110 ± 4.1 sec; $\eta = -3.29$. The larger spin-rotation contribution and a smaller Overhauser effect for $^{15}NH_4Cl$ reflect its smaller spherical shape as compared to glycine. The large energy barrier (6.7 kcal/mol)[11,22] for internal rotation about the C–N bond in glycine precludes the importance of internal spin, while the significantly larger T_{1d} for ammonium chloride again reflects its smaller size (shorter τ_c).

Conclusions

Chemical exchange has profound effects on both the nmr line shapes and relaxation times of ^{15}N contained in glycine and, presumably, in other amino acids. Exchange rate data can be obtained from modulation of the scalar interaction as well as chemical-shift averaging. Although two mechanisms of line broadening could be distinguished with glycine, it is possible with other systems that line broadening may result from both processes acting simultaneously. One way of checking this would be to monitor the proton-decoupled ^{15}N signals, which will broaden only if chemical-shift averaging is taking place. The relaxation time for ^{15}N in glycine is comparable to those observed for ^{13}C, and this provides encouragement for application of the Fourier transform technique for studying the ^{15}N resonances of amino acids at the natural-abundance level.

Acknowledgments. We thank Professors S. I. Chan and D. M. Grant for many helpful discussions during the course of this work.

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[22] R. G. C. McElroy, R. Y. Dong, M. M. Pintar, and W. F. Forbes, *J. Magn. Resonance*, **5**, 262 (1971).

Equilibrium and Kinetics of Glyconitrile Formation in Aqueous Solution

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Abstract: The equilibrium constant (K_G) for the dissociation of glyconitrile to formaldehyde and hydrogen cyanide in aqueous solution was measured relative to the known dissociation constant of lactonitrile. Over the temperature range 25–70° $\log K_G = 1.66 - 2184/T$ ($K_G = 2.1 \times 10^{-6}$ M at 25°). The enthalpy of dissociation was found by calorimetry to be +9.96 kcal mol^{-1}. The rate of addition of hydrogen cyanide to formaldehyde in dilute, acetate-buffered (pH 3.8–4.7) aqueous solution was measured between 25 and 45°. In this pH range the reaction is first order in both CN^- and unhydrated formaldehyde (at 25° $k_2 = 3.5 \times 10^5$ M^{-1} sec^{-1}, and $\Delta H^{\ddagger} = 5.02$ kcal mol^{-1}). No general acid or general base catalysis was observed. In aqueous solution at 25° the dissociation constant for mandelonitrile was found to be 5.2×10^{-3} M, and the second-order rate constant for the addition of CN^- to benzaldehyde was measured to be 1.13×10^2 M^{-1} sec^{-1}. The equilibrium and rate constants for each of the steps in the formation and dissociation of the cyanohydrins of formaldehyde, acetaldehyde, acetone, and benzaldehyde are evaluated. A plot of $\log K'$ (dissociation of the cyanohydrin to the unhydrated aldehyde) *vs.* $\Sigma\sigma^*$ gives a straight line, as do plots of $\log k_2$ and $\log k_{-2}$ *vs.* $\Sigma\sigma^*$, except for mandelonitrile where the deviations are attributed to a resonance effect. The prebiotic significance of the reaction between formaldehyde and hydrogen cyanide is discussed.

The equilibrium formation of cyanohydrins from aldehydes or ketones and hydrogen cyanide is a reaction that has been extensively studied,[1] although there have been relatively few investigations of the kinetics. However, the cyanohydrin equilibrium with formaldehyde, the simplest aldehyde, has never been investigated, although the dissociation constant is known to be very small.[2] This reaction is of importance in prebiotic chemistry, since the synthesis of purines requires free hydrogen cyanide[3] and the synthesis of sugars requires free formaldehyde.[4] The value of this equilibrium constant is relevant to the question of whether both purines and sugars could have been synthesized at the same time on the primitive earth. We have therefore investigated the equilibrium and rates of glyconitrile formation, as well as the enthalpy of the reaction.

Experimental Section

Reagents. Hydrogen cyanide was prepared from sodium cyanide and sulfuric acid and stored as the pure solid at −78°. The required amount was transferred in a vacuum line into a flask containing deaerated water. Formaldehyde was prepared by distillation of a slurry of paraformaldehyde in dilute aqueous sulfuric acid. Both solutions were standardized by methods described below, and both were stored in a refrigerator. The other chemicals used in the experiments were reagent grade and used without further purification.

The acetic acid–sodium acetate buffers were prepared by mixing appropriate volumes of standardized acetic acid and sodium acetate solutions.

The acetaldehyde solution was prepared by adding a weighed amount of vacuum distilled acetaldehyde to an appropriate quantity

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[1] C. K. Ingold, "Structure and Mechanism in Organic Chemistry," 2nd ed, Cornell University Press, Ithaca, N. Y., 1969, pp 995–999, and references therein.

[2] W. J. Svirbely and J. F. Roth, *Anal. Chem.*, **26**, 1377 (1954).

[3] J. Oro and A. P. Kimball, *Arch. Biochem. Biophys.*, **94**, 217 (1961), **96**, 293 (1962); R. Sanchez, J. P. Ferris, and L. E. Orgel, *Science*, **153**, 72 (1966); *J. Mol. Biol.*, **30**, 223 (1967); **38**, 121 (1968).

[4] A. Butlerow, *Justus Liebigs Ann. Chem.*, **120**, 295 (1861); R. Mayer, K. Runge, and H. Dreschsel, *Z. Chem.*, **3**, 134 (1963); N. W. Gabel and C. Ponnamperuma, *Nature (London)*, **216**, 453 (1967); C. Reid and L. E. Orgel, *ibid.*, **216**, 455 (1967).

of water. The aqueous solutions of benzaldehyde were prepared in a similar manner from vacuum distilled benzaldehyde.[5]

Standardization of Hydrogen Cyanide and Formaldehyde. The HCN concentration was determined, for both the freshly prepared stock solution and the kinetic runs, by adding 30 ml of the unknown solution to a known excess of 0.01 *N* $AgNO_3$ in 0.1 *M* HNO_3, filtering out the AgCN, and back titrating with 0.01 *N* potassium thiocyanate.[6]

