Kinetics of Carbamate Formation and Breakdown[1]

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Abstract: The rate law for reaction of amines with carbon dioxide is rate $= k_{amine}(R_2NH)(CO_2) + k_{amine}'(R_2NH)(OH)(CO_2)$, where the first and second terms are for uncatalyzed and hydroxide-catalyzed pathways. The latter reaction, which involves proton abstraction in the rate-determining step, is not observed with all amines. Values for k_{amine} at 10° follow the Brønsted relationship $\log k_{amine} (M^{-1} \sec^{-1}) = mpK + Y$, with values of m and Y equal to 0.43 and -1.50 for reactions of primary and secondary amines, and 0.48 and -0.20 for the reactions of hydrazine and hydroxylamine derivatives. Second-order rate constants for hydrogen ion catalyzed decarboxylation of carbamates formed from amines of pK -1.05 to approximately 5 may be fitted to a Brønsted relationship $\log k_{H^+} (M^{-1} \sec^{-1}) = 0.77pK + 3.6$ at 10°. Rates for carbamates formed from more basic amines are virtually independent of basicity and are approximately 10^8 M^{-1} $\sec^{-1}$. The rate-limiting step in carbamate formation and breakdown with weakly basic amines involves carbon–nitrogen bond formation and cleavage. It is suggested that proton transfer may be rate limiting in the synthesis and breakdown of carbamates formed from basic amines.

A carbamic acid, N-carboxybiotin, is formed as an intermediate in several enzyme-catalyzed carbon dioxide transfer reactions. To better understand the chemical and kinetic properties of this intermediate we have previously carried out a kinetic analysis of a carboxybiotin analog, N-carboxyimidazolidone.[2] We now report an extension of this work in which carbamate formation and breakdown have been studied with amines with widely varying structure and basicity.

Experimental Section

Methods. Rates of carbamate formation were determined from the product ratio, carbamate–carbonate, formed on bubbling $^{14}CO_2$ into an amine solution maintained at constant pH. Carbon dioxide of high specific activity (1 mCi/mmol) mixed with either argon or nitrogen was delivered to an amine solution maintained at 10° in a Metrohm EA 662 reaction vessel. The pH was measured using a Radiometer 202C electrode and was maintained constant by addition of concentrated base using a Radiometer SBR2/SBU1/TTA31 pH-stat assembly. The validity of pH determinations using the C electrode in highly alkaline solutions containing 1.0 M KCl was checked at 25°. In the pH range 11.2–13.2 this system was found to give readings from 0.04 to 0.11 units higher than those observed with the Radiometer G200B electrode–Radiometer PHM-4-d system. The B electrode, which is subject to smaller salt effects, could not be calibrated at 10°. Reaction mixtures were exposed to the radioactive gas for 30–120 sec depending upon the amine reactivity. During this time interval the amount of carbamate decomposition is small and is of decreasing significance with increasing amine concentration (see eq 4). After flushing the system with nitrogen for 10 sec, a 1-ml aliquot was quenched into 1 ml of a 1 M barium hydroxide solution. An aliquot of the resultant mixture was assayed for radioactivity both before and after filtration through a Millipore filter installed in a Swinny attachment on a hypodermic syringe. Barium carbamates are soluble and are separated from barium carbonate using this technique. Two precautions must be taken in the assay procedure: the barium hydroxide used for quenching must contain at least a visible trace of insoluble carbonate to obtain quantitative removal of radioactive carbonate, and the counting must not be performed for at least 15 min after addition of the quenched reaction mixture to the counting fluid to avoid a transient high background produced by barium hydroxide. Studies of carbamate decomposition were carried out with freshly prepared carbamates made in the manner described above and decomposition was initiated when the carbamate solutions were diluted either five- or tenfold into a buffer solution at the desired pH. Aliquots of these solutions were assayed for remaining carbamate by measurement of the barium hydroxide soluble radioactivity.

Dissociation constants were calculated by the logistic procedure of Reed and Berkson.[3] Concentrations of the conjugate acid–base pair were determined spectrophotometrically with imidazolidone (220 mμ) and thiosemicarbazide (248 mμ) and by potentiometric titration with aziridine. Hydrogen and hydroxide ion activities were determined from the pH and a value of 14.53 for pK_w at 10°.

