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Catalysis in Water and Ice. II.<sup>1</sup> The Reaction of Thiolactones with Morpholine in Frozen SystemsBY THOMAS C. BRUCE<sup>2</sup> AND ANTHONY R. BUTLER<sup>3</sup>

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In liquid water ( $-10$  to  $+45^\circ$ ) the reaction of morpholine with  $\delta$ -thiolvalerolactone and  $\gamma$ -thiolbutyrolactone occurs *via* general-acid ( $k_{ga}$ ) and general-base ( $k_{gb}$ ) assisted processes [*i.e.*,  $V_{H_2O} = [k_{gb}(\text{morpholine})^2 + k_{ga}(\text{morpholine})(\text{morpholine}-H^+)](\text{lactone})$ ]. Both  $k_{ga}$  and  $k_{gb}$  are associated with very small values of  $\Delta H^\ddagger$ . In frozen systems at  $-10^\circ$  the morpholinolysis of the thiolactones is not catalyzed by morpholine or its conjugate acid and an unassisted nucleophilic ( $k_n$ ) displacement occurs [*i.e.*,  $V_{ice} = k_n(\text{morpholine})(\text{lactone})$ ]. By best estimates of the maximum possible value of  $k_n$  in water that could remain undetected, the rate constant for nucleophilic attack is found to be minimally increased by  $6-7 \times 10^3$ -fold on freezing. The deuterium solvent kinetic isotope effect ( $k_n^H/k_n^D$ ) in ice was determined to be 1.6. The change of mechanism from third order to second order is opposite to that anticipated for a concentration phenomenon. The change in mechanism from processes of higher order to those of lower order is not explained by assuming that reactions are occurring in a eutectic as the pseudo-first-order rate constants are proportional to the concentration of morpholine in solution before freezing at nonconstant ionic strength. Morpholine and its hydrochloride could not be shown to be incorporated into slowly formed ice crystals. It is suggested that the ice structure itself partakes in the reaction in the step involving proton transfer or to orient the reactants so as to favor greatly  $k_n$  over  $k_{gb}$  and  $k_{ga}$  and also to accelerate the reaction associated with the former.

Two recent studies,<sup>4,5</sup> of a semiquantitative nature, have indicated that reactions may proceed much more rapidly in ice than in liquid water. The two reactions examined were the acid-catalyzed dehydration of 5-hydro-6-hydroxyuridine<sup>4</sup> and the hydrolytic opening of the  $\beta$ -lactam ring of penicillin catalyzed by imidazole and histidine.<sup>5</sup> No full explanation of the effects was attempted by the authors. In part I of the present series Butler and Bruce<sup>1</sup> examined the kinetics of eight reactions involving bimolecular and spontaneous processes in frozen systems at  $-10^\circ$ . All the reactions gave fairly clean kinetics, the bimolecular reactions exhibiting approximately tenfold rate enhancements over the same reactions in liquid water. However, the rate enhancements obtained were much smaller than those observed by the previous workers.<sup>4,5</sup> In explaining the results it was assumed that, on freezing the reaction mixture, the reactants are not incorporated into the ice crystals but remain in small liquid regions between the crystals. This was proposed to result in a concentration of the catalytic species and, therefore, an increase in the rate of reaction. Uncatalyzed reactions (*e.g.*, the spontaneous hydrolysis of acetic anhydride), in which water is one of the reactants, did not show any rate enhancement upon freezing but rather disappeared completely. This result was rationalized by considering that the freezing process lowers the concentration of one of the reactants (water) without a concomitant increase in the concentration of a catalyst. The concentration effect was also suggested by Wang who investigated several dimerization reactions in ice and found that, in the frozen system, the monomer forms solid aggregates which dimerize on irradiation with ultraviolet light.<sup>6</sup> The results obtained by Butler and Bruce (part I of this series) appeared to be without mechanistic significance but it was thought that the rate enhancements obtained on freezing might have some synthetic utility. If concentration of reactants

on freezing can account for the rate enhancements previously noted, then the higher the kinetic order of the reaction the greater should be the rate enhancement obtained. All the reactions considered in part I were either first or second order and, as a means of ascertaining if the concentration phenomenon were all that is involved in increasing the apparent rates of the second-order reactions, we have extended our studies to the effect of freezing on third-order reactions.