The formaldehyde stock solution was standardized by HCl titration of the NaOH released on the addition of $NaHSO_3$ to the formaldehyde solution.[7] The formaldehyde concentrations in dilute solutions were determined by a modification of the chromotropic acid method of West and Sen.[8] In a typical experiment, 10.00 ml of reaction solution was pipetted into 1.00 ml of 1 *M* sulfuric acid, in order to "freeze" the reaction. A 3.00-ml aliquot of this solution was diluted to 8.00 ml with 1 *M* sulfuric acid. To another 3.00-ml aliquot of the "frozen" reaction solution was added a mixture of 3.00 ml of a previously prepared standard solution of formaldehyde (4.0×10^{-4} *M*) and 2.00 ml of 1 *M* sulfuric acid. The added formaldehyde spike provided an internal calibration of color yield. The color yield was also determined with 3.00 ml of the spike solution plus 5.00 ml of 1 *M* sulfuric acid. The reagent blank consisted of 8.00 ml of 1 *M* sulfuric acid. Aliquots of 5.00 ml each of the above solutions were added to a mixture of 30 ml of concentrated sulfuric acid and 5 ml of a 1% solution of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in concentrated sulfuric acid. The resulting solutions were thoroughly mixed and allowed to stand at room temperature for precisely 0.5 hr before measurement of their absorbance with a Cary 15 spectrophotometer, using cells of 10-, 50-, or 100-mm path length. The spectrum of each solution was recorded from 700 to 400 nm. The absorbance at the 573-nm peak was corrected for the reagent blank; the absorbance between 700 and 650 nm was used to establish the base line accurately.

Formaldehyde concentrations of the unknown solutions were calculated from the color yields of the standard formaldehyde solutions. The color yield was also checked by the spiked samples.

Measurement of the Equilibrium between Lactonitrile and Glyconitrile. A solution of glyconitrile with a small excess of HCN was prepared and the excess HCN determined as described above. Known concentrations of acetaldehyde were added to aliquots of this solution and the mixtures diluted to give an initial glyconitrile concentration of 2×10^{-3} *M*, and initial acetaldehyde concentrations of between 20 and 160×10^{-3} *M*. The solutions were brought to pH 8–9 to ensure rapid attainment of equilibrium, and were allowed to remain for a minimum of 1 hr in a thermostated water bath, after which 1 *M* sulfuric acid was added to freeze the reaction. Measurements were performed at 25.0, 40.0, 55.0, and 70.0°. After acidification of each sample, the free formaldehyde was determined by the chromotropic acid method. There was no significant interference from the glyconitrile or the acetaldehyde at these concentrations. In calculating the equilibrium constant, the formaldehyde concentration was taken as the chromotropic acid value, (glyconitrile) = $(H_2CO)_{total} - (H_2CO)_{measured}$, (lactonitrile) = $(HCN)_{total}$ − (glyconitrile) − $(HCN + CN^-)_{final}$, and $(CH_3CHO)_{final} = (CH_3CHO)_{total}$ − $(\text{lactonitrile})_{final}$. The concentrations of HCN and CN^-, which were very small except in the 70° samples, were calculated by the equations

$$(HCN)_{final} = (H_2CO)_{measured}/[1 + K_{HCN}/(H^+) + (CH_3CHO)_{final}/K_L]$$

and

$$(CN^-)_{final} = K_{HCN}(HCN)_{final}/H^+)$$

Kinetic Measurements. In a typical run, 50.0 ml of buffer and 10.0 ml of standardized formaldehyde were mixed in a 125-ml glass-stoppered erlenmeyer flask. Buffer (50 ml), 10.0 ml of standardized HCN, and 80 ml of water were mixed in a 250-ml glass-stoppered erlenmeyer flask, and both flasks were left in the thermostated bath for at least 30 min. The formaldehyde solution was then added to the hydrogen cyanide solution, and 10.0-ml samples were withdrawn at appropriate intervals (2, 4, 8, 16, 32, 64, and 128 min) and added to the acidified $AgNO_3$. The bath was maintained to ±0.02° of the desired temperature. Kinetic measurements were made over a range of inital formaldehyde concentrations of 0.0388–0.524 *M*, initial hydrocyanic acid concentrations of 0.00769–0.233 *M*, pH's of 3.80–4.68, and temperatures of 25, 30, 35, 40, and 45°. The values of k_{obsd} are estimated to be accurate to 5%.

Determination of the Heat of the Reaction. In a typical experiment, 100 ml of 0.4106 *M* aqueous formaldehyde, acidified with 1.00 ml of 1 *M* HCl, was added to 100 ml of 0.4394 *M* HCN in a Dewar flask and allowed to come to thermal equilibrium. The reaction was started by adding 1.50 ml of 1 *M* NaOH. The temperature rise was measured with a Hewlett-Packard quartz thermometer, and the standard corrections were made.[9]

Kinetics and Equilibrium of Mandelonitrile Formation. The kinetics of the reaction between benzaldehyde and HCN in buffered aqueous solution at 25° were measured by following the decrease with time in the absorbance at 248 nm of aliquots of the reaction mixture (ϵ_{248} 13,200 for benzaldehyde in water; $\epsilon_{248} < 500$ for mandelonitrile). Equilibrium data were obtained from the absorbance of the kinetic experiment solutions after 15 half-lives.

Results

Equilibrium Constants. The equilibrium can be represented by the reaction

$$HOCH_2CN \rightleftharpoons H_2CO(aq) + HCN \qquad (1)$$

$$K_G = [(H_2CO)_{aq}(HCN)]/(HOCH_2CN) \qquad (2)$$

where $(H_2CO)_{aq}$ is the concentration of H_2CO + $H_2C(OH)_2$ in the equilibrium mixture. Starting with a known concentration of glyconitrile, it is possible in principle to obtain the equilibrium constant by measuring only the total cyanide concentration at equilibrium.[10] However, glyconitrile dissociates so little that this method is not reliable. We therefore mixed known concentrations of formaldehyde and hydrogen cyanide, titrated the excess HCN, and then determined the equilibrium concentration of $(H_2CO)_{aq}$ by chromotropic acid. Unfortunately, glyconitrile gives a small (~1%) but definite color yield with chromotropic acid. By plotting the absorbance of the chromotropic acid color *vs.* 1/(HCN), the color yield from glyconitrile can be estimated. The slope of this line is $K_G(HOCH_2CN)$. However, there is considerable scatter in these results. At the higher temperatures the scatter became greater, even though the concentrations of $(H_2CO)_{aq}$ were greater because of the increased dissociation. Values of the equilibrium constant at 19 and 25° determined in this way are included in Figure 1, but they cannot be considered particularly reliable.

