Acyl Transfer Reactions from and to the Ureido Functional Group. II. The Mechanisms of Aminolysis of an *O*-Acylisourea (2-Amino-4,5-benzo-6-oxo-1,3-oxazine)

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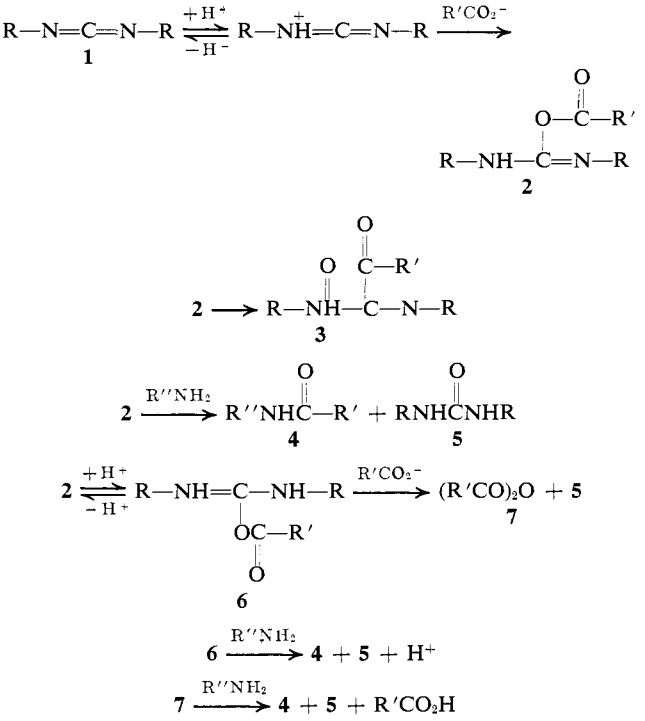
Abstract: The kinetics of reaction of representative primary and secondary amines with 2-amino-4,5-benzo-6-oxo-1,3-oxazine (**8**) [an intramolecular *o*-carboxyl addition product to a carbodiimide group], to provide *o*-ureidobenzamides, are described (30°, H_2O solvent, $\mu = 1.0$). Under the pseudo-first-order conditions of total amine concentration ($[N_T]$) $\gg$ [**8**] the aminolysis reactions were found to proceed *via* simple nucleophilic attack ($k_N[N][\mathbf{8}]$) and amine general base assisted nucleophilic attack ($k_{gb}[N]^2[\mathbf{8}]$). Only the values of k_N have been tabulated since, under the conditions of the experiments, the major portion of the reactions proceed through the path associated with this constant. The pH dependence of the aminolysis rates are readily explainable by considering reaction of amine with two (*i.e.*, **8a** and **8b**) of the four species of **8** previously shown to exist in aqueous solution. In addition the kinetics of the aminolysis reaction require the same pK_a values as those determined spectrophotometrically for interconversion of the four species of **8** (*i.e.*, **8a** $\rightleftarrows$ **8b** ($pK_{a_1} = 3.10$); **8b** $\rightleftarrows$ **8c** ($pK_{a_2} = 10.61$); **8c** $\rightleftarrows$ **8d** ($pK_{a_3} = 9.21$)]. The second-order rate constants for reaction of **8a** with primary amine free bases is related to the pK_a of the conjugate acids of the amines *via* the expression $\log k_1 = 0.75pK_a - 3.0$ when $pK_a < 9$ but tending toward a constant value of $\log k_1$ for higher pK_a's. In contrast species **8b** is far less reactive with primary amines; $\log k_2 = 0.84pK_a - 8.8$. Compound **8** may be considered as a model for the carboxylic acid adduct in the carbodiimide synthesis of amides. From the present studies rationales for certain known features of the carbodiimide synthetic procedure become evident. These include the ability to carry out the condensation in aqueous solution, the apparent greater reactivity of less basic amines etc.

One of the most versatile methods used in peptide synthesis is the coupling of suitably blocked amino acids by means of carbodiimides. The reaction is an overall dehydration, the carbodiimide **1** being converted to the corresponding urea **5**, which normally precipitates from solution. Similarly, carbodiimides have been used for the formation of aromatic and aliphatic acid amides, anhydrides and esters, and phosphate and sulfate esters. The method was first described by Khorana[2a] and by Sheehan and Hess.[2b] The synthetic procedure has many advantages, *e.g.*, reactions may be carried out in aqueous solution, and has gained wide application; several reviews, by Khorana[3] and by others,[4] list the scope of the reaction in detail. In addition to the urea (**5**), the other by-products commonly isolated are the *N*-acylurea (**3**) and anhydride (**7**); in some cases these are the major or only products.

The sequence of reactions of Scheme I has been suggested by Khorana[2a] and others[5,6] to account for the products formed; the most important point about Scheme I is the postulation of the intermediacy of the *O*-acylisourea (**2**) which may then isomerize (an O → N acyl shift, forming **3**) or react with nucleophiles to give, *e.g.*, the anhydride (**7**) or the amide (**4**). However **2** is not isolated in the course of the reaction. Attempts to isolate and characterize **2** usually yield only the *N*-acylurea **3**. This reflects the fact that the isoimide structure (RC(=O)OC=N) is rarely encountered. Unless stabilized by special structural features, a facile isomerization to the corresponding imide (C(=O)NC(=O)R) occurs. Curtin and Millar[7] have demonstrated that the anti isomer of the isoimide may, however, resist isomerization. The nitrogen lone pair is then on the side remote from the *O*-acyl group, and the

