monitored with a Leeds and Northrup Black Dot glass electrode, a Beckman calomel electrode, and a Beckman Model GS meter. Samples of reaction mixture were removed from time to time and the absorbance was measured with the Beckman DU spectrophotometer. The wave length (262.4 mμ) of the isosbestic point for the acid–base reaction of the substrate was used. Readings at this wave length gave a direct indication of the concentration of substrate and products without the necessity of knowing the fractions of the substrate in the acid and base forms. First-order rate constants were obtained from the slopes of the linear log $(A_t - A_\infty)$ *vs.* t plots, where the absorbance at infinite time, A_∞, was that of aniline and of the other ingredients (sodium hydroxide, sodium chloride, buffer) or of absorbing impurities associated with these ingredients. Trifluoroacetate ion has negligible absorbance at 262.4 mμ. The slopes were obtained by linear regression with an IBM 1620 computer. To make an approximate allowance for differences in the probable errors in the values of log $(A_t - A_\infty)$, these values were weighted in proportion to $A_t - A_\infty$.

The acid–base equilibrium constant for trifluoroacetanilide is given by $K = [C]/[[A][OH^-]]$, where [C] is the concentration of amide species bearing a single negative charge and [A] is the concentration of the uncharged species. Hydroxide concentration was calculated with the empirical relation, $-\log [OH^-] = 13.83 - pH_g$, where pH_g is the pH measured with the glass and calomel electrodes calibrated against 0.01 *M* borate. Values of [C]/[A] were determined in the usual way from absorbance measurements (245 mμ) extrapolated back to the time of mixing the nitrogen-purged amide and sodium hydroxide solutions. For five experiments at 25° and ionic strength 0.100 (sodium chloride), in which the value of [C]/[A] ranged from 0.45 to 9.0, a mean value of 4.293 was obtained for log K. The range was 0.019 log unit. This value for log K corresponds to a pK_a of 9.54, where pK_a is defined by $pK_a = pH_g - \log ([C]/[A]) = 13.83 - \log K$.

Acknowledgment. The author wishes to thank Mr. W. H. Lawton and Mr. E. A. Sylvestre, of the Eastman Kodak Co., for carrying out the operations with the IBM 1620 computer.

The Kinetics of the Hydrolysis of 6-Trichloromethylpurine in Dilute Aqueous Solutions[1]

Sasson Cohen and Nathan Dinar

Contribution from the Israel Institute for Biological Research, Ness-Ziona, Israel.
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Above pH 5, the hydrolysis of 6-trichloromethylpurine ($HPuCCl_3$) to purinoic and hydrochloric acids in dilute, aqueous solutions proceeds as follows. (1) $HPuCCl_3$ is ionized to $(PuCCl_3)^-$, the relative concentration of which depends on the pH and the pK_a of $HPuCCl_3$. (2) $(PuCCl_3)^-$ loses Cl^- in a rate-determining step, yielding a reactive intermediate, $Pu{=}CCl_2$. (3) The latter is sufficiently stable to discriminate between various nucleophiles, its relative reaction rates with water, Cl^-, or OH^- being 1, 233, and 1290, respectively. Non-ionized $HPuCCl_3$ is capable of undergoing hydrolysis, but at a rate much slower than $(PuCCl_3)^-$, the first-order rate constants being 8×10^{-7} and 1.09×10^{-3} sec.$^{-1}$, respectively. The relative stability of the CCl_3 group in $HPuCCl_3$, compared to structurally related systems, is ascribed to the double bond–no bond resonance of the molecule.