The method of determining the equilibrium constant that worked well was to study the equilibrium

$$CH_3CH(OH)CN + (H_2CO)_{aq} \rightleftharpoons HOCH_2CN + (CH_3CHO)_{aq} \qquad (3)$$

$$K_C = \frac{(HOCH_2CN)(CH_3CHO)_{aq}}{(CH_3CH(OH)CN)(H_2CO)_{aq}} = \frac{K_L}{K_G} \qquad (4)$$

where K_L is the equilibrium constant for the dissociation of lactonitrile. The values of K_L are given by Yates and Heider.[10] We have recalculated their data

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[5] L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. 1, Wiley, New York, N. Y., 1967, p 46.

[6] W. J. Svirbely and J. F. Roth, *J. Amer. Chem. Soc.*, **75**, 3106 (1953).

[7] J. F. Walker, "Formaldehyde," 3rd ed, Reinhold, New York, N. Y., 1964, p 486.

[8] P. W. West and B. Sen, *Fresenius Z. Anal. Chem.*, **153**, 177 (1956).

[9] F. Daniels, J. W. Williams, P. Bender, R. A. Alberty, C. D. Cornwell, and J. E. Harriman, "Experimental Physical Chemistry," 7th ed, McGraw-Hill, New York, N. Y., 1970, p 32.

[10] W. F. Yates and R. L. Heider, *J. Amer. Chem. Soc.*, **74**, 4153 (1952).

using the newer values of the pK_a of HCN.[11] A least-squares fit of these data gives

$$\log K_L = 4.52 - 2608/T \qquad (5)$$

We have also verified the value of K_L at 25° by preparing a lactonitrile solution with a known excess of HCN and measurring the free acetaldehyde concentration spectrophotometrically at 278 nm. The measured pK_L was 4.31, in agreement with the 4.29 recalculated value of Yates and Heider.

Table I. Equilibrium between Glyconitrile and Lactonitrile

Initial $(CH_3CHO)_{aq}$,[a] $M \times 10^3$	Final $(H_2CO)_{aq}$, $M \times 10^3$	K_c	10^4K_L[b]	10^5K_G
		25.0°, pH 9.18		
10.0	0.642	27.1		
20.1	0.849	27.8		
40.2	1.12	31.1		
		Av 28.7	0.59	0.21
		40.0°, pH 9.07		
10.0	0.618	31.1		
20.1	0.850	28.4		
40.2	1.09	27.8		
		Av 29.1	1.6	0.55
		55.0°, pH 8.99		
10.0	0.592	37.7		
20.1	0.801	34.8		
40.2	1.05	32.5		
		Av 35.0	3.7	1.1
		70.0°, pH 8.92		
10.0	0.596	46.5		
20.1	0.787	40.7		
40.2	0.936	47.1		
		Av 44.8	8.3	1.9

[a] Initial $(H_2CO)_{aq} = 2.00 \times 10^{-3}$ M, initial $(HCN)_{total} = 2.08 \times 10^{-3}$ M for all experiments. [b] From eq 5.

The experimental data, average values of K_C at different temperatures, recalculated values of K_L from eq 5, and values of K_G are given in Table I and shown in Figure 1. A least-squares fit of these data gives

$$\log K_G = 1.66 - 2184/T \qquad (6)$$

The values of K_G are estimated to be accurate to 10% because of their dependence on the values of K_L.

Enthalpy of the Reaction. The enthalpy changes for the addition of aqueous HCN to aqueous formaldehyde, in two runs with an excess of HCN, were −10.12 and −9.99 kcal mol^{-1}. Two experiments using an excess of formaldehyde yielded values of −9.99 and −9.84 kcal mol^{-1}. The average value is −9.96 kcal mol^{-1}, with an uncertainty of ±0.20 kcal mol^{-1}. This is in excellent agreement with the +9.99 kcal mol^{-1} calculated from the slope of eq 6. The average of the thermochemical and equilibrium enthalpies (+9.98 kcal mol^{-1}) was used in the following calculations. This is the enthalpy change for the reaction $HOCH_2CN = (H_2CO)_{aq} + HCN$, and also the enthalpy change for the reaction $HOCH_2CN = H_2C(OH)_2 + HCN$ since the aqueous formaldehyde is almost completely hydrated.

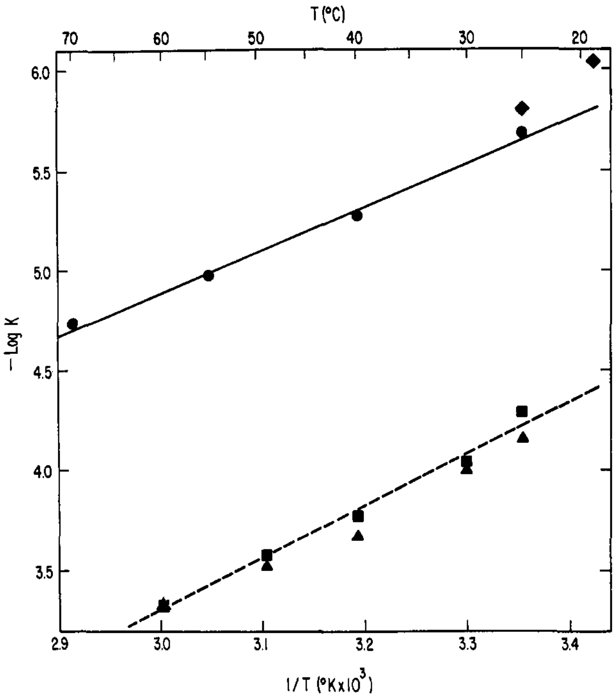

Figure 1. Negative logarithms of equilibrium constants for dissociation of glyconitrile and lactonitrile $[RCH(OH)CN \rightleftharpoons (RCHO)_{aq} + HCN]$ *vs.* $1/T$: (●) glyconitrile pK from measured equilibrium between glyconitrile and lactonitrile; (◆) glyconitrile pK from dissociation measurements; (■) lactonitrile pK (Yates and Heider data recalculated with new pK of HCN); (▲) original Yates and Heider data. The solid line is a least-squares fit of the glyconitrile data with the dissociation measurements omitted. The dashed line is a least-squares fit of the recalculated lactonitrile data.