Thiolactones are known to react with a large variety of nucleophiles and these reactions are subject to the complexities of general acid and general base catalysis.<sup>7,8</sup> A number of such reactions have been examined by Bruce and his coworkers<sup>8,9</sup> and these studies indicate that such reactions may occur *via* three distinct mechanistic routes: *viz.* direct nucleophilic attack, general-acid assisted attack, or general-base assisted attack. It is most reasonable that all three types are simultaneously operative in the reaction between one nucleophile and a thiolactone. In this case the rate expression would have the form<sup>10</sup>

$$-dL/dt = [k_n(N) + k_{ga}(N)(NH^+) + k_{gb}(N)^2](L) \quad (1)$$

The concentration of base is always much greater than that of the thiolactone so that the conditions are pseudo-first order, and eq. 1 becomes

$$k_{obsd} = k_n(N) + k_{ga}(N)(NH^+) + k_{gb}(N)^2 \quad (2)$$

Bruce and Bruno found that for a particular nucleophile generally only unassisted nucleophilic or both general-acid- and general-base-catalyzed nucleophilic attack could be detected. They found no clear way of rationalizing the class into which any base falls. It seems probable that bases, in fact, react in all three manners but that either unassisted or assisted attack

(7) P. J. Hawkins and D. S. Tarbell, *J. Am. Chem. Soc.*, **75**, 2982 (1953)(8) T. C. Bruce and J. J. Bruno, *ibid.*, **84**, 2128 (1962).(9) T. C. Bruce, J. J. Bruno, and W.-S. Chou, *ibid.*, **85**, 1659 (1963).(10) Abbreviations employed are:  $k_n$ , second-order rate constant for nucleophilic attack;  $k_{ga}$  and  $k_{gb}$ , third-order rate constants for general-acid- and general-base-catalyzed attack; (N), concentration of free base; (NH<sup>+</sup>), concentration of base in the protonated form; (N<sub>T</sub>), stoichiometric concentration of base;  $k_{obsd}$ , the experimentally determined pseudo-first-order rate constant; (L), concentration of thiolactone; superscripts H and D indicate reactions in normal water and in heavy water.(1) Part I: A. R. Butler and T. C. Bruce, *J. Am. Chem. Soc.*, **86**, 313 (1964).

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(4) W. Prusoff, *Biochim. Biophys. Acta*, **68**, 302 (1963).(5) N. H. Grant, D. E. Clark, and H. E. Alburn, *J. Am. Chem. Soc.*, **83**, 4476 (1961).(6) S. Y. Wang, *Nature*, **190**, 690 (1961).

is so much more effective than the other that the less effective mechanism plays too small a part to be detected.

In a recent study,<sup>11</sup> Fedor and Bruce examined in detail the reaction between morpholine and  $\delta$ -thiolvalerolactone and  $\gamma$ -thiolbutyrolactone. They could detect no nucleophilic term and this means that  $k_n$  must be, at least, less than 5% of  $k_{ga}$  and  $k_{gb}$  (*i.e.*, the estimated experimental limit of detection). The general acid and general base terms were found to have very small activation energies (4.37 and 1.84 kcal. mole<sup>-1</sup>, respectively) so that the rate of reaction is almost independent of the temperature in the range examined, 3–45°. Thus these two reactions are ideally suited for examining in frozen systems as the rate should not decrease greatly on lowering the temperature to -10° and, if concentration of the reactants is the only important feature accompanying freezing, then this should give rise to a very large rate enhancement since morpholine appears raised to the second power in the rate expression for  $k_{ga}$  and  $k_{gb}$  (see (1)). If such a large enhancement were to occur, then it would provide added confirmation of the explanation put forward in part I.

### Experimental

**Materials.**— $\delta$ -Thiolvalerolactone and  $\gamma$ -thiolbutyrolactone were those prepared for a previous study.<sup>11</sup> Eastman White Label morpholine was distilled over sodium and stored at 0°.