Recently it has been shown that 6-trichloromethylpurine (**1**) is capable of acylating various substrates with remarkable ease. The reaction with water or alcohols leads to purinoic acid or its esters,[2] that with amines to N-purinoylamines,[2,3] and that with phenols to *o*- or *p*-purinoylphenols.[4] The course of these reactions was rationalized by assuming the formation, from **1**, of a common, highly reactive intermediate by loss of one molecule of hydrogen chloride, similar to the formation of dichlorocarbene from chloroform.[5] These observations recall also the unusual reactivity of the trifluoromethyl group in certain trifluoromethyl indolecarboxylates[6] and in 5-trifluoromethyluracil.[7] In none of these cases, however, has the mechanisms involved been sufficiently elucidated, and it seemed of interest to investigate the kinetics of the acylation reaction with 6-trichloromethylpurine. The acylation of water will be considered first because of the relative simplicity of the treatment involved.

The hydrolysis of 6-trichloromethylpurine leads to purinoic and hydrochloric acids (eq. A) (H—Pu represents purin-6-yl, H being an imidazole hydrogen).

$$H\text{—}Pu\text{—}CCl_3 + 2H_2O \longrightarrow H\text{—}Pu\text{—}COOH + 3HCl \quad (A)$$

As a working hypothesis, we assume that reaction A proceeds stepwise in the following order.

$$H\text{—}Pu\text{—}CCl_3 + OH^- \rightleftharpoons Pu\text{—}CCl_3^- + H_2O \quad (1)$$

(1) This investigation was supported by Grants CA 06696-01 and -02, from the National Cancer Institute, Public Health Service.

(2) S. Cohen, E. Thom, and A. Bendich, *J. Org. Chem.*, **27**, 3545 (1962).

(3) S. Cohen, E. Thom, and A. Bendich, *Biochemistry*, **2**, 176 (1963).

(4) S. Cohen, E. Thom, and A. Bendich, *J. Org. Chem.*, **28**, 1379 (1963).

(5) J. Hine, *J. Am. Chem. Soc.*, **72**, 2438 (1950); J. Hine, R. C. Peek, Jr., and B. D. Oakes, *ibid.*, **76**, 827 (1954); J. Hine and A. M. Dowell, *ibid.*, **76**, 2688 (1954).

(6) J. Bornstein, S. A. Leone, W. F. Sullivan, and O. F. Bennett, *ibid.*, **79**, 1745 (1957).

(7) C. Heidelberger, D. Parsons, and D. C. Remy, *J. Med. Chem.*, **7**, 1 (1964).

$$\text{Pu—CCl}_3^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Pu=CCl}_2 + \text{Cl}^- \quad (2)$$

$$\text{Pu=CCl}_2 + 2\text{H}_2\text{O} \xrightarrow{k_w} \text{HPu—COOH} + 2\text{HCl} \quad (3)$$

In step 1, the position of the equilibrium depends on the pH of the medium and on the acid dissociation constant, K_a, of **1**. In 50% methanol, the pK_a of **1** was found to be 7.9,[2] but it is expected to be slightly lower in aqueous solutions.[8] If [H—Pu—CCl_3] be the total concentration of ionized and nonionized **1** present in solution, then at a given pH and at any time during the reaction the concentration of Pu—CCl_3^- would be given by the relation

$$[\text{Pu—CCl}_3^-] = \frac{1}{1 + \dfrac{[\text{H}^+]}{K_a}} [\text{H—Pu—CCl}_3] \quad (4)$$

The rate of disappearance of H—Pu—CCl_3, then, can be expressed by the general equation

$$\frac{d[\text{H—Pu—CCl}_3]}{dt} = -k_1 \frac{[\text{H—Pu—CCl}_3]}{1 + \dfrac{[\text{H}^+]}{K_a}} + k_2[\text{Cl}^-][\text{Pu=CCl}_2] \quad (5)$$

As the intermediate Pu=CCl_2 is assumed to be short-lived, application of the steady-state approximation leads to the following rate equation.

$$\frac{d[\text{Pu=CCl}_2]}{dt} = \frac{k_1[\text{H—Pu—CCl}_3]}{1 + [\text{H}^+]/K_a} - (k_2[\text{Cl}^-] + k_w)[\text{Pu=CCl}_2] = 0 \quad (6)$$