The enthalpy of the dissociation reaction to give aqueous unhydrated formaldehyde

$$HOCH_2CN = H_2CO + HCN \quad \Delta H^\circ = 18.4 \text{ kcal mol}^{-1}$$
$$\Delta G^\circ = 12.2, T\Delta S^\circ = 6.2 \text{ kcal mol}^{-1}$$

can be calculated from the enthalpies of the reactions

$$HOCH_2CN + H_2O = H_2C(OH)_2 + HCN \quad \Delta H^\circ = 9.98 \text{ kcal mol}^{-1}$$
$$H_2C(OH)_2 = H_2CO + H_2O \quad \Delta H^\circ = 8.4 \text{ kcal mol}^{-1} \text{ (ref 12)}$$

The ΔG° value is calculated from the value of K' (see Discussion). The enthalpies of the reactions

$$CH_3CH(OH)CN = CH_3CHO + HCN \quad \Delta H^\circ = 14.29 \text{ kcal mol}^{-1}$$
$$\Delta G^\circ = 6.2, T\Delta S^\circ = 8.1 \text{ kcal mol}^{-1}$$
$$CH_3CH(OH)CN + H_2O = CH_3CH(OH)_2 + HCN$$
$$\Delta H^\circ = 8.74 \text{ kcal mol}^{-1}$$

can be obtained from the enthalpies of the reactions[10,13]

$$CH_3CH(OH)CN = CH_3CHO(aq) + HCN$$
$$\Delta H^\circ = 11.43 \text{ kcal mol}^{-1}$$
$$CH_3CH(OH)_2 = CH_3CHO + H_2O \quad \Delta H^\circ = 5.55 \text{ kcal mol}^{-1}$$

where $CH_3CHO(aq)$ is the equilibrium mixture of hydrated to unhydrated acetaldehyde in water at 25° $[(CH_3CHO)/(CH_3CH(OH)_2) = 0.942]$.[13] The greater stability of glyconitrile over lactonitrile is due largely to the ΔH° of forming the C–CN bond with formaldehyde.

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(11) R. M. Izatt, J. J. Christensen, R. T. Pack, and R. Bench, *Inorg. Chem.*, **1**, 828 (1962). A least-squares fit of their data gives $pK_{HCN} = -8.85 + 3802/T + 0.01786T$.

(12) A. A. Zavitsas, M. Coffiner, T. Wiseman, and L. R. Zavitsas, *J. Phys. Chem.*, **74**, 2746 (1970).

(13) J. L. Kurz, *J. Amer. Chem. Soc.*, **89**, 3524 (1967).

Kinetics. The generally accepted mechanism[14] for cyanohydrin formation yields the following kinetic scheme

$$\mathrm{HCN} \overset{K_{\mathrm{HCN}}}{\rightleftharpoons} \mathrm{H^+ + CN^-} \tag{7}$$

$$\mathrm{H_2C(OH)_2} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{H_2CO + H_2O} \tag{8}$$

$$\mathrm{H_2CO + CN^-} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{{}^-OCH_2CN} \tag{9}$$

$$\mathrm{{}^-OCH_2CN + H^+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{HOCH_2CN} \tag{10}$$

where K_{HCN} is the acid dissociation constant for HCN,[11] $K_{\mathrm{F}} = k_1/k_{-1}$ is the equilibrium constant for the dehydration of methylene glycol, and $K_3 = k_{-3}/k_3$ is the acid dissociation constant of the hydroxyl of glyconitrile. Reactions 7 and 10 are taken to be fast. Under the conditions of these experiments, reaction 8 is at least ten times as fast[15] as reaction 9, so the overall rate of glyconitrile formation and HCN disappearance will be determined by the rate of reaction 9. This should be first order in unhydrated formaldehyde and first order in $\mathrm{CN^-}$

$$-\frac{\mathrm{d(HCN)}}{\mathrm{d}t} = k_2(\mathrm{H_2CO})(\mathrm{CN^-}) = \frac{k_2K_{\mathrm{HCN}}(\mathrm{H_2CO})(\mathrm{HCN})}{(\mathrm{H^+})} \tag{11}$$

where $(\mathrm{H_2CO})$ is the concentration of unhydrated formaldehyde. A term for the reverse reaction is omitted because the glyconitrile formation reaction goes to completion. When reaction 9 is the rate-limiting step, the unhydrated formaldehyde concentration can be taken as the equilibrium value[16]

$$(\mathrm{H_2CO})/(\mathrm{H_2C(OH)_2}) = K_{\mathrm{F}}$$

The value of $K_{\mathrm{F}} = 5.0 \times 10^{-4}$ at 25° given by Bell and Evans[15,17] was used. The temperature dependence of this equilibrium constant is calculated, using the heat of hydration of -8.4 kcal mol^{-1},[12] to be

$$\log K_{\mathrm{F}} = 2.857 - 1836/T \tag{12}$$

This gives $K_{\mathrm{F}} = 5.00$, 6.33, 7.93, 9.86, and 12.2×10^{-4} at 25, 30, 35, 40, and 45°, respectively. Equation 11 then becomes

$$\frac{-\mathrm{d(HCN)}}{\mathrm{d}t} = \frac{k_2K_{\mathrm{HCN}}K_{\mathrm{F}}(\mathrm{H_2C(OH)_2})(\mathrm{HCN})}{(\mathrm{H^+})} = \frac{k_2K_{\mathrm{HCN}}K_{\mathrm{F}}[(\mathrm{H_2C(OH)_2})_0 - ((\mathrm{HCN})_0 - (\mathrm{HCN}))](\mathrm{HCN})}{(\mathrm{H^+})} \tag{13}$$

where $(\mathrm{H_2C(OH)_2})_0$ and $(\mathrm{HCN})_0$ are the initial respective concentrations. Integrating gives

$$\ln\frac{(\mathrm{HCN})_0}{(\mathrm{HCN})} - \ln\frac{(\mathrm{H_2C(OH)_2})_0}{[(\mathrm{H_2C(OH)_2})_0 - ((\mathrm{HCN})_0 - (\mathrm{HCN}))]} = \frac{k_2K_{\mathrm{HCN}}K_{\mathrm{F}}[(\mathrm{H_2C(OH)_2})_0 - (\mathrm{HCN})_0]t}{(\mathrm{H^+})} = k_{\mathrm{obsd}}t \tag{14}$$

The values of k_{obsd} (the pseudo-first-order rate constant for the disappearance of cyanide) were calculated from eq 14 and the HCN titration data by a least-squares fit using a digital computer. Only experimental points up to 3 half-lives were used.