Scheme I



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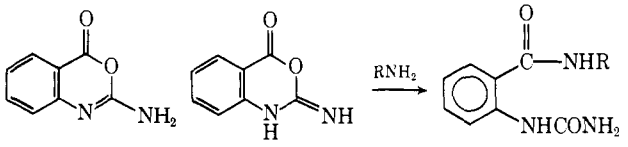
(2) (a) H. G. Khorana, *Chem. Ind. (London)*, 1087 (1955); (b) J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 77, 1067 (1955).
(3) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," Wiley, New York, N. Y., 1961.
(4) F. Kurzer and K. Douraghi-Zadeh, *Chem. Rev.*, 67, 107 (1967).
(5) H. Zahn and Schussler, *Chem. Ber.*, **95**, 1076 (1962).
(6) H. Brauniger and W. Delzer, *Pharmazie*, 20, 279 (1965).
(7) D. Y. Curtin and L. L. Millar, *Tetrahedron Lett.*, 1869 (1965).

rate of isomerization to the imide is essentially that of anti → syn conversion, the four center O → N acyl shifts occurring relatively rapidly in the syn isomer. DeTar and coworkers[8] have carried out a kinetic investigation of some carbodiimide reactions in acetonitrile and carbon tetrachloride solution. Although the results are complicated by the intervention of multiple association equilibria in the solvent system chosen for study, they agree with a scheme such as I. For example, evidence was obtained to show that the *N*-acylurea (**3**) did not arise from the reaction of the urea (**5**) with the acid anhydride and that the anhydride did not react directly with the carbodiimide (**1**) under the conditions used for the study.

An understanding of the reactivity of the *O*-acylisourea (**3**) and its partitioning amongst the various products is obviously vital to define conditions of solvent, pH, nucleophile, etc., where the yield of the desired amide **4** is maximized at the expense of the by-products. Hopefully one could then explain some of the unusual features of the reaction; *e.g.*, addition of acid is reported to have little effect (in tetrahydrofuran solution) on the amount of amide formed, and higher yields of simple amides are obtained with less, rather than more, basic amines.[9]

Lempert and Doleschall[10] have demonstrated that the cyclic benzoxazine **8**, which is a model for the isoimide **2**, may be isolated under suitable conditions.



8 **8′** **9**

Nucleophilic attack by the amino nitrogen (or by the imino nitrogen in the tautomeric form **8′**) at the carboxyl group is sterically prohibited. Kinetic studies on the hydrolysis of **8** have shown[11] that although the reactions of **8** with hydroxide ion and of protonated **8** with water are significant in basic and acidic solution, respectively, there is a large pH region (4–8) in which the hydrolysis of **8** is relatively slow ($t_{1/2}$ > 1 hr). In this study we have examined in aqueous solution the reaction of a number of representative amines with the *O*-acylisourea (**8**) to give the amides (**9**).

Experimental Section

Materials. Potassium chloride, potassium hydroxide, and potassium phosphate were reagent grade and used without further purification. The water used throughout was deionized and then twice distilled in an all-glass apparatus. The liquid amines morpholine and piperidine were purified by distilling the commercial material over sodium hydroxide using a 20-in. Teflon annular spinning band column. The morpholine obtained had bp 129°, the piperidine 105.5–106°. Both were stored under nitrogen and used immediately. Glycine (Fischer, Reagent grade) and glycylglycine (Calibochem, A grade) were used directly without further purification. The hydrochlorides of the following amines were recrystallized from ethanol or ethanol–water shortly before use: ethylamine (Eastman), mp 108–109°; L-lysine (Sigma Chemical Co.), mp 268°; 2,2,2-trifluoroethylamine (Pierce Chemical Co.); glycine ethyl ester (Aldrich), mp 145–146°; ethylenediaminedihydrochloride (Matheson Coleman and Bell).

2-Ureidobenzamide. *o*-Aminobenzamide (12.2 g, 0.10 mol) was stirred at 0° in 150 ml of 1:1 acetic acid–water and a solution of 8.1 g (0.10 mol) of potassium isocyanate was added over 1 hr. The precipitated urea (14.0 g, 85%) was filtered and washed with water. On recrystallization (from methanol–water, maintaining the temperature below 50°), the 2-ureidobenzamide had mp 180–182° dec (when heated rapidly to 170°); lit.[12] mp 184–185° (rapid heating).

The methods used for the preparation of **2-amino-4,5-benzo-6-oxo-1,3-oxazine** (**8**) and **2,4-(1*H*,3*H*)-quinazolinedione** have previously been described.[11]

Kinetic Measurements. All kinetic measurements and pK_a determinations were carried out at 30° in aqueous solution at μ = 1.0 with potassium chloride. The combined Cary 15 spectrophotometer–Radiometer pH-stat assembly used for most of the kinetic experiments has been described in detail elsewhere.[11,13]

For those aminolyses studied close (±1 pH unit) to the pK_a of the amine, the amine–amine hydrochloride solution acted as buffer. In this case the pH of the reaction solution was measured at 30° before and after a kinetic run using a Radiometer pH meter Type PHM 22 equipped with a PHA 630 scale expander. Any run showing a pH drift greater than 0.03 unit was discarded. The rates of aminolysis of 2-amino-4,5-benzo-6-oxo-1,3-oxazine were measured (in 3-ml cuvettes) using either a Cary 15 or Gilford Model 2000 spectrophotometer. Generally it was found convenient to follow the decrease in optical density at 265 nm. At pH's outside the buffer capacity range of the amine, pH was maintained constant using the pH-stat assembly described above. Use of this apparatus had the advantage that aminolyses could be studied under conditions when the amine was either essentially 0 or 100% protonated. The amine–amine hydrochloride solutions were prepared just prior to a kinetic run, by the addition of standardized hydrochloric acid or potassium hydroxide to solutions of the free amine or amine hydrochloride. A minimum of four serially diluted buffer concentrations (1.0–0.05 *M* in total amine concentration) were used in those pH regions and with those amines in which the plots of k_{obsd} *vs.* total amine concentration were strictly linear Otherwise 6–8 dilutions were used to facilitate the separation of terms first and second order in the amine concentration.

