k_{obsd}$ changed by less than 10% with buffer concentration ranges of 0.03–0.25 M. Extrapolation of plots of $k_{obsd}$ against buffer concentration, containing at least four points, to zero buffer concentration gave $k_0$, the buffer-independent rate constant for decomposition of the diazoate.

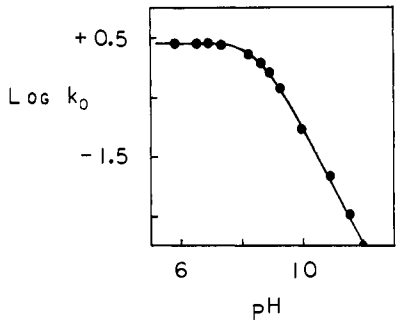
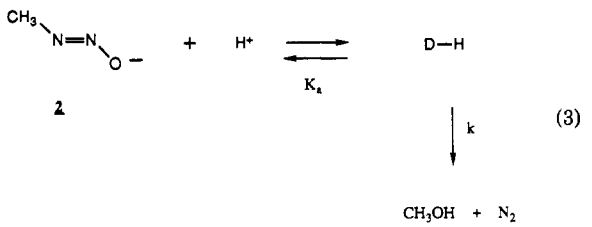



**Figure 2.** Plot of the logarithm of $k_0$, the buffer-independent rate constant for decomposition of potassium (*E*)-methanediazoate, as a function of pH at 25 °C, 1 M ionic strength ($NaClO_4$).

A plot of the logarithm of $k_0$ versus pH is presented in Figure 2. The decomposition of the diazoate is hydrogen ion independent below pH 7, with a limiting rate constant of 2.6 s$^{-1}$, but is hydrogen ion dependent above this pH. This latter observation rules out any pH-independent mechanism, such as eq 1, as the rate-limiting step for decomposition of diazoate **2**.

A mechanism consistent with the data in Figure 2 is given in eq 3 and involves the rate-limiting decomposition of a protonated form of the diazoate, D–H. The rate law for this mechanism, eq 4, gives a good fit to the data (solid curve, Figure 2) using the constants $k = 2.6$ s$^{-1}$ and $K_a = 2.30 \times 10^{-9}$ M.




(3)

$$k_0 = k/(1 + K_a/[H^+]) \quad (4)$$

If the species D–H is assumed to be the diazohydroxide, as postulated by others,[2b,4] the apparent p$K_a$ of 8.63 is not out of line with what is expected on the basis of the p$K_a$ of 7.25[11] for the stable (*E*)-benzenediazohydroxide and the observed increase of 1.2 units found upon exchange of a methyl for a phenyl group in the structurally analogous enols.[12]

However, we stress the generality of the mechanism as written in eq 3, which contains the minimal number of intermediates required by the rate law. Additional intermediates, for example, the *syn*-diazohydroxide or methanediazonium ion, are not excluded as possibilities. If the methanediazonium ion is involved, it is not likely in rapid equilibrium, relative to product formation, with **2** at high pH, on the basis of the agreement of the rate constants for disappearance of the proton signal of **2** and the chromophore of **2** in $D_2O$ (experiments above).[13]

We are currently investigating additional aspects of the aqueous reaction chemistry of compound **2** and other alkanediazoates and will report on these studies in the near future.

**Acknowledgment.** We thank Dr. George Lund of the NCI Frederick Cancer Research and Development Center for advice on the quantitation of methanol by gas chromatography and the National Institutes of Health for support under Grant CA52881.

(5) Elemental anal. Obsd: C, 11.80; H, 3.02; N, 27.07. Calcd: C, 12.24; H, 3.08; N, 28.54. $^1H$ NMR: ($CD_3OD$) (s) 3.33 ppm; ($D_2O$, 0.005–0.1 M NaOD) (s), 3.26 ppm; ($d_6$-DMSO) (s) 3.10 ppm.

(6) Lown, J. W.; Chauhan, S. M. S.; Koganty, R. R.; Sapse, A.-M. *J. Am. Chem. Soc.* **1984**, *106*, 6401.

(7) Control experiments show that the $\lambda_{max}$ of diazomethane is similar (=223 nm) but the absorbance spectrum is much more narrow. For the diazoate, $\epsilon_{223}/\epsilon_{240} = 2.2$, whereas for diazomethane, the ratio is 7.0.

(8) This rate constant is at least 100 times smaller than that obtained from the data of McGarrity and Smyth for the decomposition of diazomethane at 25 °C, 0.5 M ionic strength $NaClO_4$. McGarrity, J. F.; Smyth, T. *J. Am. Chem. Soc.* **1980**, *102*, 7303.

(9) Quantitations were made by gas chromatography. In the case of the reactions in alkaline solutions, product analysis was carried out after 7–10 half-lives of reactions. The product methanol was confirmed using $^1H$ NMR by dilution of the reaction mix at endpoint with an equal volume of $D_2O$ and recording the $^1H$ NMR spectrum, followed by spiking with authentic methanol.

(10) As well as reactions with NaOH alone, buffers used were quinuclidinol, diazabicyclo[2.2.2]octane, triethanolamine, *N*-methylimidazole, and morpholinoethanesulfonic acid. Reactions were monitored at 225, 235, or 250 nm on either a conventional spectrophotometer or an Applied Photophysics DX17MV stopped-flow spectrophotometer. Values of pH were measured with an electrode containing an internal electrolyte solution of 5 M lithium trichloroacetate. Concentrations of diazoate varied from $1 \times 10^{-4}$ to $10 \times 10^{-4}$ M.

(11) Lewis, E. S.; Hanson, M. P. *J. Am. Chem. Soc.* **1967**, *89*, 6268.

(12) Chiang, Y.; Hojatti, M.; Keeffe, J. R.; Kresge, A. J.; Schepp, N. P.; Wirz, J. *J. Am. Chem. Soc.* **1987**, *109*, 4000. Keeffe, J. R.; Kresge, A. J.; Yin, Y. *J. Am. Chem. Soc.* **1988**, *110*, 8201.

(13) Rapid disappearance of the proton signal relative to the chromophore might be expected on the basis of the observed complete proton exchange of diazomethane during hydrolysis in $D_2O$ under somewhat similar conditions (pH 13.9, 60% by volume THF) that presumably occurs through the diazonium ion. Reference 8.