PHYSICAL AND INORGANIC CHEMISTRY

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Catalysis in Water and Ice. A Comparison of the Kinetics of Hydrolysis of Acetic Anhydride, β-Propiolactone, and p-Nitrophenyl Acetate and the Dehydration of 5-Hydro-6-hydroxydeoxyuridine in Water and in Ice

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Following reports of great increases in the rates of several reactions in ice as compared to water, we have compared the rates of a series of reactions in ice at $-10°$ to the rates in water at $-10°$ (extrapolated employing values of $\Delta H\ddagger$ determined in water). The reactions investigated were: (1) spontaneous hydrolysis of acetic anhydride; (2) specific-acid catalyzed hydrolysis of acetic anhydride; (3) specific-base catalyzed hydrolysis of acetic anhydride; (4) acetate anion general-base catalyzed hydrolysis of acetic anhydride; (5) spontaneous hydrolysis of β-propiolactone; (6) the imidazole nucleophilic catalyzed hydrolysis of β-propiolactone; (7) the specific acid-catalyzed hydrolysis of p-nitrophenyl acetate; and (8) the specific-acid catalyzed dehydration of 5-hydro-6-hydroxydeoxyuridine. The spontaneous reactions were found to be completely or greatly depressed in ice as compared to water. Increases in ionic strength greatly increased the spontaneous rates in ice (up to a critical concentration) and slightly decreased the rates in water. The rate constants for the bimolecular reactions were slightly or greatly enhanced in ice as compared to water (OH^- catalysis could not be evaluated quantitatively in ice). Increase in ionic strength generally decreased the rates of the bimolecular reactions in ice. The above findings, as well as those of some preliminary n.m.r. studies, indicate a concentration phenomenon, combined with the making of the concentration of water rate limiting, to be responsible for the magnitude of the rate constants obtained in ice.

In two recent papers,[3,4] experiments of a semiquantitative nature have been described which demonstrate that certain reactions proceed at a considerably enhanced rate when occurring in ice as compared to liquid water. The two reactions studied, the acid-catalyzed dehydration of 5-hydro-6-hydroxydeoxyuridine[3] and the hydrolytic opening of the β-lactam ring of penicillin,[4] are completely unrelated. The purpose of the present study has been to establish, in a quantitative manner, the reason for the large rate enhancements obtained in ice and to determine if the effect is general. As far as the authors are aware, this is a completely new area for study. Questions investigated were: (a) Are reactions in the ice phase, involving water as a reactant, kinetically clean? (b) How do the over-all kinetics for hydrolytic reactions vary from H_2O-liquid to H_2O-ice? (c) Are hydrolytic reactions generally more facile in ice than in water.

Apart from the novel aspect of the present problem, it appeared that the investigation of reactions in the ice phase might have potential practical synthetic utility and could possibly shed some light on the mechanism of enzymatic catalysis. The feasibility of performing certain synthetic or degradative operations at low temperatures in the ice phase rather than at reflux temperatures certainly warrants investigation. The question also arises whether ice-berg structures[5-12]

formed at the active site of enzymes, by juxtaposition of lyophobic groups, could explain, at least in part, the great efficiency of enzymatic reactions by providing a more favorable medium for catalysis. Although the importance of lyophobic bonding of the substrate to enzyme has received considerable attention (see ref. 10), the possible importance of the resulting ice-berg structure in the process of catalysis has not been considered.

Possible explanations of the rate enhancements in ice include: the high mobility of the proton in ice as compared to water[13] and the concentration of reactants in regions between the ice crystals which remain liquid.

Experimental

Materials.—Acetic anhydride and sodium acetate were purified as described previously.[14,15] Aniline was distilled at atmospheric pressures and the aniline water stored away from the light. 2,6-Lutidine was purified by the method of Pritchard and Long.[16] Imidazole was Eastman White Label grade and was employed without further purification; p-nitrophenyl acetate was that prepared for a previous study.[17] Eastman White Label β-propiolactone was distilled at reduced pressure. The deoxyuridine was obtained from Cyclo Chemical Corp.

Kinetic Methods.—For the reactions in ice the following procedure was employed throughout this investigation. The compo-

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(14) V. Gold and J. Hilton, J. Chem. Soc., 838 (1955).

(15) A. R. Butler and V. Gold, ibid., 2305 (1961).

(16) J. G. Pritchard and F. A. Long, J. Am. Chem. Soc., 79, 2365 (1957).

(17) T. C. Bruice and S. J. Benkovic, ibid., 85, 1 (1963).

nents of the stock solution were prepared as described later and pipetted into a number of test tubes with neoprene lined screw caps. The test tubes, each containing between 0.5 and 5.0 ml. of the reaction mixture (depending on the reaction investigated), were placed in a circular wire basket which was then lowered quickly into a dewar flask charged with a Dry Ice–acetone mixture. The tubes were allowed to remain submerged for a period of time that ensured complete freezing. After removal from the Dry Ice–acetone bath the tubes were placed in an alcohol bath at $-10°$ and the kinetic run initiated when the tubes had warmed up to that temperature (about 15 min.). By this procedure the reaction mixtures were rapidly frozen with no separation of a supernatant solution containing the reactants due to the slow formation of ice crystals. On warming to $-10°$ the solutions remained solidly frozen. Tubes were withdrawn from the bath periodically and the solutions assayed for remaining reactant as separately described for each reaction.

Hydrolysis of Acetic Anhydride.—This reaction was followed by the aniline method of Menschutkin and Vassilief.[18] When the medium was water the technique did not differ from that used in previous studies.[19] For the ice reaction, acetic anhydride (0.2 ml.) was added to water (50 ml.) or catalyst solution and 5-ml. aliquots of the mixture dispensed into the reaction tubes. Periodically tubes were removed from the $-10°$ bath and 10 ml. of aniline water added and the tube immersed in a bath at 30° until the ice had melted (a process requiring about 45 sec.). The acetic acid formed was determined by titration of the resultant solution with 0.02 M potassium hydroxide. The use of buffers in the attempt to study hydroxyl ion catalysis rendered the titrimetric method impossible and the method of Butler and Gold[15] was used. For the reactions in ice this method was modified in essentially the same manner as the aniline method.