The values of the pseudo-first-order rate constants (k_{obsd}) were calculated either graphically [plots of log ($OD_\infty - OD_t$) *vs.* time (t)] or using programs written for an Olivetti-Underwood Programma 101 computer by Dr. Donald Tanner, formerly of this laboratory.

Product Analysis. On a preparative scale it has been shown that the reaction of the benzoxazine **8** with a variety of primary and secondary amines gives the corresponding amides **9** in high yield.[10] Although no detailed product analysis was carried out in this study for all the amines used, it was shown that in one case (with ammonia as the amine) the amide was the primary aminolysis product of both **8a** and **8b**. The ammonolysis of **8** was initially carried out in 0.5 *M* total ammonia concentration at pH 9.50. Under these conditions reaction is essentially with **8b**. On completion of ammonolysis, the pH was rapidly adjusted to 10.50 and the recyclization of the *o*-ureidobenzamide (**9**, R = H) formed in the initial experiment [to 2,4-(1*H*,3*H*)-quinazoline] was followed by repetitive scans of the ultraviolet. The spectral changes observed were identical with those observed on cyclization of an authentic sample of the amide **9** (R = H), as was the rate constant calculated from this date.[14] Since the cyclization of **9** (R = H) is not subject to general base catalysis,[14] the rate was unaffected by the presence of ammonia. In a similar manner, by carrying out the ammonolysis of **8** at pH 6.0, it was shown that **9** (R = H) was also formed from **8a**.

Results

The rates of aminolyses of **8** by representative primary and secondary amines have been measured at 30° in aqueous solution (μ = 1.0). The kinetics were studied

(8) D. F. DeTar and R. Silverstein, *J. Amer. Chem. Soc.*, **88**, 1013, 1020 (1966); D. F. DeTar, R. Silverstein, and F. F. Rogers, *ibid.*, **88**, 1024 (1966).

(9) A. Buzas, F. Canac, C. Egnell, and P. Freon, *C. R. Acad. Sci.*, 260, 2249 (1965); A. Buzas, C. Engell, and P. Freon, *ibid.*, **252**, 896 (1961).

(10) K. Lempert and G. Doleschall, *Monatsch. Chem.*, **95**, 950 (1964).

(11) A. F. Hegarty and T. C. Bruice, *J. Amer. Chem. Soc.*, **92**, 6561 (1970).

(12) W. A. Jacobs and M. Heidelberger, *ibid.*, **39**, 2437 (1917).

(13) T. C. Bruice and J. R. Maley, *Anal. Biochem.*, **34**, 275 (1970).

(14) A. F. Hegarty and T. C. Bruice, *J. Amer. Chem. Soc.*, **92**, 6575 (1970).

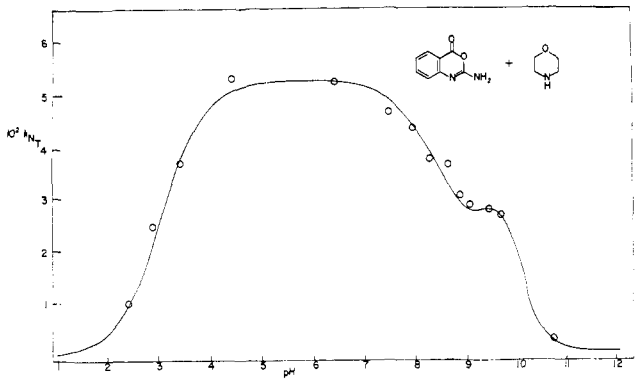


Figure 1. Plot of apparent second-order rate constants (calculated for total amine concentration; l. mol^{-1} sec^{-1}) for reaction of morpholine with **8** (H_2O, 30°, μ = 1.0). Points are experimental and the curve generated from eq 5.

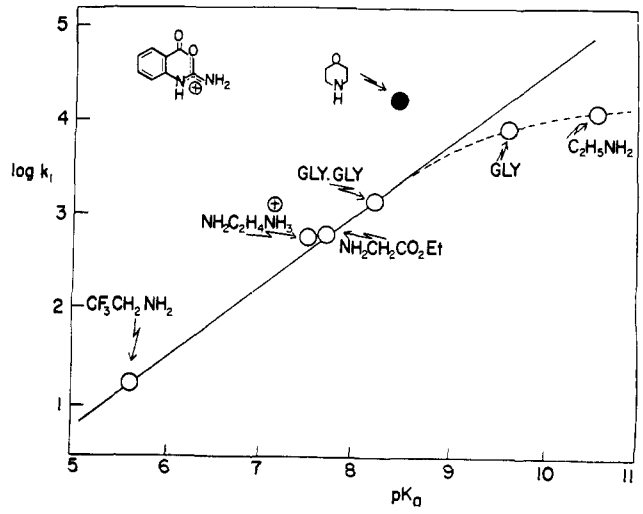


Figure 2. Brønsted plot for reaction of amines with the indicated ionic species of **8** (k_1 in l. mol^{-1} sec^{-1}).

under pseudo-first-order conditions by maintaining the amine at all times in a large excess relative to **8**. For primary amines, plots of the observed pseudo-first-order rate constants (k_{obsd}) *vs.* the free amine concentration (at constant pH) exhibited upward curvature indicating a term of greater order than one in amine. For these cases k_{obsd} was found to be related to the free amine concentration and could be expressed by a sum of terms *via* eq 1, where [N] refers to the free (unprotonated)

$$k_{obsd} = k_{hydr} + k_N[N] + k_{gb}[N]^2 \quad (1)$$

amine concentration (*i.e.*, $K_a = [N][H^+]/[NH^+]$, and $[N_T] = [N] + [NH^+]$). The constant k_{hydr} is the hydrolytic (*i.e.*, amine independent) rate at the given pH. The constants k_N and k_{gb} are best separated by rearrangement of eq 1 to provide 2. A secondary plot of

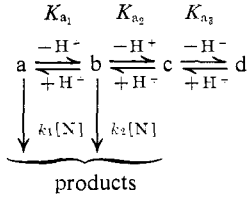
$$\frac{k_{obsd} - k_{hydr}}{[N]} = k_N + k_{gb}[N] \quad (2)$$

$(k_{obsd} - k_{hydr})/[N]$ *vs.* [N] then gives k_N (the second-order rate constant for reaction of the substrate with free amine) as intercept and k_{gb} (nucleophilic attack assisted by the amine as a general base) as slope. Only the k_N values are quoted in this study; the contribution of k_{gb} was in all cases small and could be ignored with some secondary amines (*e.g.*, morpholine) or at low amine concentration.