The values of k_2 were then calculated from eq 14 using the values of k_{obsd}, K_{HCN}, and K_{F} and the pH values. No corrections were made for the effect of ionic strength on the HCN dissociation equilibrium; this is equivalent to using the activity of $\mathrm{CN^-}$ instead of the concentration in the kinetic equation when the pH is taken to be the activity of $\mathrm{H^+}$. The pH at 25° of the 1:1 buffer consisting of 0.05 *M* acetic acid and 0.05 *M* sodium acetate was taken as 4.679[18a] (the pH meter relative to a potassium hydrogen phthalate standard gave a pH of 4.650). The temperature dependence of the pH of this solution was calculated by the equation

$$\mathrm{pH}_T - \mathrm{pH}_{25°} = \mathrm{p}K_T - \mathrm{p}K_{25°} \tag{15}$$

where pH_T is the pH at temperature T and $\mathrm{p}K_T$ is the pK of acetic acid[18b] at temperature T. The pH's of the 2:1, 4:1, and 8:1 acetic acid–sodium acetate buffers (all 0.05 *M* in sodium acetate) were calculated by subtracting 0.301, 0.602, and 0.903, respectively, from the pH of the 1:1 buffer. Table II gives values

Table II. Rate Constants for the Reaction of Formaldehyde with Hydrogen Cyanide in Aqueous Solution

Run no.	Temp, °C	pH	(HOAc)/(NaOAc)[a]	$(\mathrm{H_2C(OH)_2})_0$,[b] *M*	10^4k_{obsd}, sec^{-1}	$10^{-5}k_2$, M^{-1} sec^{-1}
1	25.0	4.68	1	0.0388	1.34	3.50
2	25.0	4.68	1	0.0776	3.23	3.45
3	25.0	4.68	1	0.1939	8.75	3.35
4	25.0	4.68	1	0.349	14.56	3.01
5	25.0	4.38	2	0.1939	4.44	3.39
6	25.0	4.38	2	0.349	7.41	3.06
7	25.0	4.08	4	0.291	2.86	2.85
8	25.0	4.08	4	0.524	4.66	2.53
9	25.0	3.78	8	0.291	1.38	2.75
10	25.0	3.78	8	0.524	1.94	2.12
11	30.0	4.68	1	0.291	22.0	3.26
12	30.0	4.08	4	0.291	5.25	3.12
13	30.0	3.78	8	0.291	2.64	3.15
14	35.0	4.08	4	0.291	9.94	3.64
15	35.0	3.78	8	0.291	4.72	3.45
16	40.0	4.09	4	0.291	18.64	4.18
17	40.0	3.79	8	0.291	9.29	4.15
18	45.0	4.10	4	0.1164	13.73	5.19
19	45.0	3.80	8	0.1164	6.80	5.16

[a] (NaOAc) = 0.050 *M*, ionic strength = 0.050 *M* for all runs. [b] $(\mathrm{HCN})_0 = 0.233$ *M* for all runs.

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[14] A. Lapworth, *J. Chem. Soc.*, **83**, 995 (1903); **85**, 1206 (1904); W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).

[15] R. P. Bell and P. G. Evans, *Proc. Roy. Soc., Ser. A*, **291**, 297 (1966).

[16] The $(\mathrm{H_2CO})$ is given more precisely by the steady-state assumption as

$$(\mathrm{H_2CO}) = \frac{K_{\mathrm{F}}(\mathrm{H_2C(OH)_2})}{1 + [k_2K_{\mathrm{HCN}}(\mathrm{HCN})/k_{-1}(\mathrm{H^+})]}$$

Using this expression in eq 13 and integrating give eq 14 except for a small correction factor on the second term on the left side of the equation. Using the corrected equation changed the rate constants in these experiments by less than 1%. At pH values of 6 and higher depending on the reactant concentrations, reaction 8 can become rate limiting rather than reaction 9.

[17] The absolute value of K_{F} is relatively uncertain. The values for k_2 can be recalculated as more accurate values of K_{F} become available by multiplying by the factor $K_{\mathrm{F}}^{\mathrm{new}}/K_{\mathrm{F}}^{\mathrm{old}}$.

[18] (a) H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold, New York, N. Y., 1958, p 712; (b) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd ed, Butterworths, London, 1959, p 517.

of k_{obsd} and k_2 for 19 kinetic runs, conducted over the range of temperatures, pH's, and initial formaldehyde concentrations. Figure 2 shows the pseudo-first-order reaction kinetics for a typical experiment.

The values of k_2 are constant within the experimental error except for a 30% decrease in the 25° series at high acetic acid and formaldehyde concentrations. Thus run 10 contains 0.4 *M* acetic acid and 0.5 *M* hydrated formaldeyde compared with 0.05 *M* acetic acid and 0.04 *M* hydrated formaldehyde in run 1. This suggested that elevated concentrations of acetic acid and/or formaldehyde were decreasing the rate. We tested this medium effect by adding ethylene glycol and ethanol to the reaction mixture (Table III). A comparison of runs 27 and 28 with run 23 shows that the addition of organic solvents reduced the value of k_2 to approximately the same extent as an equivalent concentration of acetic acid plus formaldehyde. However, the situation is complicated by the reaction of formaldehyde with alcohols[19] to form hemiacetals. The data are not sufficiently extensive or accurate to separate the medium effect and the hemiacetal equilibria.