Hydrolysis of p-Nitrophenyl Acetate.—A dioxane solution of p-nitrophenyl acetate (0.5 ml.) was diluted to 50 ml. with dilute hydrochloric acid and 2-ml. aliquots distributed into screw-capped test tubes. Half the aliquots were frozen and stored at $-10°$ while the others were stored at $+5°$. To determine the amount of p-nitrophenol formed after a timed interval, the frozen samples were melted at 30° and 2 ml. of 1.0 M phosphate buffer (pH 7.1) added. The absorbance of this solution at 400 mμ was then measured. For the samples at 5° the procedure was the same without the thawing of the sample. To obtain the infinity reading the mixture of buffer and reaction mixture were allowed to stand for 8 hr. before the absorbance was measured.

Hydrolysis of β-Propiolactone.—The disappearance of the lactone was followed by a modification of the hydroxamate method of Lippman and Tuttle.[20] For the development of the hydroxamate–ferric ion complex, the stock solution of hydrochloric acid was 3 N and the ferric chloride 5% in 0.1 N hydrochloric acid. The aqueous stock hydroxylamine was prepared by neutralizing a 28% hydroxylamine hydrochloride solution with an equal volume of 14% sodium hydroxide solution. The neutralized hydroxylamine solution was buffered with an equal volume of a solution made by mixing 4 parts of 0.1 M sodium acetate solution and 1 part of 0.1 M acetic acid.

Imidazole buffers were prepared of constant ionic strength (0.1 M with KCl) and the pH adjusted to between 7.22 and 8.32 at 5°. Each buffer was diluted with 0.1 M potassium chloride solution to give a series of solutions of constant pH and varying imidazole content. β-Propiolactone (0.02 ml.) was added to 25 ml. of each imidazole buffer solution and 1-ml. aliquots pipetted into screw-capped tubes. For the runs at $-10°$ the standard freezing procedure was employed. To determine the lactone remaining at time t, a tube was removed from the constant temperature bath, thawed at 30° for 2 min., and 2 ml. of buffered hydroxylamine added. This was allowed to stand for 5 min. when 2 ml. of ferric chloride solution was added and the absorbance at 540 mμ measured. The pseudo-first-order rate constants were obtained from the slope of the plot of log D_0/D_t vs. t. Apart from the thawing, the same procedure was used for the reactions at 5 and 20°.

Product analysis for this reaction was carried out by thin layer chromatography. Microscope slides were coated with silica suspended in chloroform and allowed to dry in the air. Six slides were spotted with the following mixtures: (a) imidazole buffer, (b) solution obtained by hydrolyzing β-propiolactone at room temperature in above imidazole buffer, (c) same as (b) but reaction allowed to proceed at $-10°$, (d) solution of β-propiolactone in water, (e) solution of 1-methyl-5-hydroxymethylimidazole, and (f) solution of β-hydroxypropionic acid. After developing the chromatogram with 95% ethanol the slides were exposed to iodine vapor and the R_f values of any spots developed measured.

Dehydration of 5-Hydro-6-hydroxydeoxyuridine.—The above compound was prepared by the irradiation by ultraviolet light

of a solution of 2′-deoxyuridine.[21] A 0.002 M solution of deoxyuridine (20 ml.) was irradiated in a 10-cm. petri dish placed 5 cm. from a SL.2537 ultraviolet lamp for 75 min. This was then diluted to 50 ml. with water. Reaction mixtures were prepared by diluting 5 ml. of this and a measured quantity of hydrochloric acid and any salt to 25 ml. For the reaction in ice, 2-ml. aliquots of the reaction mixture in screw-capped tubes were frozen and the reaction followed by thawing and measuring the absorbance at 260 mμ. For the reactions at 30° the reaction mixture was placed in a thermostated cuvette inside a Zeiss spectrophotometer and the absorbance determined at timed intervals.

Apparatus.—All spectrophotometric determinations were carried out with a Zeiss PMQII spectrophotometer. pH measurements were made with a Radiometer Model 22 pH meter by use of a Radiometer a-K 2021 C combined calomel–glass electrode. The n.m.r. studies were carried out on a Varian instrument with a low temperature probe at Bell Telephone Laboratories, N. J.

Results[22]

Hydrolysis of Acetic Anhydride.—The kinetic equation 1 describes the hydrolysis of acetic anhydride in water.[23]

$$- \mathrm{d(Ac_2O)}/\mathrm{d}t = [k_0 + k_{\mathrm{H}^\oplus}(\mathrm{H}^\oplus) + k_{\mathrm{OH}^\ominus}(\mathrm{OH}^\ominus) + k_{\mathrm{OAc}^\ominus}(\mathrm{AcO}^\ominus)](\mathrm{Ac_2O}) \quad (1)$$

where k_0 is the kinetic constant for spontaneous solvolysis, a reaction associated with a deuterium solvent isotope effect $(k^\mathrm{H}/k^\mathrm{D})$ of about 3 and, therefore, probably representing a case of general-base catalysis.[15,24]

$$(2)$$
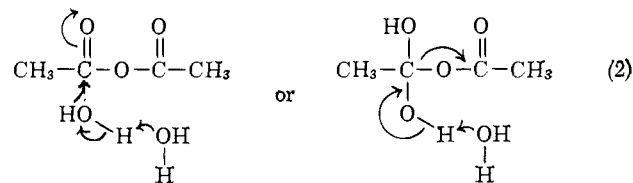
The constants $k_{\mathrm{H}^\oplus}$, $k_{\mathrm{OH}^\ominus}$, and $k_{\mathrm{OAc}^\ominus}$ are second-order rate constants for the specific-acid, specific-base and acetate anion general-base catalyzed hydrolysis.