The reaction between the secondary amine, morpholine, and **8** was investigated over the pH range 2–11. The observed rate constants at a given pH followed the simple relationship seen in eq 3; in Figure 1, the k_{N_T}

$$k_{obsd} = k_{N_T}[N_T] \quad (3)$$

values obtained are plotted as a function of pH. A theoretical fit of the data was obtained assuming the kinetic scheme shown in eq 4; the species a, b, c, and d



(4)

designate the various forms of the substrate in solution; the equilibrium constants K_{a_1}, K_{a_2}, K_{a_3} have the values 7.96 × 10^{-4}, 2.43 × 10^{-11}, and 6.17 × 10^{-10}, respectively.[11] Using these known constants and the acid dissociation constant of the conjugate acid of morpholine (K_{a_4} = 2.09× 10^{-9}) the values of k_1 and k_2 which

$$k_{N_T} = \frac{k_1 K_{a_4} a_H}{(a_H + K_{a_1})(a_H + K_{a_4})} + \frac{k_2 a_H^2}{a_H^2 + K_{a_2} a_H + K_{a_2} K_{a_3}} \times \frac{K_{a_4}}{a_H + K_{a_4}} \quad (5)$$

best fit the observed kinetic data are calculated as 1.60 × 10^4 M^{-1} sec^{-1} and 6.3 × 10^{-2} M^{-1} sec^{-1}, respectively. The solid line in Figure 1 is theoretical having been derived from eq 5 employing these constants.

Below *ca.* pH 7, aminolysis occurs essentially entirely with species A of the substrate. Then eq 5 reduces to eq 6. The rates of aminolysis of several amines were

$$k_{N_T} = \frac{k_1 K_{a_4} a_H}{(a_H + K_{a_1})(a_H + K_{a_4})} \quad (6)$$

determined in the "plateau" region (pH 4–7). The resultant second-order rate constants (k_1) for the reaction of the free amine with species a are listed in Table I. A Brønsted plot of these data is provided in Figure 2. The solid line drawn through the points for trifluoroethylamine, ethylenediamine monocation, gly-

Table I. Second-Order Rate Constants (k_1) for the Aminolyses[a] of Species a of **8** (30°, μ = 1.0)

Amine	k_1, l. mol^{-1} sec^{-1}	pK_a[b]
Trifluoroethylamine	17.2	5.63
Ethylaminediamine, monocation	565	7.53
Glycine ethyl ester	600	7.75
Glycylglycine	1,290	8.25
Glycine	7,500	9.63
Ethylamine	11,000	10.69
Morpholine	16,000	8.68

[a] Calculated from data obtained in the "plateau" region pH 4–7. [b] At 30°, μ = 1.0 [see T. C. Bruice, A. Donzel, R. W. Hufmann, and A. R. Butler, *J. Amer. Chem. Soc.*, **89**, 2106 (1967); T. C. Bruice, J. J. Bruno and W. S. Chou, *ibid.*, **85**, 1659 (1963); M. J. Gregory and T. C. Bruice, *ibid.*, **89**, 2327 (1967)].

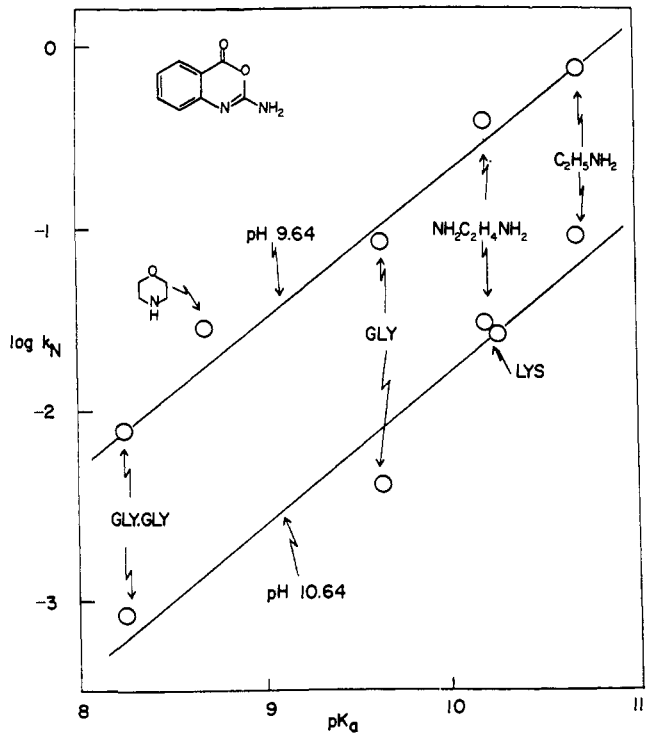


Figure 3. Brønsted plots for rates of reaction of free amine species at pH 9.64 and 10.64 with **8** (second-order rate constants in l. mol^{-1} sec^{-1}; rate constants corrected for small contribution of reaction with **8a**—see text).