Table III. General Acid–General Base and Medium Effects on Cyanohydrin Reaction Rate

Run no[a]	(NaOAc), *M*	Ionic strength, *M*	$10^4 k_{obsd}$, sec^{-1}	$10^{-5} k_2$, M^{-1} sec^{-1}
20	0.00500	0.050	2.36	3.32
21	0.0100	0.050	2.46	3.46
22	0.0250	0.050	2.39	3.36
23	0.0500	0.050	2.42	3.40
24	0.0850	0.085	2.31	3.38
25	0.0100	0.010	2.41	3.08
26[b]	0.0500	0.050	2.36	3.32
27[c]	0.0500	0.050	1.77	2.49
28[d]	0.0500	0.050	2.13	2.99

[a] Temperature = 25.0°, pH 4.65, (HOAc)/(NaOAc) = 1.0, $(H_2C(OH)_2)_0$ = 0.0572 *M*, $(HCN)_0$ = 0.00769 *M* for all runs. For runs 20–22, $NaNO_3$ was added to increase ionic strength. [b] Formaldehyde–buffer and hydrocyanic acid–buffer solutions were kept separately for 18 hr at 25.0° before start of run. [c] Reaction solution was 0.89 *M* in ethylene glycol. [d] Reaction solution was 0.56 *M* in ethanol.

Table III also shows the effect of changing the ionic strength at a constant acetic acid/sodium acetate ratio on the value of k_{obsd}. Runs 21, 25, and 26 show no significant change in the value of k_{obsd} over the ionic strength range of 0.01–0.085 *M* (run 26 has a higher acetic acid concentration than runs 21 and 25, so a small medium effect may be present). In calculating these values of k_2, the pH was taken to be independent of ionic strength; it can be shown[6] that the addition of an inert salt to an acetic acid–sodium acetate buffer lowers the pH, but the salt effect on the pH is counterbalanced by the salt effect on the dissociation of the HCN. Therefore, no change in the rate is predicted from a change in the ionic strength. There appears to be no significant effect of increasing the concentration of acetic acid and sodium acetate at constant buffer ratio and ionic strength (runs 20–24). General acid and general base catalysis might be measurable at buffer concentrations higher than used in this study, but such catalysis would be complicated by medium effects. Svirbely and Roth[6] found no observable general acid or general base catalysis of cyanohydrin formation with acetone or acetaldehyde and only a small general catalysis with propionaldehyde. There is a general acid and a general base catalysis of the dehydration of formaldehyde,[15] but our experiments have been run under conditions where the dehydration of formaldehyde is not rate limiting.

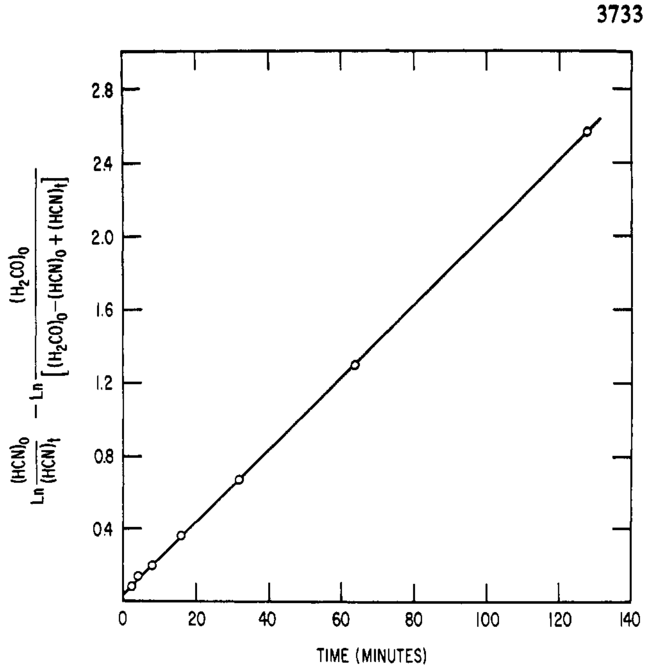

Figure 2. Typical kinetic run: ln $(HCN)_0/(HCN)_t$ − ln $(H_2CO)_0/[(H_2CO)_0 - (HCN)_0 + (HCN)_t]$ *vs.* time for run 3.

A complex of formaldehyde and acetic acid has been reported[20] and this could affect the kinetics. Runs 23 and 26 show that there is no significant difference in the rate with preincubation of the formaldehyde and acetic acid for 18 hr. This shows that the acetic acid–formaldehyde complex is not an important species in these solutions or that the complex is formed very slowly or decomposes rapidly.

Heat of Activation. A least-squares fit of the k_2 values gives an Arrhenius activation energy of 5.61 kcal mol^{-1} for the 4:1 buffer runs (5.77 kcal mol^{-1} for the 8:1 buffer runs). This corresponds to $\Delta H^{\ddagger}$ = 5.02 kcal mol^{-1} and $\Delta S^{\ddagger}$ = −16.9 eu at 25°. It should be noted that this value of the heat of activation for reaction 9 is very sensitive to the value chosen for the heat of hydration of formaldehyde.

Kinetics and Equilibrium of Mandelonitrile Formation in Aqueous Solution. The dissociation constant for the reaction

$$C_6H_5CH(OH)CN \rightleftharpoons C_6H_5CHO + HCN \qquad (16)$$

was found to be 5.2 × 10^{-3} at 25°. This is close to the value of 4.4 × 10^{-3} at 20° in 95% ethanol.[21]

The kinetics were run as a psuedo-first-order reaction at 25° using acetate buffers ((benzaldehyde) = 1.0 × 10^{-4} *M*, (HCN) = 6.1 × 10^{-2} *M*). The k_{obsd} were 1.84 × 10^{-4} and 3.71 × 10^{-4} sec^{-1} at pH values of 4.68 and 4.98, respectively. This gives values of

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[19] E. G. Sander and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 6154 (1968).

[20] Reference 7, p 345.

[21] J. W. Baker, G. F. C. Barrett, and W. T. Tweed, *J. Chem. Soc.*, 2831 (1952).