By substituting in eq. 5 for [Pu=CCl_2] its value derived from eq. 6, one obtains the following relationship.

$$\frac{d[\text{H—Pu—CCl}_3]}{dt} = -\frac{k_1[\text{H—Pu—CCl}_3]}{(1 + [\text{H}^+]/k_a)(1 + [\text{Cl}^-]k_2/k_w)} \quad (7)$$

The term $k_2[\text{Cl}^-]/k_w$ can be neglected if $k_w/k_2 \gg 3 \times 10^{-3}$, and the observed rate constant of reaction A (k^0_{obsd}), at a given pH and in the absence of added chloride, may be related to k_1 by the following equation.

$$k^0_{obsd} = \frac{k_1}{1 + [\text{H}^+]/K_a} \quad (8)$$

Experimental

6-Trichloromethylpurine[2] was purified by passage of its chloroform solution through a column of acid-washed alumina. The eluate, concentrated under reduced pressure and chilled to −5°, gave crystals which were separated by filtration and dried from solvent under reduced pressure.

Rate Measurements. The rate of appearance of acid in solutions of **1** in the pH range 5–10 was determined by pH-Stat titration with a Radiometer automatic assembly comprising a 0.5-ml. piston buret (SBU 1), a Tritrigraph (TTT 1), and a recorder (SBR 2 c). A freshly prepared solution of **1** (2.0–2.5 mg.) in 0.2% methanol–water (10.0 ml.) or in 0.2% methanol–water containing a known concentration of KCl, was placed in a thermostated vessel (25.0° ± 0.1), equipped with a magnetic stirrer and calomel and glass electrodes, and was automatically titrated with 0.1 *N* potassium hydroxide solution at a preset pH value. The titration was continued until no more base was consumed, and the volume of titrant at any given time was read from the titration curve recordings. Since 1 mole of **1** liberates 4 equiv. of acid

$$k_{obsd}(t - t_0) = \ln \frac{4ac_0 - x_0}{4ac_0 - x}$$

where c_0 is the initial concentration of **1**, and x_0 and x are the equivalents of acid formed during time t_0 and t, respectively; a is a factor which, theoretically, should have a value between 1 and 1.25 and depends on the pH and the second dissociation constant of purinoic acid.[3] The value of a was found by dividing the total quantity of base consumed in a titration by the theoretical quantity calculated according to reaction A. The calculation of k^0_{obsd} at pH 9.0 is given in Table I as a general example.

Table I. Determination of k^0_{obsd} at pH 9.0 and at 25.0°[a]

x, mequiv./l.	$t - t_0$, sec.	$4ac_0 - x$, mequiv./l.	$A = (4ac_0 - x_0)/(4ac_0 - x)$	$k^0_{obsd} \times 10^3$, sec.$^{-1}$
1.43	60	3.298	1.064	1.03
1.81	180	2.918	1.202	1.02
2.14	300	2.588	1.356	1.03
2.44	420	2.288	1.533	1.02
2.71	540	2.018	1.738	1.02
3.25	840	1.478	2.373	1.04
3.66	1140	1.068	3.284	1.05
3.95	1440	0.778	4.509	1.05
4.16	1740	0.568	6.176	1.05
4.51	2640	0.218	16.09	1.05
4.63	3540	0.098	35.80	1.01
			Av. k^0_{obsd} =	1.04×10^{-3} sec.$^{-1}$

[a] $c_0 = 1.105 \times 10^{-3}$ mole/l.; $a = 1.07$; $4ac_0 = 4.728 \times 10^{-3}$ mole/l.; $x_0 = 1.22 \times 10^{-3}$ equiv./l.; $4ac_0 - x_0 = 3.508 \times 10^{-3}$ equiv./l.; $t_0 = 60$ sec. c_0 = initial concentration of 6-trichloromethylpurine; x = concentration of acid formed after time t; x_0 = concentration of acid at time t_0.