**Kinetic Methods.** (i) **Reactions in Ice at -10°.**—Morpholine was used as its own buffer and the buffers were made up by adding a known volume of standard hydrochloric acid to a weighed quantity of morpholine and diluting to 1 l. The ratio of protonated to unprotonated morpholine does not change on cooling so there is no need to consider changes in the heat of ionization. For the reaction mixture 0.2 ml. of an ethereal solution of the thiolactone was added to 50 ml. of the buffer to make the thiolactone *ca.* 10<sup>-4</sup> M. Aliquots (3 ml.) of this mixture were pipetted into a number of screw cap tubes and then frozen by immersing in a Dry Ice-acetone bath. They were then transferred to an alcohol bath at -10° and the kinetic run was initiated when the samples had warmed up to this temperature (about 10 min.). The reaction was followed by removing tubes at timed intervals, melting the contents by immersing the tubes in a bath at 30°, and measuring the optical density of the resulting solution (at 239.5 m $\mu$  for the thiolvalerolactone and 237 m $\mu$  for the thiolbutyrolactone). In this manner the disappearance of the thiolactone was followed and first-order plots were obtained by plotting  $\log(O.D._\infty/O.D._t)$  against time.

Serial dilution of the buffer with water allowed variation of the concentration of free morpholine without changing the ratio of free to protonated base. No attempt was made to keep the ionic strength constant as the addition of potassium chloride was found to lead to a substantial decrease in the rate of reaction.<sup>12</sup> Three different buffers were used and the pH's were routinely examined before and after the reaction. As the pH in ice could not be determined, 30° was arbitrarily selected as a reference temperature for pH measurement and no variation of pH was detected during the course of a run or by serial dilution. Conditions were, in all cases, pseudo-first order, but the concentration of morpholine was low enough that, after melting, no reaction occurred during the brief time needed for measuring the optical density.

(ii) **Reactions in Water at -10°.**—If aliquots of the reaction mixture are cooled to -10° they do not always freeze. Nucleation with an ice crystal or violent shaking will induce freezing but, if care is taken, it is possible to follow the course of the reaction in water at -10°. To obtain reasonable rates the concentration of morpholine was made much higher than that used for the reactions in ice and the reaction was arrested by adding 1 ml. of 2 M hydrochloric acid to each 3-ml. aliquot of the reaction mixture. After warming to room temperature the

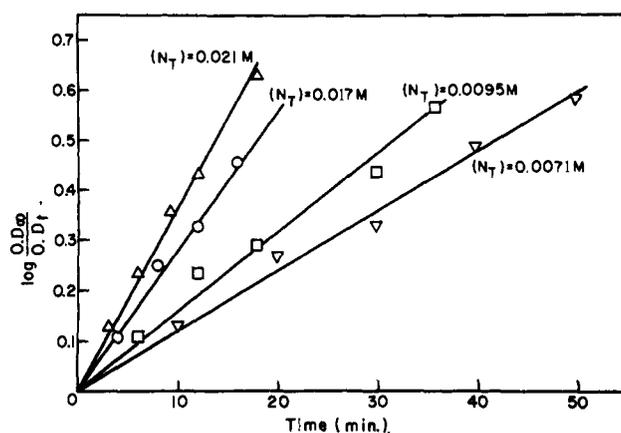


Fig. 1.—The first-order reaction between morpholine and  $\delta$ -thiolvalerolactone in ice at -10°;  $(NH^+)/N = 0.69$ .

optical density of the solution was measured as described previously. In order to facilitate comparisons with the same reactions in ice, no attempt was made to keep the ionic strength constant. In the aliquots which froze, the reaction was over almost instantaneously and these samples provided the infinity readings for the first-order plots.

**Apparatus.**—All spectrophotometric determinations were carried out with a Zeiss P MQ II spectrophotometer. pH measurements were made with a Radiometer Model 22 meter, fitted with a Radiometer scale expander, by use of a Radiometer a-K 2021 combined calomel-glass electrode. The bath at -10° was a Blue M Model MR-3210A modified with a Precision electronic relay and thermoregulator.