At ambient temperatures k_0 is of such a value compared with the products $k_{\mathrm{H}^\oplus}(\mathrm{H}^\oplus)$ and $k_{\mathrm{OH}^\ominus}(\mathrm{OH}^\ominus)$ that it may be obtained from the rate of hydrolysis of acetic anhydride in unbuffered aqueous solutions. The rate constant at $+5°$ is 4.17×10^{-2} min.$^{-1}$.[25] In ice at $-10°$ spontaneous hydrolysis is reduced, exhibiting a rate constant of about 1.4×10^{-3} min.$^{-1}$. The true rate constant in ice at $-10°$ may be smaller for, as will be seen later in this paper, the presence of some acetic acid produced by anhydride hydrolysis before freezing may materially increase the rate of the reaction. Thus, the spontaneous hydrolysis of acetic anhydride at $+5°$ proceeds 30 times faster than the same reaction in ice at $-10°$. From Gold's value of the energy of activation[25] for k_0 it is possible to calculate the rate of reaction at $-10°$ in water, assuming no ice formation. The rate constant thus obtained is 1.33×10^{-2} min.$^{-1}$, so that the observed rate of reaction at $-10°$ in ice is, at least, one-ninth the calculated value for water at $-10°$.

The addition of potassium chloride lowers the value of k_0 in water.[23] For the reaction in ice at $-10°$ the effect is more complex. Below 0.02 M potassium chloride the rate of spontaneous hydrolysis increases with increasing salt, but above 0.02 M the rate is independent of the concentration of potassium chloride (see Table I). In the presence of 0.035 M acetic acid the value of k_0 is 3.1×10^{-3} min.$^{-1}$ and with 0.0487 M dioxane k_0 is 4.01×10^{-3} min.$^{-1}$.

(21) A. M. Moore and C. H. Thompson, *Science*, **122**, 594 (1955).

(22) Abbreviations employed: Im, imidazole free base; Im$_T$, total imidazole; [lut$_T$], total lutidine; p-NPA, p-nitrophenyl acetate; Ac$_2$O, acetic anhydride; OAc°, acetate ion; D, optical density; a_H, hydrogen ion activity as determined with the glass electrode; K_a', experimentally determined dissociation constant; T, volume of titer.

(23) M. Kilpatrick, *J. Am. Chem. Soc.*, **50**, 2891 (1928).

(24) C. A. Bunton, N. A. Fuller, S. G. Perry, and V. J. Shiner, *Chem. Ind.* (London), 1130 (1960).

(25) V. Gold, *Trans. Faraday Soc.*, **44**, 506 (1948).

(18) Menschutkin and Vassilief, *Zh. Fiz. Khim.*, **21**, 192 (1889).

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(20) F. Lippman and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

TABLE I

SALT EFFECT ON THE RATE OF SPONTANEOUS ACETIC ANHYDRIDE
HYDROLYSIS

(KCl), M	$10^3 k_{obsd}$, min.$^{-1}$	(KCl), M	$10^3 k_{obsd}$, min.$^{-1}$
0.00	1.4	0.06	4.7
.01	2.3	.10	4.9
.02	4.3	.20	4.2
.04	4.4		

The value of $k_H\oplus$ was determined by experiments employing 0.02 to 0.10 M hydrochloric acid as buffer. The kinetics of the acid-catalyzed reaction in ice were found to be clearly first order over at least two half-lives (see Fig. 1). The value of $k_H\oplus$ in water is fairly small
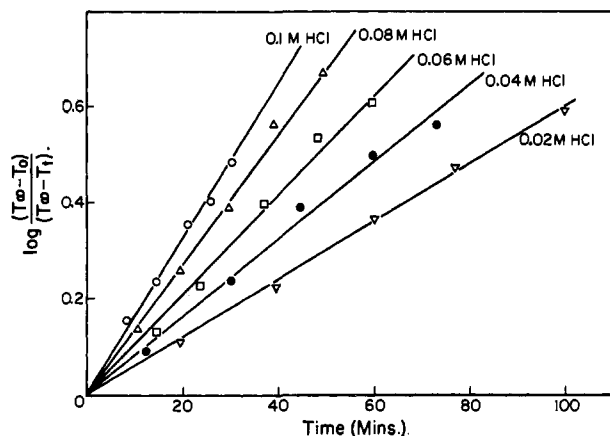
Fig. 1.—The first-order hydrolysis of acetic anhydride in the presence of hydrochloric acid at −10° in ice.

but in ice at −10° it is increased. Preliminary experiments showed that there is a large negative salt effect, so that in the first series of experiments the ionic strength was maintained at a constant value of 0.1 M by the addition of potassium chloride. Under these conditions a linear relationship was found between acid concentration and the rate of reaction (see Fig. 2) and the slope of this plot gave a value for $k_H\oplus$ of 0.19 l. mole^{-1} min.$^{-1}$. Similar determinations were made at 5 and 25°, using the same concentration of acid and the same ionic strength, providing the values $k_H\oplus$ (at 5°) = 5.1 × 10^{-2} and $k_H\ominus$ (at 25°) = 0.41 l. mole^{-1} min.$^{-1}$. Thus, the value of $k_H\oplus$ is 3.7 times greater in ice at −10° than in water at +5°. From the values of $k_H\oplus$ at 5 and 25° the activation energy was calculated to be 17.2 kcal. mole^{-1}. Employing this value, the rate constant for the acid-catalyzed reaction at −10° in water was calculated to be 8.7 × 10^{-3} l. mole^{-1} min.$^{-1}$, so that the value of $k_H\oplus$ in ice at −10° is 22 times greater than the calculated value for water at −10°.