Table IV. Rate and Equilibrium Constants for Reaction of Aldehydes and Acetone with Cyanide in Aqueous Solution at 25°

Aldehyde	K	K'	K_{hyd}	K_3[a]	K_2	k_2, M^{-1} sec^{-1}	k_{-2}, sec^{-1}
Formaldehyde	2.18×10^{-6}	1.09×10^{-9}	5.0×10^{-4}	2.0×10^{-11}	3.29×10^{-8}	3.50×10^{5}	1.15×10^{-2}
Acetaldehyde	5.65×10^{-5}	2.74×10^{-5}	0.94[b]	3.2×10^{-12}	5.16×10^{-3}	6.87×10^{2}	3.54
Acetone	7.16×10^{-2}	7.16×10^{-2}	7.2×10^{2}[c]	6.3×10^{-13}	6.85×10^{1}	3.97	2.72×10^{2}
Benzaldehyde	5.14×10^{-3}	5.14×10^{-3}	[d]	4.4×10^{-12}	0.705	1.14×10^{2}	8.24×10^{1}

[a] Reference 24. [b] Reference 13. [c] J. Hine and R. W. Redding, *J. Org. Chem.*, **35**, 2769 (1970). Value is for 33°. [d] Benzaldehyde is taken to be not significantly hydrated as is the case for *p*-chlorobenzaldehyde.[22]

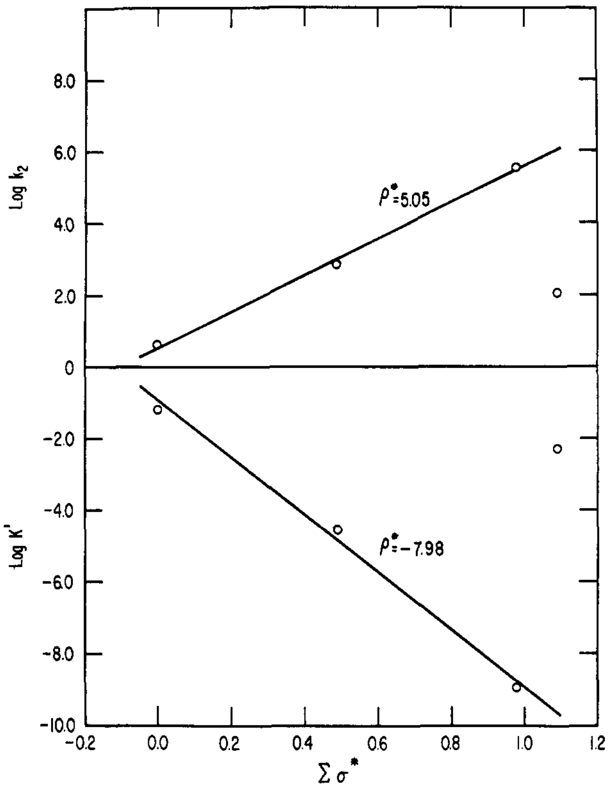

Figure 3. (Upper plot) Log k_2 *vs.* $\Sigma\sigma^*$. (Lower plot) Log K' *vs.* $\Sigma\sigma^*$. $\Sigma\sigma^*$ formaldehyde = 0.98, $\Sigma\sigma^*$ acetaldehyde = 0.49, $\Sigma\sigma^*$ acetone = 0.00, and $\Sigma\sigma^*$ benzaldehyde = 1.09.

k_2 in the equation

$$-\mathrm{d}(\mathrm{C_6H_5CHO})/\mathrm{d}t = k_2(\mathrm{C_6H_5CHO})(\mathrm{CN^-}) \quad (17)$$

of 1.13×10^2 and 1.14×10^2 M^{-1} sec^{-1}, respectively.[22]

Discussion

It is interesting to compare the equilibria for the unhydrated aldehydes

$$\mathrm{RCH(OH)CN = RCHO + HCN} \quad (18)$$

$$K' = \frac{\mathrm{(RCHO)(HCN)}}{\mathrm{(RCH(OH)CN)}} \quad (19)$$

where (RCHO) is the concentration of the unhydrated aldehyde. K' is related to the measured equilibrium constant (K) by

$$K' = KK_{\text{hyd}}/(1 + K_{\text{hyd}}) \quad (20)$$

where

$$K_{\text{hyd}} = \frac{\mathrm{(RCHO)}}{\mathrm{(RCH(OH)_2)}} \text{ and } K = \frac{\mathrm{(RCHO)_{aq}(HCN)}}{\mathrm{(RCH(OH)CN)}}$$

We can also consider the equilibrium

$$\underset{\substack{|\\ \mathrm{O^-}}}{\mathrm{RCHCN}} = \mathrm{RCHO + CN^-} \quad (21)$$

$$\frac{\mathrm{(RCHO)(CN^-)}}{\mathrm{(RCH(O^-)CN)}} = K_2 = \frac{k_{-2}}{k_2} \quad (22)$$

where (RCHO) is the concentration of the unhydrated aldehyde. We have

$$K_2 = K'K_{\mathrm{HCN}}/K_3 \quad (23)$$

and

$$k_{-2} = k_2K_2 = k_2K'K_{\mathrm{HCN}}/K_3 \quad (24)$$

The values of the various equilibrium and rate constants for the formaldehyde, acetaldehyde, acetone, and benzaldehyde cyanohydrin reactions are given in Table IV. Figure 3 shows a plot of log K' and log k_2 *vs.* $\Sigma\sigma^*$. It can be seen that the K' values give a good fit for the formaldehyde,[23] acetaldehyde, and acetone cases ($\rho^* = -7.98$) but not for benzaldehyde. This is to be expected since the phenyl ring of benzaldehyde contributes more than an inductive effect. The resonance between the carbonyl group and the phenyl ring stabilizes the benzaldehyde carbonyl over what would be expected on the basis of only an inductive effect, thereby shifting the equilibrium of eq 18 to the right. A plot (not shown) of log K *vs.* $\Sigma\sigma^*$ does not give a straight line, as would be predicted from eq 20. The plot of log k_2 *vs.* $\Sigma\sigma^*$ gives a good fit ($\rho^* = 5.05$); a plot (not shown) of log k_{-2} *vs.* $\Sigma\sigma^*$ gives an equally good fit ($\rho^* = -4.46$, $\Sigma\sigma^* = 2.66$, 3.15, and 3.64 for the cyanohydrins of acetone, acetaldehyde, and formaldehyde, respectively).[24] A plot of log K_2 *vs.* $\Sigma\sigma^*$ (not shown) also gives a good fit (ρ^* -9.51). In all cases benzaldehyde does not fit because of resonance (and steric) effects. The data of Table IV also show that the inductive effects of the substituents on the value of K_2 manifest themselves about equally on the rate of formation of the cyanohydrin anion and the rate of its decomposition. The