### Results

In ice at -10° the reaction between  $\delta$ -thiolvalerolactone and morpholine was found to follow fairly good first-order kinetics and the rate of reaction is a function of the concentration of morpholine in the buffer before freezing (see Fig. 1). The plots obtained are not as good as those obtained by Fedor and Bruce,<sup>11</sup> using liquid water solvent, but this is hardly surprising. They found that the nucleophilic term is zero in the range 3–45° so that the rate eq. 2 simplifies to

$$k_{obsd} = k_{ga}(N)(NH^+) + k_{gb}(N)^2 \quad (3)$$

For any one buffer  $(NH^+)/N = \text{constant } (p)$

$$k_{obsd} = (N)^2(pk_{ga} + k_{gb})$$

and Fedor and Bruce obtained good linear plots of  $k_{obsd}$  vs.  $(N)^2$  for each buffer.

An examination of the data obtained showed that this is not true for the reaction in ice and, indeed, such a plot exhibits downward curvature. However, a good linear plot is obtained for  $k_{obsd}$  vs. the first power of the free morpholine concentration and the points from all three buffers employed fall on the same straight line (see Fig. 2). The amount of free morpholine present in the ice was assumed to be the same as that present in the solution before freezing and equals the amount of base left unneutralized by the addition of acid. This result clearly indicates that the general-acid and general-base mechanistic pathways have completely disappeared and have been replaced by direct nucleophilic attack. If there is a small and undetected nucleophilic term even in water, then this result may really mean that nucleophilic attack is greatly increased, rather than replacing the other mechanisms. The value of  $k_n$  from the curve in Fig. 2 is 6.7 l. mole<sup>-1</sup> min.<sup>-1</sup>.

(11) L. R. Fedor and T. C. Bruce, *J. Am. Chem. Soc.*, **86**, 4117 (1964).

(12) For a full discussion of this effect see Part I.

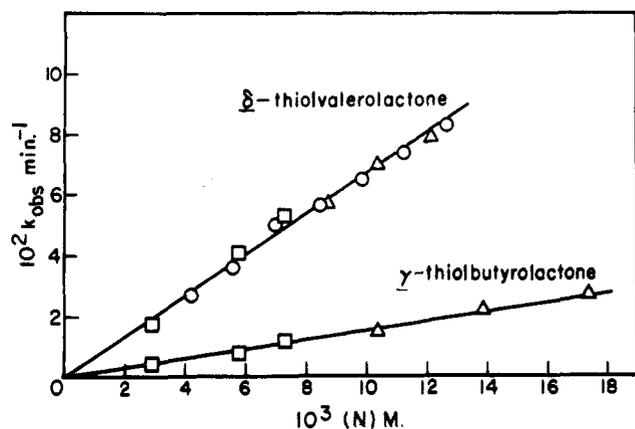


Fig. 2.—The pseudo-first-order rate constants for the reaction of morpholine with  $\delta$ -thiolvalerolactone and  $\gamma$ -thiolbutyrolactone in ice at  $-10^\circ$  plotted against concentration of free morpholine; O,  $(\text{NH}^+)/(\text{N}) = 0.69$ ;  $\Delta$ ,  $(\text{NH}^+)/(\text{N}) = 0.22$ ;  $\square$ ,  $(\text{NH}^+)/(\text{N}) = 2.12$ .

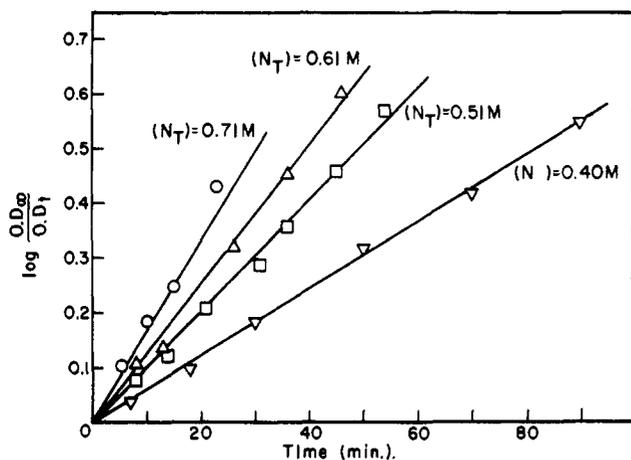


Fig. 3.—The first-order reaction between morpholine and  $\delta$ -thiolvalerolactone in water at  $-10^\circ$ ;  $(\text{NH}^+)/(\text{N}) = 1.07$ .