Addition of potassium chloride decreases the acid-catalyzed reaction, and if the ionic strength is not kept constant there is no direct relationship between the observed rate and the acid concentrations (see Table II). The first two results in Table II show that the salt effect of the acid is the same as that for potassium chloride. From the figures in the fourth column of Table II it is seen that the catalytic coefficient increases as the ionic strength is lowered. The last result is a deviation from this general trend. Clearly, the rate enhancement obtained on freezing when the acid concentration is very low is much greater than that observed when the ionic strength is maintained at 0.1 M. The salt effect for the acid-catalyzed reaction in water is small.[23] Using 0.055 M acid the reaction in ice is 27 times faster at −10° than in water at +5° and 161 times faster than the calculated rate at −10° in water.
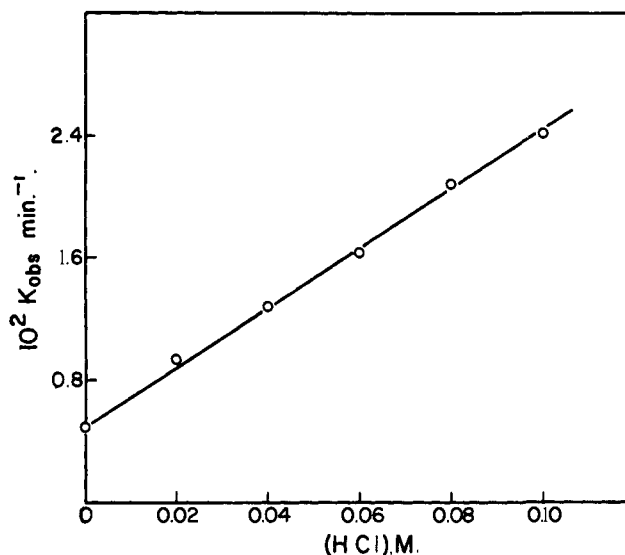
Fig. 2.—The observed pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of acetic anhydride at −10° plotted against hydrochloric acid concentration.

The value of k_{OH^-} is difficult to determine owing to the magnitude of the constant and the necessity of employing buffered systems. Carboxylates and amines interfere owing to the formation of mixed anhydrides[26] or acetyl derivatives. A phosphate buffer was tried for the reaction at 5° but nonlinear first-order plots were obtained, an effect noted by other investigators using these buffers.[27] It was found, however, that a

TABLE II

SALT EFFECT ON THE ACID-CATALYZED ACETIC ANHYDRIDE
HYDROLYSIS

Ionic strength,[a] M	(HCl), M	$10^2 k_{obsd}$, min.$^{-1}$	$k_H\oplus$, l. mole^{-1} min.$^{-1}$
0.20	0.20	2.94	0.13
.20	.055	1.06	.12
.10	.060	1.66	.19
.055	.055	2.42	.36
.020	.020	1.41	.49
.018	.018	1.24	.47
.010	.010	0.92	.69
.005	.005	.91	1.40
.002	.002	.33	0.80

[a] Computed from the total HCl + KCl present.

hindered amine, 2,6-lutidine, was suitable for this study. Following Pritchard and Long's procedure,[16] lutidine perchlorate was dissolved in water, half neutralized with potassium hydroxide, and potassium chloride added to make the ionic strength 0.1 M. This mixture was then diluted to give a number of solutions of varying buffer concentration but constant pH (7.1 at 5°) The rate of reaction in each solution was determined and the results are shown in Fig. 3. From the intercept the value of $k_{OH}\ominus$ obtained is 6.2 × 10^5 l. mole^{-1} min.$^{-1}$, which agrees well with the only previously determined value of 4.4 × 10^6 l. mole^{-1} min.$^{-1}$ at 25° due to Skrabal.[28]

Serious problems accompany attempts to determine $k_{OH}\ominus$ in ice. The value of the pK_a' of lutidine in ice is not known, and therefore it is not possible to be certain of the hydrogen ion activity in the buffered solutions once frozen. In addition, the autoprotolysis constant of water in the ice phase at −10° remains open to question (see following discussion). For this reason the

(26) S. L. Bafna and V. Gold, J. Chem. Soc., 1406 (1953).

(27) H. K. Hall, J. Am. Chem. Soc., 79, 5439 (1957).

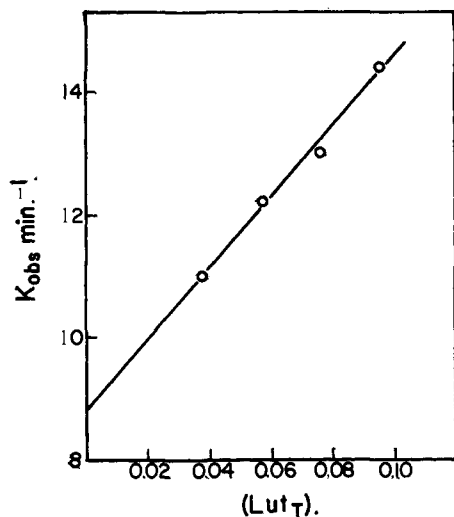(28) A. Skrabal, Monatsh., 43, 493 (1922).

Fig. 3.—The observed pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of acetic anhydride at 5° plotted against concentration of lutidine buffer.

hydroxyl ion concentration would not be known with certainty even if the hydrogen ion activity were. In addition, experiments showed that the linear relationship between lutidine concentration and the value of k_{obsd} did not persist in ice. As a qualitative effect, obtained by using identical solutions at -10 and 5°, it was observed that the reaction proceeded a little more slowly in ice at -10° than in water at $+5$°. If the pH is unchanged or increased this would represent a reversal of previous trends. It is probable that the pH increases on freezing since amines possess sizable and positive heats of ionization. However, it has been suggested[13] that the autoprotolysis constant of ice is only about 10^{-20} and if this were so, then an enormous increase in the catalytic efficacy of the hydroxyl ion would be indicated. However, as Eigen and de Maeyer[13] point out, the above value of the autoprotolysis constant is open to some doubt so that, for the present, there is no unambiguous conclusion to be drawn from these results.

The acetate ion is a fairly effective catalyst in the hydrolysis of acetic anhydride and its action has been subject to full investigations by Kilpatrick[23] and by Butler and Gold.[15] The mechanism is of the general-base type (as in (2) where an $OAc^{\ominus}$ replaces a water molecule). In the present study it was found that in ice at -10° the catalytic effect of the acetate ion is enhanced. As in previous studies, a little acetic acid was added to remove any hydroxyl ions and the ionic strength was maintained at 0.1 M. Inspection of Fig. 4 reveals that acetate catalysis follows good pseudo-first-order kinetics in ice and the rate constant obtained was 0.184 l. mole^{-1} min.$^{-1}$. The corresponding rate constant at 5° was found to be 0.067 l. mole^{-1} min.$^{-1}$ showing that the ion is 2.7 times more effective as a catalyst at -10° in ice than at 5° in water. From the results of Kilpatrick and of Butler and Gold the energy of activation for acetate catalysis was found to be 11.8 kcal. mole^{-1}. Using this value, a figure of 1.7 $\times$ 10^{-2} l. mole^{-1} min.$^{-1}$ is obtained for the calculated rate constant for acetate ion catalysis in water at -10°. Thus, the observed rate constant at -10° in ice is 11 times greater than the calculated value for water at -10°.