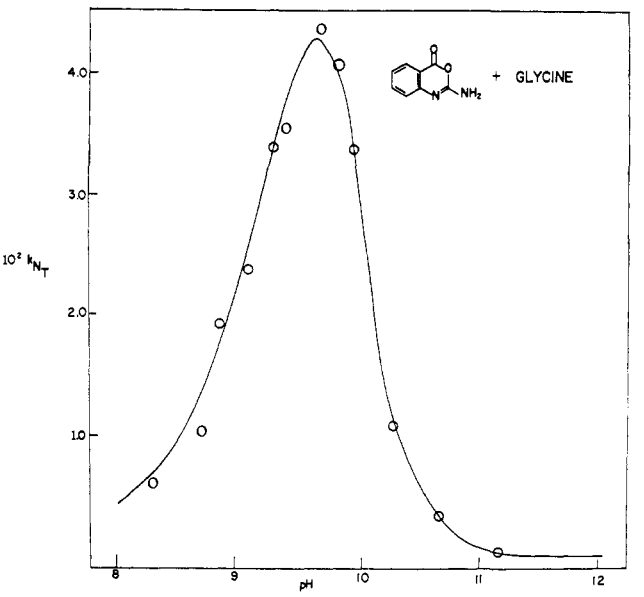


Figure 4. Plot of apparent second-order rate constants (calculated for total amine concentration; l. mol^{-1} sec^{-1}) for reaction of glycine with species of **8** as controlled by pH. Points are experimental and the curve generated from eq 5.

cine ethyl ester, and glycylglycine has slope (β) = 0.75. The more basic amines, glycine and ethylamine show negative deviation and the secondary amine morpholine shows a positive deviation from this line.

The reaction of several amines with **8** was also investigated at pH 9.64 and 10.64. From the observed rate constants the rate constants for reaction with species a (Table I) were subtracted thus providing a set of constants for reaction with any other ionic species of **8** (see Table II; k_N represents the second-order constant

Table II. Second-Order Rate Constants for the Reaction of Unprotonated Amines with Species b of **8**[a]

Amine	pK_a	k_N,[b] M^{-1} sec^{-1} pH = 9.64	pH = 10.64
Glycylglycine	8.25	8.0×10^{-3}	8.2×10^{-4}
Morpholine	8.68	2.4×10^{-2}	
Glycine	9.63	8.8×10^{-2}	3.8×10^{-3}
Ethylenediamine	10.18	3.9×10^{-1}	3.17×10^{-2}
Lysine	10.25		2.80×10^{-2}
Ethylamine	10.69	7.6×10^{-1}	9.3×10^{-3}
Brønsted β		0.82	0.84

[a] 30°; μ = 1.0; pH 9.64 and 10.64. [b] Correction has been made for reaction with protonated 2-amino-4,5-benzo-6-oxo-1,3-oxazine (pK_a = 3.10).

for reaction between free amine and b present at a given pH). Brønsted plots of the log of these rate constants *vs.* the pK of the corresponding amine (Figure 3) are essentially parallel; an average Brønsted β of 0.84 can be calculated for aminolysis of species b.

The reactions of two further amines, glycine and piperidine, were also studied as a function of pH in the pH region where b is the principal reactive species. With morpholine as nucleophile, as a consequence of the positive deviation from the Brønsted plot shown for species a (Figure 2) but not for b (Figure 3), reaction with a was significant at all pH's; the observed rate constants were therefore not highly dependent on the values of K_{a_2} and K_{a_3}. A small difference in the spectrophotometrically and kinetically determined pK_a's might therefore go unnoticed. This difficulty did not arise with glycine. The k_{N_T} values showed a "bell-shaped" dependence on pH (Figure 4). This curve has a maximum at pH 9.63, which is the pK_a of glycine. Consequently if a simple single protonation–deprotonation of the substrate were occurring, this should also have a pK_a at 9.63 [since for a bell-shaped curve, $pH_{max} = {}^1/_2 (pK_{a_1} + pK_{a_2})$].[15] A satisfactory fit of the data in Figure 4 for reaction of glycine with b could not be obtained using eq 7, the "normal" equation for a bell-

$$k_{N_T} = \frac{k_\gamma K_{a_1} a_H}{a_H^2 + K_{a_1} a_H + K_{a_1} K_{a_2}} \qquad (7)$$

shaped profile, with $pK_{a_1} = pK_{a_2} = 9.63$. The reason for this is clear from Figure 5 in which the rate constants for unprotonated amine (k_N) are plotted against pH. The sigmoid curve is not that for a single pK, but for two overlapping pK's with $pK_{a_2} = 10.61$, $pK_{a_3} = 9.21$ (these values are identical with those previously obtained from hydrolytic data[11]). Using these values a best fit of the observed data was sought by variation of k_2. For glycine, $k_2 = 0.12$ M^{-1} sec^{-1} was used in

$$\begin{array}{ccccc} & & K_{a_2} & K_{a_3} & \\ & & -H^+ & -H^+ & \\ b & \rightleftharpoons & c & \rightleftharpoons & d \\ & & +H^+ & +H^+ & \\ & & \downarrow k_2[N] & & \\ & & p & & \end{array}$$

$$k_N = \frac{k_2 a_H^2}{a_H^2 + K_{a_2} a_H + K_{a_2} K_{a_3}} \qquad (8)$$

(15) R. A. Alberty and V. Massy, *Biochem. Biophys. Acta*, **13**, 347 (1954).

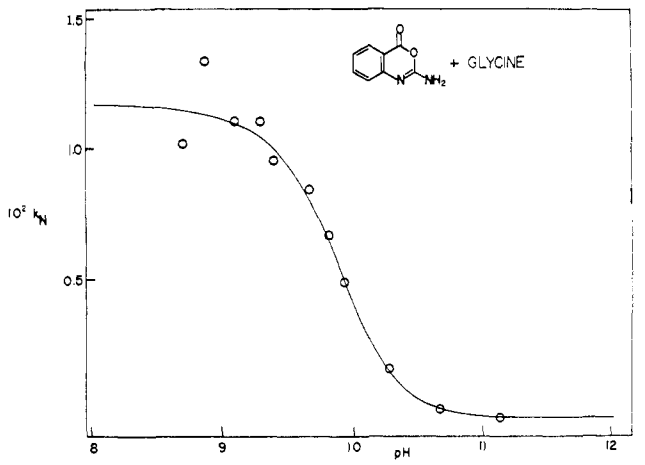


Figure 5. Plot of true second-order rate constant (l. mol^{-1} sec^{-1}) for reaction of glycine free base with species of **8** as controlled by pH. Points are experimental and the line theoretical having been generated from eq 8.