The reaction between  $\gamma$ -thiolbutyrolactone and morpholine behaves similarly and fairly good first-order plots for the reaction in ice at  $-10^\circ$  are obtained. Again the value of  $k_{\text{obsd}}$  is a function of the first power of the free morpholine concentration (see Fig. 2), showing that in ice at  $-10^\circ$  nucleophilic attack is the only detectable mechanism of reaction. The value of  $k_n$  in this case is  $1.56 \text{ l. mole}^{-1} \text{ min.}^{-1}$ .

In view of the unexpected nature of these results it became relevant to examine the course of the reaction in water at  $-10^\circ$ . As was anticipated, the results gave good first-order plots, the only difficulty being that frequently not enough samples remained unfrozen to provide sufficient points for a rate constant to be obtained. A typical set of results are shown in Fig. 3. Three different buffers were used and the results obtained, using 11 different concentrations of morpholine, clearly indicate that the value of  $k_{\text{obsd}}$  is not directly proportional to the concentrations of free morpholine. Instead the dependence is the same as that found by Fedor and Bruce<sup>11</sup> for the reaction carried out between  $3$  and  $45^\circ$  and a plot of  $k_{\text{obsd}}$  vs.  $(\text{N})^2$  is shown in Fig. 4. The three straight lines correspond to the three different buffers employed. The fact that all the lines pass through the origin shows that there is no detectable nucleophilic term, a fact to be expected from the results of Fedor and Bruce. The slope of each

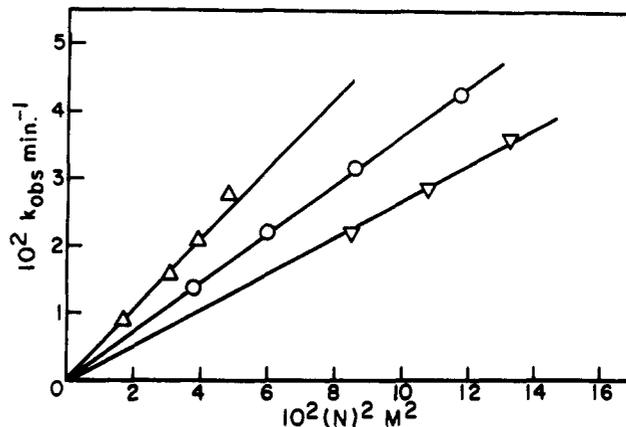


Fig. 4.—The observed pseudo-first-order rate constants for the reaction of morpholine with  $\delta$ -thiolvalerolactone in water at  $-10^\circ$  plotted against concentration of free morpholine raised to the second power; O,  $(\text{NH}^+)/(\text{N}) = 1.07$ ;  $\Delta$ ,  $(\text{NH}^+)/(\text{N}) = 3.54$ ;  $\nabla$ ,  $(\text{NH}^+)/(\text{N}) = 0.43$ .

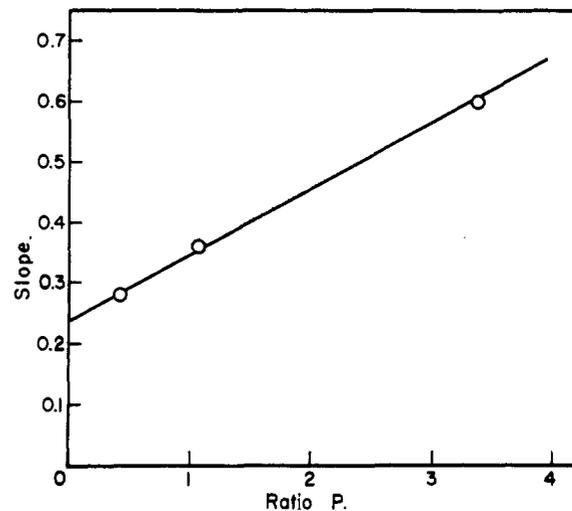


Fig. 5.—The slopes of the lines in Fig. 4 plotted against the ratio of protonated to free morpholine ( $p$ ) for the reaction in water at  $-10^\circ$ .