As in the case of hydrogen ion catalysis, the addition of potassium chloride reduces the catalytic efficacy of the acetate ion. This is illustrated in Table III.
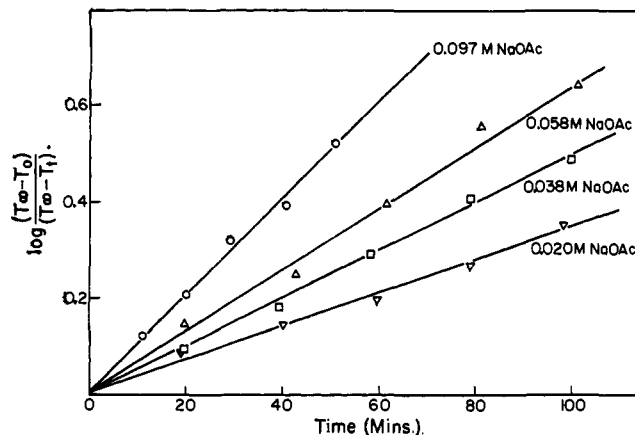

Fig. 4.—The first-order hydrolysis of acetic anhydride in the presence of acetate ion at -10° in ice.

Hydrolysis of p-Nitrophenyl Acetate.—This reaction is not as subject to specific-acid catalysis as is the hydrolysis of acetic anhydride,[29] and it seemed significant, because of this, to examine the reaction in the ice phase. The reaction in ice was found to follow fairly good first-order kinetics and to be slightly faster than in water at $+5$° (k_{H^+}(at -10°)/k_{H^+}(at 5°) = 1.6 at 0.04 and 0.08 M hydrochloric acid).

TABLE III
SALT EFFECT ON THE ACETATE-CATALYZED HYDROLYSIS OF ACETIC ANHYDRIDE

(AcONa), M	Ionic strength, M	k_{OAc^-}, l. mole^{-1} min.$^{-1}$
0.057	0.057	0.26
	.100	.19
0.084	.084	.25
	.100	.19

Hydrolysis of β-Propiolactone.—Grant, et al.,[4] reported that the β-lactam ring in penicillin was catalytically hydrolyzed by histidine in ice but was stable at 38° in aqueous histidine buffers. In relation to this finding, the imidazole-catalyzed hydrolysis of β-propiolactone was investigated. Nitrogen bases have been shown to react with β-propiolactone via nucleophilic attack at the alkyl or acyl carbons[30] to give either
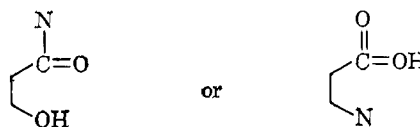
where N is the nucleophile. The N-acylimidazoles are known to be very labile[31] to hydrolysis whereas N-alkylimidazoles are stable. Thin layer chromatography on silica of reaction mixtures (at 25 and -10°) showed imidazole as the only amine present at the completion of the reaction at both room temperature and -10°. Nothing was detected which had an R_f value corresponding to that of an N-alkylimidazole. It may be concluded, therefore, that the imidazole catalysis of β-propiolactone occurs via nucleophilic attack on the acyl carbonyl or via general-base catalysis. The observed deuterium solvent isotope effort (k_H/k_D) of 1.2 at 20°, determined in this study, suggests that the correct mechanism is that of nucleophilic catalysis. The spontaneous solvolysis of β-propiolactone is known to involve alkyl–oxygen fission[32] and has a solvent isotope

(29) E. Tommila and C. N. Hinshelwood, J. Chem. Soc., 1801 (1938).

(30) T. L. Caresham, J. F. Jansen, F. W. Shaver, R. A. Banbert, and F. T. Fiedorek, J. Am. Chem. Soc., **73**, 3168 (1951).

(31) M. L. Bender, Chem. Rev., **60**, 53 (1960); T. C. Bruice, Brookhaven Symp. Biol., **15**, 52 (1962).

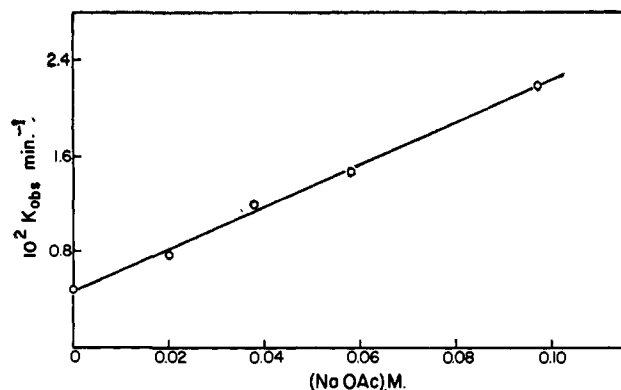(32) F. A. Long and M. Purchase, J. Am. Chem. Soc., **72**, 3267 (1950).

Fig. 5.—The observed pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of acetic anhydride at $-10°$ plotted against acetate concentration.
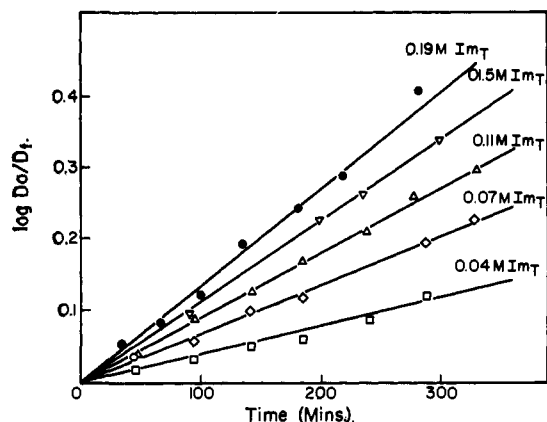

Fig. 6.—The first-order hydrolysis of β-propiolactone in the presence of imidazole buffer at $5°$ (pH 7.50).

effect of 1.15.[33] The rate of spontaneous solvolysis at $25°$ was determined by the method described in the Experimental section and the result was identical with that reported by Long and Purchase using a titrimetric method.