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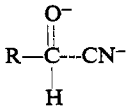
(22) J. P. Kuebrich, R. L. Schowen, M. Wang, and M. E. Lupes [*J. Amer. Chem. Soc.*, **93**, 1214 (1971)] report a k_2 value of 1.6×10^4 M^{-1} sec^{-1} at 34° in methanol. We have also measured the rate of mandelonitrile formation in methanol with a 1:1 acetic and sodium acetate buffer. Our measured pseudo-first-order rate constant for the disappearance of benzaldehyde in methanol (2.4×10^{-4} sec^{-1} with 6×10^{-2} M HCN) is only 33% greater than that which we measured for the corresponding reaction in water. The much larger difference between our value of k_2 in water and the k_2 value of Kuebrich, *et al.*, in methanol is apparently due to the large changes in the pK values of acetic acid, hydrogen cyanide, and the OH of mandelonitrile on going from water to methanol.

(23) When $-\log K'$ is added to Sander and Jencks'[19] plot of log K_0 *vs.* γ for equilibrium additions of nitrogen, oxygen, and sulfur nucleophiles to formaldehyde, the point lies on the extension of their line through the nitrogen nucleophiles, rather than the line through the oxygen and sulfur nucleophiles.

(24) P. Ballinger and F. A. Long, *J. Amer. Chem. Soc.*, **82**, 795 (1960). The σ^* for CN is taken as 3.64 and ρ^* as 1.42.

overall equilibrium in aqueous solution (K), in turn, is determined by the value of K_2, the acidity of the cyanohydrin hydroxyl (K_3) and the extent of hydration of the free aldehyde. The almost equal and opposite values of ρ^* for k_2 and k_{-2} are consistent with a transition state lying half-way along the reaction coordinate from reactants to product.



$$R{-}\overset{\overset{O^-}{\|}}{\underset{H}{C}}{-}{-}CN^-$$

Prebiotic Significance. The great stability of glyconitrile and the expected stability for glycinenitrile show that glycolic acid and glycine could have been synthesized in the primitive oceans under very dilute conditions. Thus, if the free HCN concentration were, for example, 2×10^{-5} M, then 90% of the formaldehyde would have been in the form of glyconitrile. Conversely, if the free formaldehyde concentration were 2×10^{-5} M, then 90% of the cyanide would also have been in the form of glyconitrile. Once the nitrile has been hydrolyzed to the amide or the acid, the carbon–carbon bond is stable. Therefore, glycolic acid and glycine synthesis could have taken place at great dilution in the primitive oceans.

The proposed prebiotic synthesis of adenine requires high concentrations of hydrogen cyanide,[3] while the proposed prebiotic syntheses of sugars require high concentrations of formaldehyde.[4] The great stability of glyconitrile shows that the two type of compounds could not have been synthesized at the same time on the primitive earth unless there was a mechanism to concentrate the formaldehyde and hydrogen cyanide in different areas. It is more plausible to think that the adenine was synthesized during one period and the sugars during another period. Since sugars are decomposed rapidly on the geological time scale even at neutral pH and low temperatures, the likely sequence of reactions is

adenine and guanine synthesis from HCN
↓
pentose synthesis from H_2CO
↓
nucleoside synthesis from purines and pentoses
↓
further polymerization steps

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Reaction of a Six-Membered Cyclic Sulfonate Ester, β-(2-Hydroxy-3,5-dinitrophenyl)ethanesulfonic Acid Sultone, with the Active Site of Papain[1]

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Contribution from the Department of Chemistry, University of Chicago, Chicago, Illinois 60637. Received October 26, 1972

Abstract: Rate constants for the sulfonylation of the thiol group at the active site of papain by the reactive aromatic six-membered sultone β-(2-hydroxy-3,5-dinitrophenyl)ethanesulfonic acid sultone (**2d**) to give an enzymatic thiolsulfonate species **5** have been measured over the range pH 3.5–9.6. The rate data obtained below pH 7 have been interpreted as indicating that, as with specific substrates, reaction occurs with Cys-25 in the thiol form assisted by general base catalysis by the basic form of a group in the enzyme with p$K_a \cong 4$. At higher pH values, nucleophilic displacement at the sulfonyl group of the sultone by the thiolate form of Cys-25 becomes predominant. The stoichiometric reaction of papain with **2d** to produce an enzyme-bound dinitrophenolate chromophore can be used for the titration of the active site of the enzyme. Reporter group titration of the dinitrophenolate chromophore present in the thiolsulfonate species **5** indicates that the ionization of the phenolic hydroxyl is significantly perturbed by that of a group of similar pK_a at the active site of papain. Most importantly, it has been found that at pH 5.2, recyclization to generate the sultone **2d** *via* the nucleophilic attack of the phenolic hydroxyl group in **5** on the sulfonyl function competes effectively with hydrolysis, the first observation of this kind for an enzyme other than a serine proteinase.

A variety of labile cyclic compounds, including lactones, sultones, cyclic sulfate and phosphate esters, react readily with the active sites of proteolytic enzymes. For example, the serine proteinase, α-chymotrypsin (α-CT), reacts with the family of compounds **1**–**2**.[2] In each case, as illustrated in eq 1 for the five-membered cyclic esters (where E·S represents the Michaelis complex), the group Y undergoes nucleophilic attack by the active site serine with formation of the acyl, sulfonyl, sulfuryl, or phosphoryl enzyme, as well as a new phenolic hydroxyl group.

The presence of this new group at the enzyme active site gives rise to two types of unusual reactions. First, regeneration of active enzyme may take place by a recyclization reaction (step k_{-2}) under conditions where kinetic control allows reformation of **1**, rather than **4** which is often thermodynamically more favored.

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(1) A preliminary account of this work has appeared: P. Campbell and E. T. Kaiser, *Biochem. Biophys. Res. Commun.*, **4**, 866 (1972).

(2) (a) E. T. Kaiser, *Accounts Chem. Res.*, **3**, 145 (1970), and references therein; (b) E. T. Kaiser, T. W. S. Lee, and F. P. Boer, *J. Amer. Chem. Soc.*, **93**, 2351 (1971); (c) G. Tomalin, M. Trifunac, and E. T. Kaiser, *ibid.*, **91**, 722 (1969).