line is given by the expression  $(pk_{\text{ga}} + k_{\text{gb}})$  and Fig. 5 shows a plot of the slope against  $p$  for the three different buffers. The slope of this is  $k_{\text{ga}}$  and the intercept  $k_{\text{gb}}$  and the values obtained are  $0.10$  and  $0.25 \text{ l. mole}^{-1} \text{ min.}^{-1}$ , respectively. It is clear, then, that the behavior of the system when cooled to  $-10^\circ$  in remaining liquid is quite normal. The values obtained for  $k_{\text{ga}}$  and  $k_{\text{gb}}$  by extrapolation of the data of Fedor and Bruce are  $0.18$  and  $0.39 \text{ l. mole}^{-1} \text{ min.}^{-1}$ , respectively, which are in good agreement with the experimentally determined values. However, if the system freezes there is a radical change in mechanism from general-acid- and general-base-assisted to solely nucleophilic attack. While it is true that at low concentrations of nucleophile the nucleophilic pathway becomes relatively more important, it is impossible that, for a value of  $k_n$  which is too small to detect at morpholine concentrations of around  $0.2 \text{ M}$ , this should be the sole mechanism apparent at morpholine concentrations of  $0.01 \text{ M}$  (i.e., the concentration used in the ice experiment.) At ambient temperatures the reaction between  $\gamma$ -thiolbutyrolactone and morpholine shows the same pattern as that for  $\delta$ -thiolvalerolactone so one can assume that in water at  $-10^\circ$  the mechanism is general-acid/general-base assisted attack. The fact that in

ice at  $-10^{\circ}$  the only term is nucleophilic means that freezing again brings about a radical change of mechanism

As well as changing the mechanism, freezing accelerates the reaction. Comparison of rates are really meaningless owing to the change in kinetic order. However, some indication may be obtained from the following calculation: using a half-neutralized morpholine buffer with  $\delta$ -thiovalerolactone, 0.343 *M* free morpholine in water at  $-10^{\circ}$  gives rise to the same pseudo-first-order rate constant as does 0.0063 *M* free morpholine in ice at  $-10^{\circ}$ . It may be said, then, that the effective increase in rate on freezing is 50-fold. The corresponding figure for the  $\gamma$ -thiolbutyrolactone reaction, comparing the rates at 30 and at  $-10^{\circ}$  in ice, is fifty. It is more meaningful to attempt to compare the size of nucleophilic terms but, as they are too small to be detected in water, we may calculate only the minimal increase in  $k_n$ . An analysis of the accuracy of the work of Fedor and Bruce suggests that the value of  $k_n$  at  $-10^{\circ}$  for the thiolvalerolactone reaction obtained by extrapolation must be less than 0.001 l. mole $^{-1}$  min. $^{-1}$ , assuming that the rate is halved for every  $10^{\circ}$  decrease in temperature. This means that the increase in  $k_n$  on freezing must be at least 6,000-fold. The corresponding figure for the thiolbutyrolactone is 7000. Whichever figure one takes as the more meaningful, there is obviously a very considerable increase in the rate of reaction on freezing.

Water and morpholine are miscible in all proportions. It is most reasonable to assume that if a mixture of morpholine and water is cooled slowly to  $-10^{\circ}$  then pure ice crystals will separate until the composition of the concentrated supernatant liquid is such that it remains unfrozen at  $-10^{\circ}$ . If the reactants are all concentrated into this liquid phase then, by knowing its composition, we can calculate what the rate of reaction would be. A series of samples containing various quantities of morpholine (which had been half-neutralized with hydrochloric acid) and water were immersed in the alcohol bath at  $-10^{\circ}$ . After cooling, each sample was nucleated with an ice crystal and violently shaken. The critical concentration for freezing at  $-10^{\circ}$  was found to be about 2.9 *M* total morpholine. If the reaction between the thiolvalerolactone and morpholine did occur in a liquid phase of this composition by only nucleophilic attack, and taking the estimated maximum possible value of  $k_n$  in water at  $-10^{\circ}$ , then the rate would be *ca.* 0.003 min. $^{-1}$ , which is much smaller than any rate for the reaction in ice actually observed. It was impossible to determine the rate of reaction in a buffer of this composition owing to its extreme rapidity. This suggests that the general-acid/general-base mechanisms are operative, as in mixtures of slightly lower morpholine content used in the experiments on the rate of reaction in water at  $-10^{\circ}$ , and that the third-order terms are not replaced by a solely nucleophilic pathway. The composition of this liquid phase must be independent of the initial concentration of morpholine, only its volume changing. The reaction, therefore, cannot be occurring in this liquid phase as the rate *does* depend upon the initial concentration of morpholine. Again, the presence of such a liquid phase offers no explanation for the change of mechanism on freezing. The above analysis does not affect the con-