At $-10°$ in ice no spontaneous hydrolysis of the lactone could be detected over a 5-hr. period. The spontaneous hydrolysis of the lactone in water is known to be depressed by the addition of salt[32] but, as with the spontaneous hydrolysis of acetic anhydride, this effect is reversed in ice and in 0.1 M potassium chloride at $-10°$ hydrolysis of the lactone was found to occur with a first-order constant of 2.3×10^{-4} min.$^{-1}$.

In imidazole buffer at $5°$ the hydrolysis of β-propiolactone is greatly accelerated and in the presence of excess imidazole good pseudo-first-order kinetics are obtained (Fig. 6). The pseudo-first-order rate constants were found to be proportional to the concentration of the neutral conjugate base form of the imidazole (Fig. 7). The value of the second-order rate constants at $5°$, determined from experiments carried out at two pH values, was found to be 3.28×10^{-2} 1. mole^{-1} min.$^{-1}$ and that at $20°$, determined at one pH, was 0.131 1. mole^{-1} min.$^{-1}$. The concentration of free imidazole is given by the product $(\mathrm{Im_T})K_a'/(K_a' + a_\mathrm{H})$ and K_a' was determined at both temperatures by the method of half neutralization. From the second-order rate constants at 5 and $20°$ an approximate heat of reaction of 14.9 kcal. mole^{-1} was obtained.

For the reaction in ice at $-10°$ the situation is more complex. The reaction was found to follow good first-order kinetics to at least three half-lives but, if the concentration of free imidazole is assumed to be the same
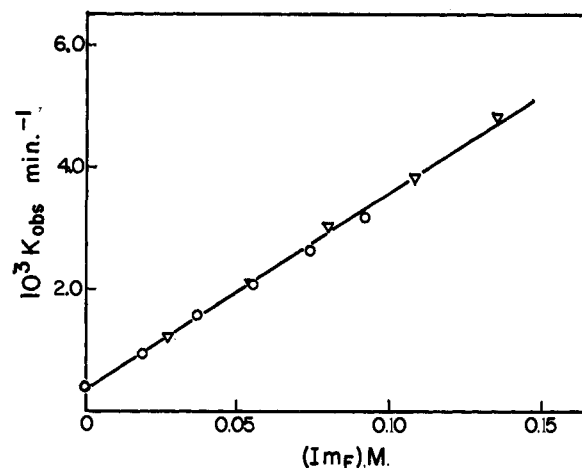
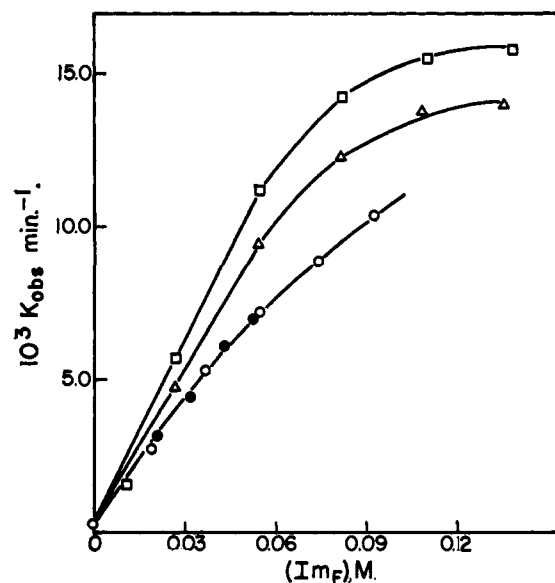(33) A. R. Butler and V. Gold, J. Chem. Soc., 2212 (1962).

Fig. 7.—The observed pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of β-propiolactone at $5°$ plotted against concentration of free imidazole ($\mathrm{Im_F}$): O, pH 7.50; ∇, pH 7.99.

Fig. 8.—The observed pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of β-propiolactone at $-10°$ in ice plotted against concentration of free imidazole as measured at $5°$: $\triangle$, pH 7.99; $\square$, pH 8.32; O, pH 7.50; ●, pH 7.22.

as in water at $5°$, then k_{obsd} is not a linear function of the free imidazole concentration. This is shown in Fig. 8 for four different imidazole buffers. However, both K_a and a_H will change on freezing. Taking the known heat of ionization of imidazole,[34] the value of pK_a' at $-10°$ should be 7.87. If we assume that a_H has decreased on freezing by a factor of 1.11 then the ratio $(\mathrm{Im_F})/(\mathrm{ImH}^+)$ is modified in such a way that, for low concentrations of imidazole, there is a single linear relationship between free imidazole concentration and k_{obsd}. The justification for this is that the mechanism of reaction, if it is the same as that in water, necessitates such a relationship and the figure of 1.11 is really a parameter which can, however, be given some theoretical significance. The factor of 1.11 could represent the change in the ratio $(\mathrm{Im})/(\mathrm{ImH}^+)$ caused by concentration of the buffer on freezing water out as ice. Examination of the equation defining K_a'

$$K_a' = (\mathrm{Im})a_\mathrm{H}/(\mathrm{ImH}^+)$$

indicates that concentration of the system will result in a decrease in a_H, and this, coupled with the change in

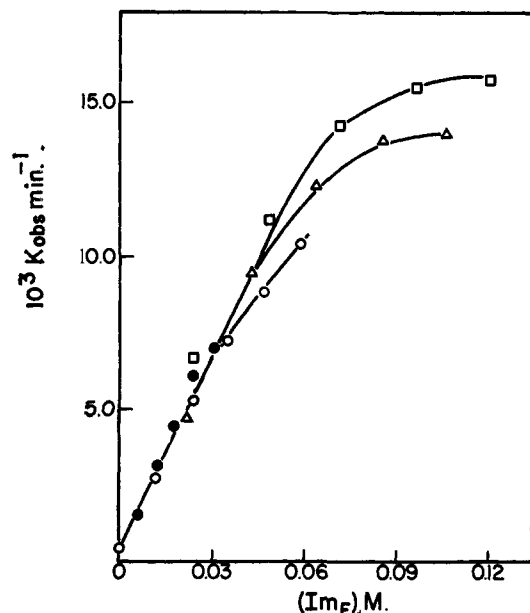(34) T. C. Bruice and S. J. Benkovic, J. Am. Chem. Soc., 86, 418 (1964).