The pH-Stat procedure was not applicable to solutions of pH value higher than 11.0. Therefore, recourse was taken to a spectrophotometric measurement of the rate of disappearence of 6-trichloromethylpurine. A solution of potassium hydroxide, or potassium hydroxide and potassium chloride, of known concentration (25.0 ml.), previously maintained at 25°, was added rapidly to a solution of **1** (10.0 mg.) in methanol (0.05 ml.). The mixture was placed in a water bath at 25° and 1.0-ml. aliquots were withdrawn periodically and added rapidly to 0.5 ml. of 1 *N* HCl solution. The residual 6-trichloromethylpurine was extracted by shaking the acidified solution with chloroform (10.0 ml.), and the optical density of the solution was read at 278 mμ, where **1** has a maximum. Beer's law is obeyed over the range 0–50 μg./ml. Purinoic acid does not interfere. The rate of the reaction was then found from the relation

$$\ln (D - D_\infty) = \ln (D_0 - D_\infty) - k_{obsd}t$$

(8) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen and Co., Ltd., London, 1962; K. C. Ong, R. A. Robinson, and R. G. Bates, *Anal. Chem.*, 36, 1971 (1964).

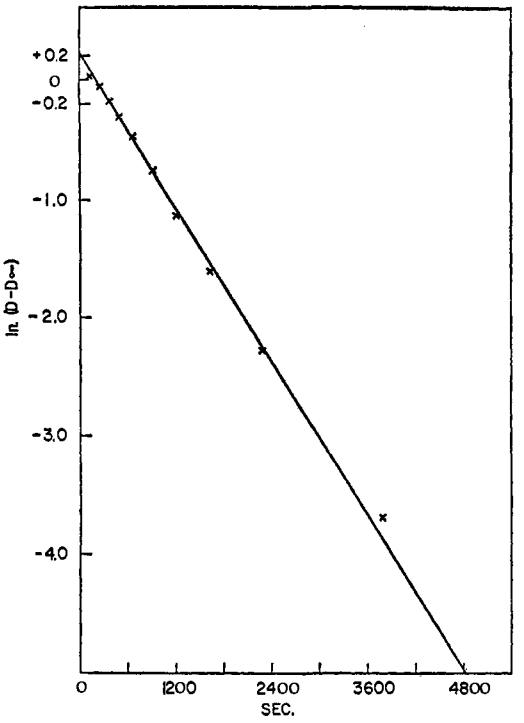


Figure 1. Determination of k^0_{obsd} by the spectrophotometric method at pH 13. $-k^0_{obsd} = \Delta\{\ln (D - D_\infty)\}/\Delta t = 1.08 \times 10^{-3}$ sec.$^{-1}$.

where D_0, D, and D_∞ are the optical densities at time 0, t, and infinity, respectively. A plot of ln $(D - D_\infty)$ against t gives a straight line, the slope of which is equal to $-k_{obsd}$. An example of the calculation of k_{obsd} by the spectrophotometric method is given in Figure 1. The spectrophotometric method was also used for the determination of k^0_{obsd} at pH values lower than 5. In this case, **1** was dissolved in perchloric acid solution of known strength and the rate was calculated by taking $D_\infty = 0$.

Results and Discussion

In the absence of added chloride, the dependence of k^0_{obsd} on the pH of the medium is given graphically in Figure 2. At pH 10 there is no further significant increase in rate, and k^0_{obsd} becomes almost equal to $k_1 = 1.09 \times 10^{-3}$ sec.$^{-1}$. By solving for K_a in eq. 8, the pK_a of 6-trichloromethylpurine is found to be 7.70 ± 0.05 (Table II). These results are in agreement with a mechanism such as the one formulated in steps 1 and 2. However, the conclusive evidence for the existence of step 2 lies in a demonstration of its reversibility.