clusions drawn in part I of this series, since in the reaction considered there the ionic strength was kept constant so that the volume of the liquid phase would be unaltered and the concentration of the catalyst in this phase would, therefore, be dependent on the buffer concentration before freezing.

The possible involvement of a water molecule in nucleophilic attack is best investigated by a consideration of the deuterium isotope effect. For the nucleophilic term, a deuterium solvent kinetic isotope effect ( $k^H/k^D$ ) of *ca.* 1.2 is expected, caused merely by changing the reaction medium from normal to heavy water.<sup>13,14</sup> For the reaction of the  $\delta$ -thiolvalerolactone the general-acid and general-base terms have deuterium solvent kinetic isotope effects of 4.1 and 4.3, respectively, at  $30^{\circ}$ , values which clearly differentiate them from a nucleophilic term.<sup>11</sup> The deuterium isotope effect was determined for the reactions in the frozen system. For both thiolactones the reaction was faster in frozen H<sub>2</sub>O than in frozen D<sub>2</sub>O by a factor of 1.6. This is somewhat higher than the ratio for nucleophilic terms in water but it is known that isotope effects generally are greater at lower temperatures.<sup>15</sup> What it may indicate is that a water molecule is involved in the rate-determining step for the reaction in ice at  $-10^{\circ}$ , although a figure of 1.6 is impossible to interpret unambiguously.

Evidence has been presented to show that, for the runs in ice at  $-10^{\circ}$ , the reaction does not occur in a liquid phase which remains unfrozen at  $-10^{\circ}$ . The reaction may, therefore, occur within the ice crystals and to test this possibility we determined whether morpholine is incorporated into ice crystals on freezing a morpholine solution. The method used was essentially that of Zaromb and Brill.<sup>16</sup> A 3-l. sample of a morpholine buffer (of concentration similar to that used for the runs in ice) was placed in an insulated glass container and allowed to stand in a cold room at  $-20^{\circ}$ . After 24 hr., half the solution had frozen. Small clear crystals were selected, melted slightly, and then dried with a piece of filter paper. The crystals were then completely melted. The solution before freezing, the melted crystals, and the supernatant liquor after freezing were analyzed for free morpholine (by titration with standard acid) and protonated morpholine (by determining the chloride present by titration with standard silver nitrate). The results are shown in Table I.

TABLE I  
EFFECT OF FREEZING ON THE COMPOSITION OF A MORPHOLINE BUFFER

	[N], mole/l.	[NH <sup>+</sup> ], mole/l.
Before freezing	0.0124	0.0107
Supernatant liquor	.0156	0.0136
Ice crystals	.0004	None detected

According to Zaromb and Brill one can expect up to 20% apparent incorporation of solute into ice crystals owing to solution being retained in the boundary regions of the crystals. Therefore, the small amount of morpholine detected in the crystals in these experiments is probably due to this effect. Admittedly the method of freezing used in this experiment is com-

(13) C. A. Bunton and V. J. Shiner, *J. Am. Chem. Soc.*, **83**, 42 (1961).

(14) C. A. Bunton and V. J. Shiner, *ibid.*, **83**, 3207 (1961).

(15) R. P. Bell, J. A. Fendley, and J. R. Hulett, *Proc. Roy. Soc. (London)*, **A235**, 453 (1956).

(16) S. Zaromb and R. Brill, *J. Chem. Phys.*, **24**, 895 (1956).

pletely different from that in the kinetic runs, where freezing in a Dry Ice-acetone bath is complete within about 3 min. However, freezing the reaction mixture more slowly by using a sodium chloride-ice freezing mixture at  $-24^{\circ}$  rather than Dry Ice-acetone at  $-72^{\circ}$  was found to have no effect on the rate of reaction. Slow freezing was used in these experiments only to allow the formation of large crystals, which could be easily separated from the supernatant liquor. We must conclude, therefore, that the reactions examined in this study occur in a liquid phase outside the ice crystals but that the composition of that liquid phase is not that of the eutectic at  $-10^{\circ}$ .