Fig. 9.—The observed pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of β-propiolactone at $-10°$ plotted against the calculated concentration of free imidazole: O, pH (at 5°) 7.50; ●, pH (at 5°) 7.22; △, pH (at 5°) 7.99; □, pH (at 5°) 8.32.

pK_a', changes the $(Im)/(ImH^+)$ ratio. The leveling off of the plots of Im_F vs. k_{obsd} at high Im_T is difficult to understand. That it is, in part, a function of the catalyst concentration is self-evident. Simple complex formation would not appear to be the correct explanation as the experimental results do not fit a Michaelis–Menten nor a Lineweaver–Burk plot. As yet, the effect is unexplained.

The second-order rate constant obtained from the linear portion of the curve in Fig. 9 is 0.22 l. mole⁻¹ min.⁻¹ which means that imidazole is about seven times more effective as a catalyst at $-10°$ in ice as at $+5°$. Employing the determined activation energy for the reaction of imidazole with β-propiolactone, the anticipated second-order rate constant at $-10°$ in water was calculated to be 7.1×10^{-3} l. mole⁻¹ min.⁻¹ so that the rate constant determined for the reaction at $-10°$ is 31 times the calculated value.

A single experiment was attempted with the imidazole at very low concentration (0.010 M), comparable with that of the lactone. Under these conditions it is impossible to buffer the imidazole so the initial rate was measured, giving a value of 1.23 l. mole⁻¹ min.⁻¹. This is 173 times faster than the calculated rate at $-10°$. In this case the initial rate means the rate when the first tube was withdrawn. In fact, at least 20 min. elapsed between mixing and this withdrawal so that a larger factor may be operative. In unbuffered imidazole some $OH^\ominus$ ion will be present, but Long and Purchase[32] showed that β-propiolactone hydrolysis is not very sensitive to catalysis by these ions.

Dehydration of 5-Hydro-6-hydroxydeoxyuridine.— This is one of the original reactions studied in ice, but no rigorous kinetics were attempted.[3] At 30° and constant ionic strength (0.1 M) the dehydration reaction was found to follow good first-order kinetics (Fig. 10) and k_{obsd} is proportional to the concentration of hydrochloric acid, giving a second-order rate constant of 0.0343 l. mole⁻¹ min.⁻¹. The salt effect was found to be very small.

For the reaction in ice at $-10°$ the kinetics were fairly good and gave reasonable first-order plots. The results are given in Table IV; no attempt was made to keep the ionic strength constant. Clearly, there is no
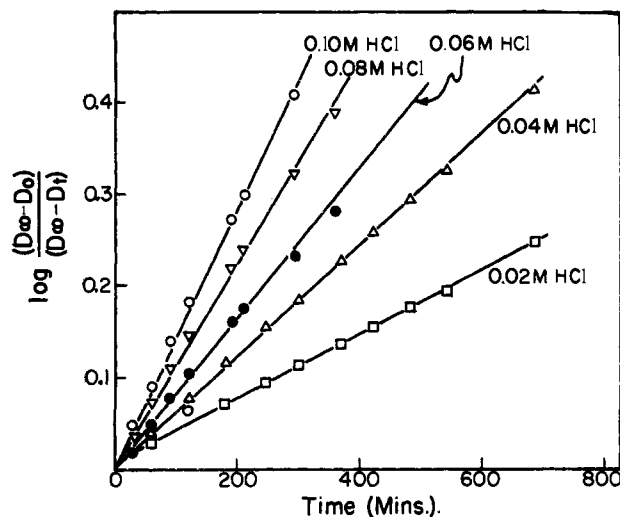

Fig. 10.—The first-order dehydration of 5-hydro-6-hydroxydeoxyuridine in the presence of hydrochloric acid at 30°.

proportionality between k_{obsd} and the concentration of acid. The addition of even a small amount of potassium chloride has a dramatic effect on the rate of reaction. This is illustrated in Table V. The results do

TABLE IV

DEHYDRATION OF 5-HYDRO-6-HYDROXYDEOXYURIDINE

(HCl), M	0.002	0.004	0.006	0.010
$10^4 k_{obsd}$, min.⁻¹	7.9	7.0	6.5	8.0

TABLE V

SALT EFFECT ON THE ACID-CATALYZED DEHYDRATION OF 5-HYDRO-6-HYDROXYDEOXYURIDINE

(HCl), M	(KCl), M	$10^4 k_{obsd}$, min.⁻¹	(HCl), M	(KCl), M	$10^4 k_{obsd}$, min.⁻¹
0.01	0.00	8.0	0.01	0.03	4.1
.01	.01	4.6	.01	.04	4.2
.01	.02	5.1	.01	.06	2.1

not fall into a very clear pattern, but as a qualitative statement we can say that the addition of potassium chloride radically lowers the rate of reaction. If the ionic strength is increased to 0.1 M by the addition of salt there is no detectable reaction. Using 0.002 M acid the reaction is 12 times faster at $-10°$ than 30°, but at higher acid concentrations the ratio is smaller.

Discussion

The present paper is the first occasion on which the kinetics of reactions in frozen aqueous solution have been reported. All the reactions investigated followed pseudo-first-order kinetics in water. Quite surprisingly the reactions were found to be, on the whole, cleanly pseudo-first order in ice at $-10°$.