Table II. Calculation of the pK_a of 6-Trichloromethylpurine from k^0_{obsd} at Various Hydrogen Ion Concentrations

pH	$k^0_{obsd}/(k_1 - k^0_{obsd})$	log $k^0_{obsd}/(k_1 - k^0_{obsd})$	pK_a
6.5	0.058	−2 + 0.77	7.73
7.0	0.211	−1 + 0.32	7.68
7.5	0.535	−1 + 0.73	7.77
7.7	0.975	−1 + 0.99	7.71
8.0	2.28	0.36	7.64
8.5	7.03	0.84	7.66
9.0	21.2	1.33	7.67
		Av. pK_a = 7.70 ± 0.05	

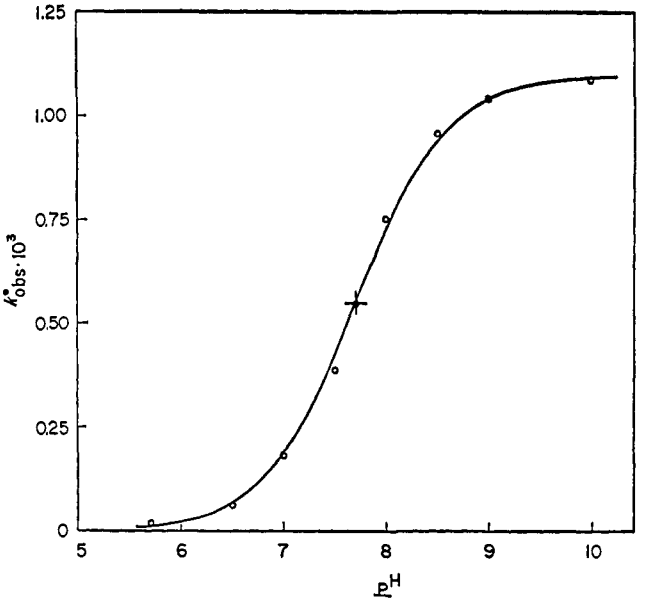


Figure 2. Dependence of k^0_{obsd} on the pH.

The effect of known concentrations of added chloride on the observed rate of the reaction at various pH values is given in Table III and Figure 3.

Table III. Effect of Added Chloride on the Rate of Hydrolysis of 6-Trichloromethylpurine

[Cl$^-$], mole/l.	$k^0_{obsd} \times 10^3$, sec.$^{-1}$	pH	$k^0_{obsd} \times 10^3$,[a] sec.$^{-1}$	k^0_{obsd}/k_{obsd}
0.011	0.995	9.0	1.04	1.04
0.042	0.895	9.0	1.04	1.16
0.042	0.607	8.0	0.725	1.19
0.042	0.360	7.5	0.421	1.17
0.110	0.710	9.0	1.04	1.47
0.123	0.476	8.0	0.725	1.52
0.123	0.267	7.5	0.421	1.58
0.184	0.576	10.0	1.09	1.89
0.184	0.062	6.7	0.099	1.60
0.210	0.525	9.0	1.04	1.98
0.210	0.360	8.0	0.725	2.01
0.210	0.242	7.5	0.421	1.74
0.310	0.304	8.0	0.725	2.38
0.310	0.180	7.5	0.421	2.34
0.424	0.400	9.0	1.04	2.60
1.05	0.230	9.0	1.04	4.52
1.05	0.143	8.0	0.725	5.07
1.05	0.088	7.5	0.421	4.78
3.11	0.135	9.0	1.04	7.72
3.11	0.034	7.5	0.421	11.4

[a] Calculated values.

The observed decrease in the rate of the reaction must be a consequence of the recombination of the reactive intermediate (Pu=CCl_2) with chloride.