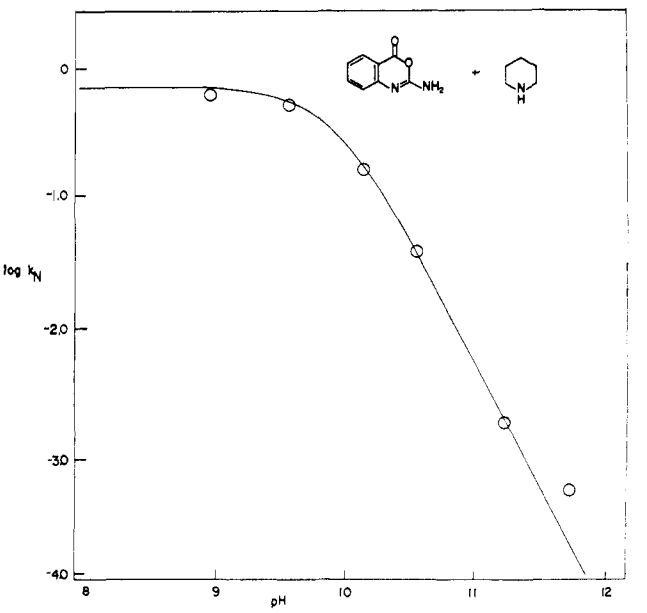


Figure 6. Plot of the log of the true second-order rate constant for reaction of morpholine free base with the species of **8** as controlled by pH. Points are experimental and the curve generated from eq 8.

eq 8 to compute the theoretical plot in Figure 5. This also fits the k_{N_T} values observed (Figure 4), when allowance is made for protonated amine. Below pH 9, the major reaction was with a; therefore at pH's below 9 there is considerable inaccuracy (with consequent scatter observed in Figure 5) in the data for reaction with b.

Equation 8 predicts that at very low a_H values k_N will be proportional to a_H^2, *i.e.*, a plot of log k_N *vs.* pH should have slope −2.0 at high pH. This condition cannot be rigorously tested with glycine since k_N rapidly becomes less than k_{hydr} (the hydrolytic constant) as the pH is raised. A more reactive amine, piperidine, was therefore used and a plot of log k_N *vs.* pH is provided in Figure 6. Clearly, as required by eq 8 (from which the theoretical line was drawn with $k_2 = 0.70$ M^{-1} sec^{-1}), at high pH the rate constants decrease with the square of the hydrogen ion concentration.

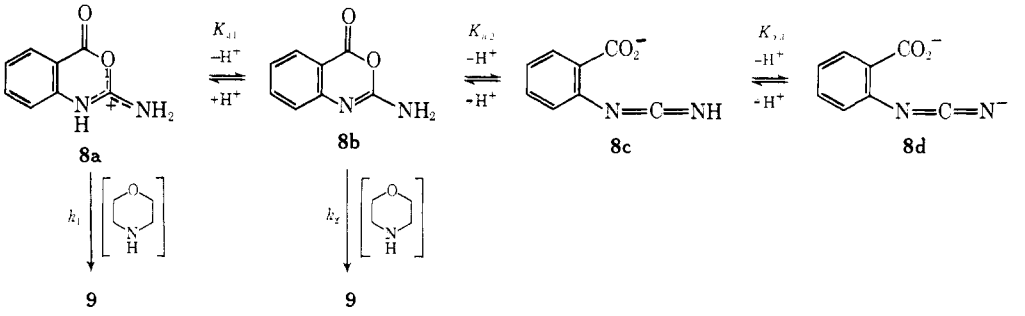
Discussion

The *O*-acylisourea (**8**) has been established, *via* spectrophotometric titration in aqueous solution,[11] to exist in protonated (**8a**), neutral (**8b**), monoanionic (**8c**), and dianionic (**8d**) forms. The determined pK_a values are: **8a** ⇄ **8b** (pK_{a_1} = 3.10); **8b** ⇄ **8c** (pK_{a_2} = 10.61); **8c** ⇄ **8d** (pK_{a_3} = 9.21). Since $pK_{a_2} > pK_{a_3}$ the mole fraction of **8c** never exceeds 10%, regardless of the pH.

If one considers that the substrate (**8**) exists in four forms and the amine in two (protonated and free) then it is obvious that a complex dependency of rate on pH might be anticipated. For the purpose of visualizing the species involved in the aminolysis of **8** the apparent second-order rate constant (k_{N_T}, eq 3 and 5) based on the total concentration of amine as free base and conjugate acid is plotted *vs.* pH (Figure 1). Morpholine was chosen as the amine because reaction of this secondary amine with **8** showed no dependency upon the second power of amine concentration. The reaction sequence of Scheme II is shown to fit the data best.

Reaction of amine with 8a suffices to rationalize k_{N_T} of Figure 1 between pH 1.0 and 8.0. Reaction of amine with both **8a** and **8b** accounts for k_{N_T} between pH 8 and 12. The theoretical line in Figure 1 was computed using the spectrophotometrically determined pK_a's for substrate (*loc. cit.*) and Scheme II. No reaction between the amine and the anionic species **8c** and **8d** was detected. Below pH 3 essentially all of **8** is as **8a** so that further reducing the pH merely decreases the rate by making the concentration of free amine limiting. In the pH region *ca.* 4–7, the rate of morpholinolysis of **8** is essentially independent of pH giving the "plateau" in Figure 1. Since the region lies between (but relatively far from) the pK_a's of both **8a** and the amine (pK_a = 8.68), the major species in solution are **8b** and protonated amine. Increasing the pH by one unit in this region increases the concentration of free amine tenfold but there is no observable change in the

Scheme II



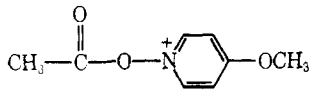
overall rate since the concentration of **8a** decreases tenfold. At pH 7, essentially all **8** is as **8b** and further increase in pH decreases k_{N_T} because (as the pK_a of the amine is approached) **8a** becomes limiting.