Consideration of the whole problem of reactions in ice immediately raises the question of the homogeneity of the frozen samples. The quick freezing technique employed in this study was used to assure, as far as possible, complete homogeneity. Nevertheless, it would appear that the rate enhancements noted in this study can be explained on the basis of a concentration of reacting species in liquid regions between the ice crystals. The spontaneous reactions, which involve the substrate and water as the sole reactants (the hydrolyses of acetic anhydride and β-propiolactone), should be unaffected by concentration of the substrate on freezing. The large observed depression in the spontaneous rates is best accounted for by the effect of lowering the temperature and (primarily) the water concentration becoming limiting. Addition of potassium chloride or dioxane should increase the size of the liquid regions and there-

fore increase the rate by making the reaction medium more aqueous. However, once the addition of the salt has brought about sufficient melting to make the water concentration not limited, no further change in the rate would be expected on further increase of salt concentration. All the experimental results obtained in this study agree with these expectations. In ice at $-10°$ the spontaneous hydrolyses of acetic anhydride and β-propiolactone are both depressed. The addition of potassium chloride is found to increase the spontaneous rates until a rate is reached which is essentially independent of the concentration of salt. It is known, in support of this explanation, that foreign substances are not in general incorporated into ice crystals.

All the bimolecular reactions studied showed enhanced reaction rates in ice, presumably due to the concentration of the catalyst in the liquid regions. However, the addition of a salt, which would increase the size of the liquid regions and thus dilute the reactants, lowers the rate of reaction. Clearly, the lower the concentration of electrolytes (KCl, HCl), the smaller will be the liquid regions and the greater the concentration effect. The data show that as the catalyst concentration is lowered the rate enhancement as determined by the catalytic coefficient increases. However, at very low HCl concentration the rate of reaction decreases as the water concentration becomes limiting. The very great rate enhancements reported by Prusoff[3] and by Grant, et al.,[4] are larger than those obtained in the present study where the ionic strength was as high as 0.1 M. Our more detailed study did not confirm the very large values obtained by Prusoff but, in most cases studied, large rate enhancements were obtained when catalytic coefficients were determined at very low ionic strength.

The n.m.r. spectra of pure water, 0.01 M aqueous potassium chloride, and 0.05 M aqueous potassium chloride were determined at -5, -10, and $-25°$. The spectra seem to indicate the presence of regions of liquid water in ice crystals when an electrolyte is present. The spectrum of ice has no distinctive features but with the 0.01 M salt solution two sharp peaks of similar height appear at all temperatures. One of these disappears in the 0.05 M solution at -5 and $-10°$, and at
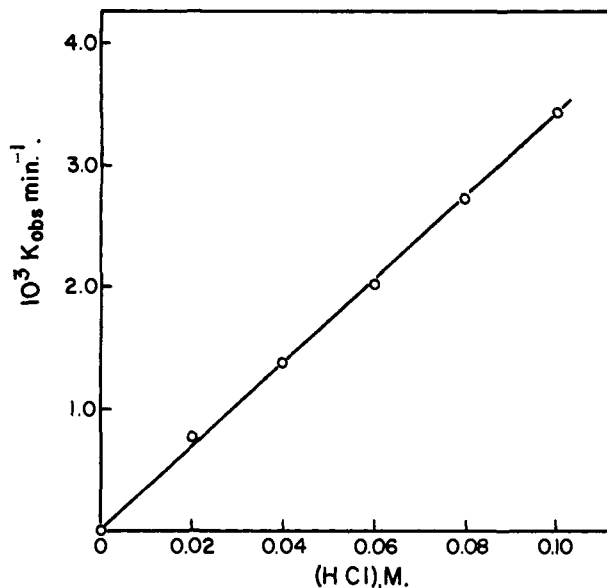

Fig. 11.—The observed pseudo-first-order rate constants for the dehydration of 5-hydro-6-hydroxydeoxyuridine at $30°$ plotted against concentration of hydrochloric acid.

$-25°$ the spectrum is indistinguishable from that of pure ice. A complete explanation of these results has yet to be developed and, clearly, further study is necessary.

If the explanation of the rate enhancements and depressions found on moving from water to ice are simply concentration effects, no new mechanistic factors are introduced. The phenomenon may be of some synthetic interest and further studies in that direction are being carried out.
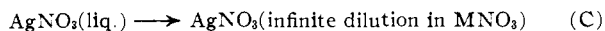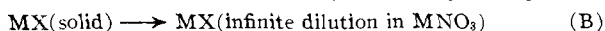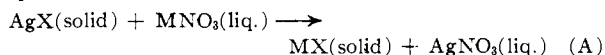

Solutions of Solids in Molten Reciprocal Salt Systems

BY MILTON BLANDER AND E. B. LUCHSINGER

Electromotive force measurements were made to obtain standard heats of solution (ΔH_{soln}) and solubility products (K_{sp}) of AgI and AgBr in molten alkali nitrates ($NaNO_3$, KNO_3, or mixtures of the two). The measured values are in good agreement with values of ΔH_{soln} and $-RT \ln K_{sp}$ calculated from a cycle similar to one proposed by Flood, Førland, and Grjotheim and shown in text equations A, B, and C. Our results confirm the general usefulness of the cycle in making predictions, a priori, in molten salt systems of this class.

Introduction

A prediction of the standard heats of solution (ΔH_{soln}) and solubility products (K_{sp}) of solids in molten reciprocal salt systems may be made by the use of a simple cycle first proposed by Flood, Førland, and Grjotheim (FFG).[1] As an example, for the dissolution of solid silver halides (AgX) in molten alkali nitrates (MNO_3) one method of writing the cycle consists of the three steps

$$AgX(solid) + MNO_3(liq.) \longrightarrow$$
$$MX(solid) + AgNO_3(liq.) \quad (A)$$

$$MX(solid) \longrightarrow MX(infinite\ dilution\ in\ MNO_3) \quad (B)$$

$$AgNO_3(liq.) \longrightarrow AgNO_3(infinite\ dilution\ in\ MNO_3) \quad (C)$$

with processes such as A generally leading to the largest contribution to the estimated values of the standard heat of solution or of the standard free energy of solution ($-RT \ln K_{sp}$) of AgX.[1,2] Where direct measurements are not available, good estimates of the relatively small changes of the standard heat or free energy changes in the processes B and C may be made by comparison with similar mixtures.

In essence, the use of this cycle, with the aid of generally available thermodynamic data on the pure com-

(1) H. Flood, T. Førland, and K. Grjotheim. Z. anorg. allgem. Chem., **276**, 289 (1954).

(2) M. Blander, J. Braunstein, and M. D. Silverman, J. Am. Chem. Soc., **85**, 895 (1963).