Although the absolute value of k_2 cannot be found from the available data, the ratio k_2/k_w which represents the *mass law constant*[9] (or α) can be calculated from the following relation

$$\frac{k^0_{obsd}}{k_{obsd}} = \frac{k_2}{k_w}[Cl^-] + 1 = \alpha[Cl^-] + 1 \qquad (9)$$

(9) L. C. Bateman, M. G. Church, E. D. Hughes, C. K. Ingold, and N. A. Taher, *J. Chem. Soc.*, 979 (1940).

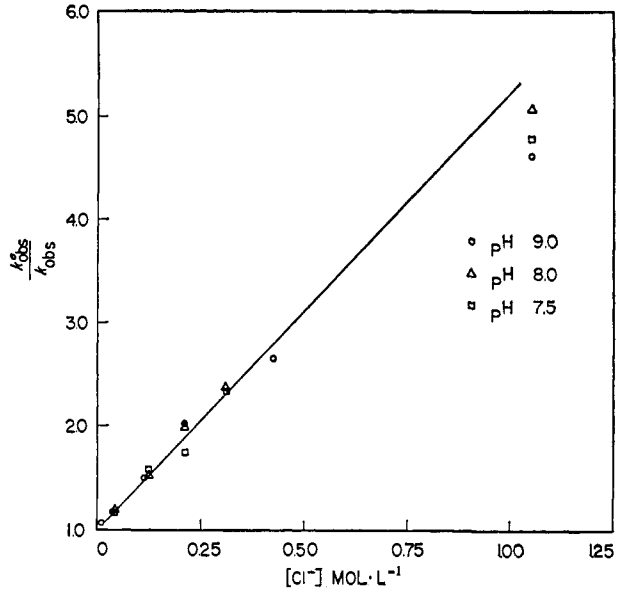


Figure 3. Dependence of k^0_{obsd}/k_{obsd} on $[Cl^-]$. $\alpha = ((k^0_{obsd}/k_{obsd}) - 1)/[Cl^-] = 4.2$ l. mole^{-1}.

where k^0_{obsd} and k_{obsd} are the observed rates in the absence and presence of added chloride, respectively, under exactly equal conditions of pH and concentration of **1**. A plot of k^0_{obsd}/k_{obsd} against chloride concentration gives a straight line the slope of which is $\alpha = 4.21$ mole^{-1} (Figure 3). At relatively high chloride concentration, *e.g.*, in 1 *M* KCl solution, secondary effects become important enough to cause an appreciable deviation of the experimental values from the plot described by eq. 9.

One such effect could be an increase in the ionization constant of **1** with increasing ionic strength.

Two salient features emerge from the above findings: (1) the reaction of chloride with the reactive intermediate is an equilibrium-governed process (since a plot of k_{obsd}/k^0_{obsd} against log $[Cl^-]$ would give a sigmoid curve), and (2) the effect of added chloride on the observed rate seems to be independent of hydroxide ion concentration in the pH region below pH 10.

Of course, this last observation does not necessarily imply that no reaction occurs between hydroxide ion and the reactive intermediate. It only means that in the presence of low concentrations of OH^- (*e.g.*, below 10^{-4} *M*), among the possible reaction pathways open to $Pu{=}CCl_2$ that with water is the major one. In order to obtain a measure of the contribution of hydroxide in the hydrolysis of **1**, eq. 8 may be extended to include a term for hydroxide and chloride

$$k_{obsd} = \frac{k_1}{\left(1 + \frac{[H^+]}{K_a}\right)\left(1 + \frac{k_2[Cl^-]}{k_w + k_{OH^-}[OH^-]}\right)} \quad (10)$$

where k_{OH^-} is the rate constant of the reaction of $Pu{=}CCl_2$ with hydroxide. At pH values higher than 10, eq. 10 becomes

$$k_{obsd} = \frac{k_1}{1 + \frac{k_2[Cl^-]}{k_w + k_{OH^-}[OH^-]}} = \frac{k_1}{1 + \frac{[Cl^-]}{\frac{k_{OH^-}[OH^-]}{k_w} + 1}}$$