The second-order rate constants (k_1) for the reaction of **8a** with the various amines listed in Table I were determined from the observed rate constants in the plateau region (pH 4–7). The more weakly basic amines fit the Brønsted relation of eq 9. The pro-

$$\log k_1 = 0.75pK_a - 3.0 \qquad (9)$$

tonated species **8a** is, therefore, highly reactive toward amines. For example, a 1.0 *M* solution of glycine at pH 3.0 contains only 2.3×10^{-7} *M* free glycine, but $t_{1/2}$ for the glycinolysis of **8a** under these conditions can be calculated as just 12 min.

As the amine base strength is increased, the k_1 values calculated for the amines no longer follow eq 10 and the plot k_1 *vs.* pK_a of the amine shows downward curvature (Figure 2). Jencks and Gilchrist[16] have also recently noted similar curvature in Brønsted plots; it was particularly marked in reactions between the most reactive amines and substrates. In fact, with the most reactive substrate studied, 1-acetoxy-4-methoxypyridinium ion **10**, increase in base strength above pK_a 10 (for primary amines) had little effect on the aminolysis rate. Not surprisingly, **10** and **8a** also show other similarities. Thus the pK_a of the conjugate acid of the



10

leaving groups in **10** and **8a** are *ca.* 2 and 0, respectively, and secondary amines in both cases show significant positive deviation from the Brønsted relationship. In fact, even though both plots of log k_1 for aminolysis of **8a** and **10** *vs.* pK_a of the amine are curved, the individual log k_1 values when plotted against one another (Figure 7) give a straight line, none of the amines seriously deviating. That the slope of this line is greater than (rather than equal to) unity is due to the fact that the curvature in the Brønsted plots occurs at slightly different pK_a for the two substrates.

Several similarities between the aminolyses of **8a** and carbodiimide-mediated amide-formation reactions (Scheme I) are now apparent. The compound **8a** may be considered as a model for the intermediate **2** in the carbodiimide reaction. Similarities in the aminolysis of **8a** and the carbodiimide reaction follow with the appropriate rationale for the reactivity of **8a**: (i) the aminolysis of **8a** may be carried out in aqueous solution because the competing hydrolysis of **8a** is minimal over a large part of the pH range in which the aminolysis reactions are maximal; (ii) a change in the pH of the solution within this region [*ca.* pH 4 to $(pK_{amine} - 1)$] does not affect the rate of aminolysis; (iii) since the Brønsted β value for reaction of free amine with **8a** is 0.75 (see eq 10), it follows that the apparent second-order rate constants, k_{N_T}, for less basic amines are *larger* than those for more basic amines in the plateau region. This is due to the fact that the product of the

(16) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968).

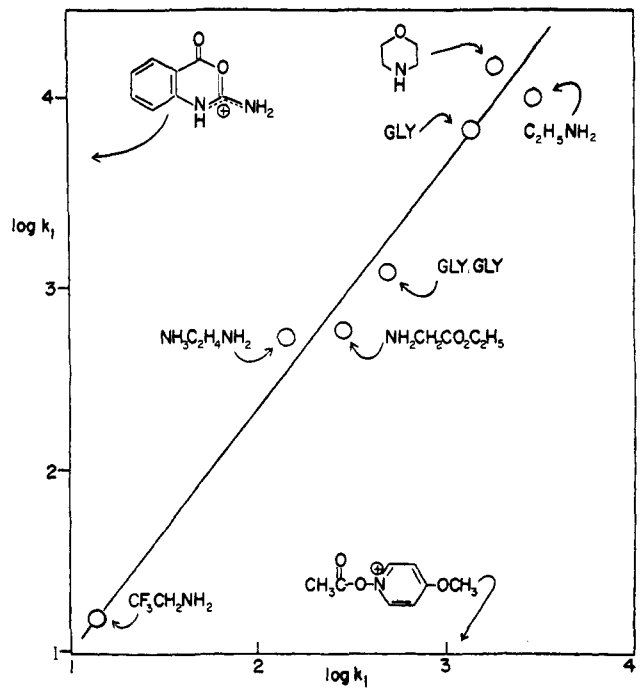
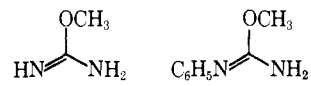


Figure 7. Plot of the log values of the true second-order rate constants (l. mol^{-1} sec^{-1}) for reactions of amines with **8a** *vs.* the corresponding values for aminolysis of 1-acetyoxy-4-methoxypyridinium ion.

concentration of reactant species (*i.e.*, **8a** and free amine base) is greater for an amine of low pK_a and that $\beta <$ 1.0 so that the decreasing concentration of free amine is not compensated by the increase in its nucleophilicity with increasing pK_a. The curved Brønsted plot even accentuates this feature. For example, in a competitive reaction between equal concentrations of ethylamine (pK_a = 10.69) and trifluoroethylamine (pK_a = 5.63) at, say pH = 4.0, 99.5% of the aminolysis product can be calculated to result from trifluoroethylamine attack on **8a**. If amines with pK_a *lower* than **8a** itself were to be used, however, this advantage would be gradually lost so that at pH 4, ethylamine will react with **8a** equally rapidly as will an amine of pK_a *ca.* 0 (and more rapidly than with an amine of even lower pK_a). The efficiency of the *O*-acylisourea intermediate **2** as an acyl transfer agent depends on the extent and position of the plateau region for this compound. The model system **8** is probably less readily protonated than is the intermediate normally occurring in carbodiimide reactions, **2**, where R = cyclohexyl. From consideration of the pK_a's of the conjugate acids of *O*-methylisourea **11** (9.8) and



11 **12**

its *N*-phenyl derivative **12** (7.3), the *O*-acyl group in **8** would appear to have a large effect in reducing basicity (pK_a of **8a** = 3.10). The low basicity of **8a** probably also reflects the possible resonance stabilization of the cyclic free base **8b**. The termination of the plateau region at high pH is normally dictated by the pK_a of the amine used.