### Discussion

The concentration effect, which worked well as a rationale for the reactions studied in part I, appears to be entirely inadequate to explain the effects associated with the two systems examined in this study. Any tenable theory must explain three facts: (1) the dependence of rate on the composition of the buffer before freezing, (2) the change of mechanism on freezing, and (3) the large increase in the rate of reaction. The concentration effect at nonconstant ionic strength, to give a liquid phase of such composition that it remains liquid at  $-10^{\circ}$ , does not explain the first of these observations, as has been argued above. Nor does it explain the change of mechanism on freezing. It does offer an explanation for the rate enhancements. However, if we neglect for the moment the change of mechanism on freezing, then the size of these enhancements is wrong. The above experiments showed that such a liquid phase would be 2.9  $M$  in total morpholine (*i.e.*, 1.45  $M$  in free morpholine). So that, taking the determined values of  $k_{ga}$  and  $k_{gb}$  at  $-10^{\circ}$  in water, the observed rate constant ( $= k_{ga}(N)(NH^+) + k_{gb}(N)^2$ ) should be 0.79  $\text{min.}^{-1}$ . None of the observed rate constants for the reaction in ice at  $-10^{\circ}$  is as large as this. They are one-tenth as large, or less, so the concentration effect at nonconstant ionic strength, without change of mechanism, predicts a rate enhancement larger than that observed, thus making this explanation inconsistent with all three criteria.

The main problem associated with finding the correct explanations is that details of the heterogeneity of the system are not known. Although the reactants

are not incorporated into the actual structure of the ice, the rapid freezing may leave them in the form of microinclusions within the ice crystals and the reactions must occur within these microinclusions. Nucleophilic attack is not a more favorable pathway than those involving assisted attack. Obviously, assisted attack necessitates a large  $\Delta S^*$  value as there are three, rather than two, molecules in the transition state, but there is compensation for this in a lowering of the  $\Delta H^*$  value owing to the presence of the catalysts.<sup>17</sup> Therefore, some special condition must exist within the microinclusions to make the nucleophilic pathway the more favorable one. It may be that the reactants become oriented in such a way that nucleophilic attack occurs without assistance and this mechanism completely replaces the others. Alternatively, the surface of the ice crystal may replace the second molecule of free morpholine or the protonated morpholine molecule, acting as a general acid and/or general base. The size of the deuterium isotope effect tends to support this possibility. It is well known that the proton mobility in ice is much greater than in liquid water<sup>18</sup> and the general-acid- and general-base-catalyzed steps must involve a proton transfer. Further speculation about this mechanism is difficult until the exact heterogeneity of the ice sample is known but, with reactant concentrations in the range  $10^{-2}$ – $10^{-4}$   $M$ , such an investigation poses many problems. Further studies on this subject are in progress.<sup>18a</sup>

**Acknowledgment.**—This work was supported by a grant from the National Science Foundation. We should like to express our thanks to Professor W. P. Jencks for a stimulating discussion.

(17) T. C. Bruce and S. J. Benkovic, *J. Am. Chem. Soc.*, **86**, 418 (1964).

(18) M. Eigen and L. DeMaeyer, *Proc. Roy. Soc. (London)*, **A247**, 505 (1958).

(18a) NOTE ADDED IN PRESS.—An alternate explanation of the change of kinetic order on freezing might involve the complete conversion of the substrate to a complex with morpholine. This complex could then be converted into product *via* general-base catalysis by a molecule of morpholine. This possibility is not supported by the drastic decrease of the deuterium solvent isotope effect which accompanies freezing and would require the disappearance of general-acid but not general-base catalysis by morpholine on freezing.

There is also a possibility that the presence of a small amount of ether, added as solvent for the thiolactones, in the mixtures used for the kinetic runs may affect the kinetics. In the slowest runs it was possible to shake the thiolactone (with no ether present) with the morpholine buffer, filter off any undissolved thiolactone, and then freeze. Under these conditions the reaction was found to go somewhat faster but the kinetic order remained unchanged.