It can be shown that

$$\frac{k_{OH^-}}{k_w} = \frac{1}{[OH^-]}\left(\frac{\alpha[Cl^-]k_{obsd}}{k_1 - k_{obsd}} - 1\right)$$

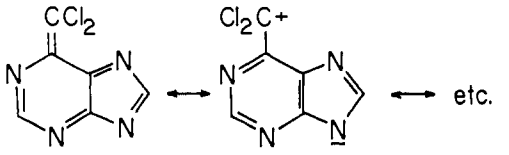
The experimental values of k_{OH^-}/k_w found for different concentrations of chloride and hydroxide are given in Table IV.

Table IV. Determination of k_{OH^-}/k_w

$[OH^-]$, mole/l.	$[Cl^-]$, mole/l.	$k_{obsd} \times 10^3$, sec.$^{-1}$	k_{OH^-}/k_w, l. mole^{-1}	k_{OH^-}/k_2, l. mole^{-1}
0.1	0.3	0.79	23.2	5.5
0.05	0.5	0.55	22.8	5.4
0.2	0.6	0.76	24.0	5.7
		Av. $k_{OH^-}/k_w = 23.2 \pm 0.6$ l. mole^{-1}		

If one takes the molarity of water as 55.5 *M*, then the relative reaction rates of $Pu{=}CCl_2$ with water, chloride, and hydroxide are 1, 233, and 1290, respectively.

The ability of $Pu{=}CCl_2$ to discriminate between water and nucleophilic anions gives an indication of its stability, which seems to be of an order similar to that reported for the benzhydryl carbonium ion in aqueous acetone.[10] The ratio of the nucleophilic constants of hydroxide to chloride found in the present study (1.31) is very close to the ratio of the values of these constants (1.38) given by Swain and Scott[11] for other substrates. The high reactivity of $Pu{=}CCl_2$ toward water (in comparison with the tritylium ion)[10] may be a consequence of charge localization in the molecule. That is, among the many resonance forms that could be written for $Pu{=}CCl_2$, those bearing a positive charge on the alkyl carbon are statistically favored.



At low pH values, there is a marked departure of k^0_{obsd} from the values predicted from eq. 8 (see Table V).

Table V

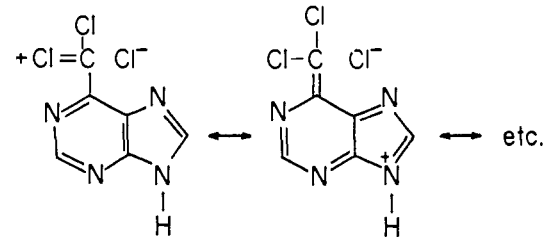
pH	k^0_{obsd}, sec.$^{-1}$	k^0_{obsd}, sec.$^{-1a}$
1.25	8.0×10^{-7}	3.8×10^{-10}
1.90	8.5×10^{-7}	1.7×10^{-9}

[a] Calculated from eq. 8.

The value of 8×10^{-7} sec.$^{-1}$ is therefore taken as the rate of hydrolysis of nonionized **1**. **1** is most probably protonated in solutions of pH less than 1,[2] but the effect of such a protonation on the hydrolysis rate within the pH range 1–2 seems to be negligible. It is perhaps significant that this rate is comparable to that reported for benzodifluorochloride,[12] but much lower than that for benzotrichloride.[13] The difference in the

(10) C. G. Swain, C. B. Scott, and K. H. Lohmann, *J. Am. Chem. Soc.*, 75, 136 (1953).
(11) C. G. Swain and C. B. Scott, *ibid.*, 75, 141 (1953).
(12) J. Hine and D. E. Lee, *ibid.*, 74, 3182 (1952).
(13) J. Hine and D. E. Lee, *ibid.*, 73, 22 (1951).

reactivity of the CCl_3 group between the two systems could be explained by a greater ability of the purine ring structure, compared to benzene, to contribute to the double bond–no bond resonance[14] of the molecule, thus leading to stronger C–Cl bonds.