Aminolysis of 8b. At pH's 9–11, the concentration of the protonated species **8a** is extremely low and aminoly-

sis of **8b** by the strongest amine bases becomes the major reaction. At pH 10.64 and 9.64, Brønsted plots of the log of the second-order rate constants (k_N) for reaction between **8** and the free amine (Figure 3) are parallel (slope = 0.84). These particular pH values were chosen for study because they are on the ascending and descending limbs of the bell-shaped pH profile which describes the hydrolysis of **8**. That β values are identical at pH 10.64 and 9.64 is anticipated for the proposed[11] hydrolytic scheme in which **8b** is the reactive species in both the ascending and descending portions of the pH–rate profile. Just as the reactivity of the electron deficient positively charged **8a** with amines is similar to that of **10**, the reactivity of the neutral species, **8b**, lies between that of phenyl acetate and *p*-nitrophenylacetate. Note also that morpholine, a secondary amine, fits on the Brønsted plot for the reaction of primary amines with **8b** (Figure 3), whereas in the reaction with **8a** morpholine exhibits a positive deviation from the Brønsted plot for primary amines (Figure 2). Though the reason for this reactivity pattern is not known, it is shared in the reaction of morpholine with phenyl acetate and **10**, respectively.[16,17] The relationship of pK_a of the amine conjugate acid to k_N at pH values 10.64 and 9.64 leads to the expression $\log k_2 = 0.84pK_a - 8.8$.

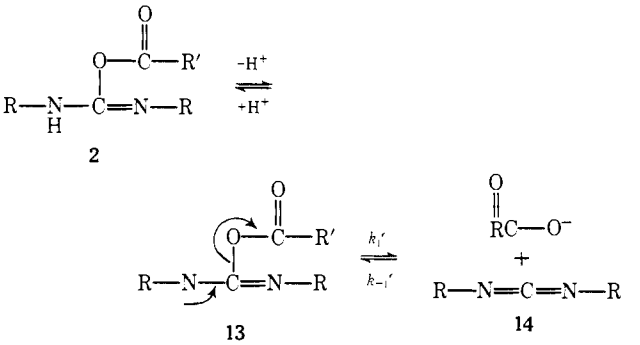
Although the Brønsted plots of k_N in Figure 3 are parallel, the rates of aminolysis at pH 9.64 are *ca.* tenfold greater than those at pH 10.64. In Figure 5 there is plotted the second-order rate constants, k_N, for reaction of glycine free base with total **8** *vs.* pH. Though the plot of Figure 5 is sigmoid, a theoretical fit requires two ionization constants and these prove to be identical with the spectrophotometrically determined[11] values for the ionization of **8b** and **8c**. From the theoretical fit of the points of Figure 5 it is logically deduced that **8b** is the reactive species and that reaction of amine with **8c** and **8d** cannot be detected (eq 8). Note that it was not possible to make such a clear distinction between **8b** and **8c** as the reactive species in the hydrolysis of **8**.[11]

Extra support for the kinetic scheme of eq 8 was obtained with piperidine where at high pH, $k_N \propto a_H^2$ (Figure 6). Though the concentration of **8b** decreases very rapidly beyond pH 10, because $pK_{a_2} > pK_{a_3}$, the aminolysis could be studied over a relatively large pH range due to the great nucleophilicity of this more basic amine ($pK_a = 11.05$).

(17) T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *J. Amer. Chem. Soc.*, **89**, 2106 (1967).

In designing a synthetic reaction one is concerned with the yield of product as a function of pH (*i.e.*, k_{N_T}) rather than the specific rate constant for reaction of any species (k_N, k_1, k_2, etc). The variation of k_{N_T} with pH for glycine is given in Figure 4; the curve shows a maximum at pH 9.63, decreasing at lower pH due to protonation of the amine and at higher pH because **8b** is converted to the inactive **8c** and **8d**. The more basic amine, piperidine, gives a similar type of plot with a maximum at pH 9.9. With the more basic amines, therefore, the most rapid reaction occurs, not in the plateau region (pH 4–7), but *ca.* pH 9.6–9.9. In this latter region reaction of weaker amines, such as trifluoroethylamine, with **8** is negligible. Therefore, in a competitive situation involving a strong and weak base, the product from attack by either can be obtained in high yield merely by choosing the optimal pH for the reaction. At pH 9.9 the hydrolysis of the substrate is, of course, maximal ($t_{1/2}$ is *ca.* 3 min) so that some hydrolysis inevitably accompanies aminolysis at this pH.

It is difficult to predict with certainty how closely the reactions of the *O*-acyl intermediate **2** (where R = cyclohexyl) will parallel those of **8**. Thus, **2** should be less acidic than **8b**, and its pK_a should therefore be >10.61. Moreover the proton loss is reversible from **8b** since both ring opening of the initially formed anion and the retrograde intramolecular addition of the benzoate anion to the carbodiimide are both rapid processes. With **2**, however, dissociation of the anion **13** might be equally rapid (k_1' large) but carboxylate



anion attack on the neutral carbodiimide (an intermolecular reaction) is almost certainly relatively slow (k_{-1}' slow), by comparison with its reaction with hydroxide ion.

Acknowledgment. This work was supported by grants from the National Science Foundation and the National Institutes of Health.