(14) J. Hine, *J. Am. Chem. Soc.*, **85**, 3239 (1963).

Examples of resonance stabilization resulting from the presence of an electron-withdrawing group on the same carbon atom with a resonance electron-donating group have been given by Hine and Rosscup.[15]

The purine ring system may be unusual in that it is able to act both as electron acceptor and electron donor; in the present case, the latter effect may predominate. Generally, however, purine is considered as a π-excessive heteroaromatic because it embodies an electron releasing setting (=CH—NH—CH=).[16]

Acknowledgment. The authors wish to thank Dr. E. D. Bergmann and Dr. A. Bendich for their continued interest in this work.

(15) J. Hine and R. J. Rosscup, *ibid.*, 82, 6115 (1960).
(16) A. Albert, "Heterocyclic Chemistry," Oxford University Press, London, 1959.

Reactions of Nucleophilic Reagents with Phosphoramidate[1]

William P. Jencks and Mary Gilchrist

Contribution No. 363 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received February 18, 1965*

The rates of the reactions of a number of compounds with the phosphoramidate monoanion show only a small dependence upon the basicity of the nucleophilic reagent: the Brønsted β-value is 0.22 for substituted pyridines, and other amines react at similar rates. In concentrated solutions there is a significant accumulation of the phosphorylated tertiary amine in the reactions of phosphoramidate with pyridine, 4-methylpyridine, triethylenediamine, and N-methylimidazole. The rate of hydrolysis of the phosphorylated 4-methylpyridine is independent of pH and occurs with a half-time of about 5 min. at 25°; the product of the reaction with N-methylimidazole is much more stable. The reaction with hydroxylamine gives predominantly the O-phosphorylated product, which is probably the same as the compound which is formed from adenosine triphosphate and hydroxylamine in the presence of pyruvate kinase and bicarbonate. Fluoride reacts predominantly with the species of phosphoramidate which carries no net charge, but also reacts slowly with the monoanion. Several phosphorylated diamines which have two available sites for protonation on the leaving group undergo rapid hydrolysis at slightly acidic pH.

In recent studies of the reactions of nucleophilic reagents with acetyl phosphate, it was found that glycine, aniline, imidazole, N-methylimidazole, and morpholine attack the carbonyl group, while pyridine, fluoride, triethylenediamine, and trimethylamine attack at phosphorus.[2,3] Although there appears to be a tendency for primary amines to attack preferentially at carbon and for tertiary amines to attack at phosphorus, this distinction is not clear-cut and there is no obvious reason for the order of nucleophilic reactivities. In the hope of clarifying this situation, we have examined the reactivity of a series of nucleophilic reagents toward phosphoramidate, which is not subject to the complication of nucleophilic attact at positions other than the phosphorus atom.

There is a considerable amount of information available regarding nucleophilic reactivity toward fully substituted phosphate compounds,[4] but there are only scattered data for ionized phosphates, which are the phosphate compounds of principal biological interest.[2,3,5–8] Phosphoramidates have been widely utilized for synthetic work, especially for the synthesis of pyrophosphates,[9] and the synthesis of a substituted pyrophosphate has been shown to be a bimolecular reaction.[10] Phosphoramidate has been shown to undergo nucleophilic reactions with imidazole, pyridines, and fluoride, and the reactions with pyridine and 4-methylpyridine proceed at rates which differ by only 30%.[5–8,11] An enzyme preparation from *E. coli* has been shown to catalyze phosphoryl transfer from a

(1) Supported by grants from the National Science Foundation and the National Institute of Child Health and Human Development of the Public Health Service (HD-01247).
(2) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, 83, 4393, 4400 (1961).
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