Materials. Amines were purified by one or more crystallizations or by distillation. The latter procedure was found to be inadequate with triethylamine since the distilled material was found to react with labeled carbon dioxide to give a radioactive barium hydroxide soluble product; purification was, therefore, accomplished by recrystallizing the hydrochloride. No purification was undertaken with either hydrazine or aziridine. The former compound was obtained as a water-white liquid from Fluka and was used directly. Characterization of aziridine, which is highly toxic, was accomplished by measuring the nucleophilic reactivity of this material with *p*-nitrophenyl acetate at pH 9.65 and 30°. The second-order rate constant obtained in these studies equal to 2.0 × 10^3 M^{-1} min^{-1} is in good agreement with the value 1.7 × 10^3 M^{-1} min^{-1} determined previously.[4] Primary and secondary amines formed as hydrolysis or polymerization products could be expected to react more slowly with nitrophenyl acetate.[5] Aziridine is stable at high pH, and the second-order rate constant for reaction with nitrophenyl acetate was unaffected when a concentrated solution of amine was incubated at pH 12 and 0° for 90 min. Titration of aziridine at 25° and ionic strength 1.0 with KCl gave a theoretical titration curve with a pK of 8.16 (lit.[6] pK = 8.01, 30°).

Results

Rates of Aminolysis. The assay used for measurement of the rates of reaction of amines with carbon dioxide is based upon an analysis of the products formed in a competition between hydroxide ion and amine for added carbon dioxide. The product ratio from this competition should be determined by the relationship

$$\frac{\text{carbamate}}{\text{carbonate}} = \frac{k_{amine}(\text{amine})}{k_{OH}(\text{OH})} \qquad (1)$$

From the product ratio, k_{amine} can be calculated by using a value for k_{OH} equal to 2.4 × 10^3 M^{-1} sec^{-1} at 10°.[7] This technique, which was first used in studies of carbon dioxide aminolysis by Faurholt,[8] has been modified

(1) Supported by a grant from the National Institutes of Health (GM 11820).
(2) M. Caplow and M. Yager, *J. Am. Chem. Soc.*, **89**, 4513 (1967).
(3) W. M. Clark, "Oxidation–Reduction Potentials of Organic Systems," Williams and Wilkins Co., Baltimore, Md., 1960, p 149.
(4) Reference *j* in Table I.
(5) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 1778 (1960).
(6) L. R. Fedor, T. C. Bruice, K. L. Kirk, and J. Meinwald, *ibid.*, **88**, 108 (1966).
(7) (a) J. A. Sirs, *Trans. Faraday Soc.*, **54**, 201 (1958). (b) Hydroxide was calculated as activity, which was determined from the pH.
(8) C. Faurholt, *J. Chim. Phys.*, **22**, 1 (1925).

Figure 1. Product ratio obtained in the reaction of hydrazine with carbon dioxide at pH 13.70.

Figure 2. Hydroxide ion dependence of the second-order rate constant for reaction of benzylamine with carbon dioxide.

by the use of isotopic carbon dioxide. This increases the analytical sensitivity over that previously obtainable with a gravimetric assay and the range of experimental conditions for which this technique is applicable is thereby widened. Thus it is possible to study reactions at the very low amine concentrations required for obtaining interpretable results at low pH's (see eq 1). Furthermore, the miniscule consumption of amine during the reaction permits an analysis in which it is assumed that the concomitant hydrolysis and aminolysis at constant pH are pseudo-first-order processes irrespective of the amine concentration. Rate constants for aminolysis have been determined from a graphical representation of eq 1 and the linear relationship between the product ratio and amine concentration predicted therein is illustrated in Figure 1. Equation 1 was found, however, to require an additional term since values of k_{amine} were found to increase with increasing pH. This was interpreted in terms of an hydroxide-catalyzed aminolysis reaction which is incorporated into eq 2. Values of k_{amine} were determined

$$\frac{carbamate}{carbonate} = \frac{k_{amine}(amine) + k_{amine}'(amine)(OH)}{k_{OH}(OH)} \quad (2)$$

at each of several pH's from plots similar to the one shown in Figure 1, and k_{amine} in eq 2 was determined from the intercept in plots of the observed second-order rate constant vs. the hydroxide ion activity; third-order rate constants, k_{amine}, were determined from the slope. Figure 2 shows a plot of the observed second-order rate constant for the reaction of benzylamine with carbon dioxide as a function of the hydroxide ion activity. Values of k_{amine} and k_{amine}' for 15 amines are summarized in Table I. In several cases a k_{amine}' reaction could not be detected because the experimental scatter apparently exceeded the contribution of this reaction to the rate. No attempt was made to detect a term in the rate law for aminolysis proportional to more than the first
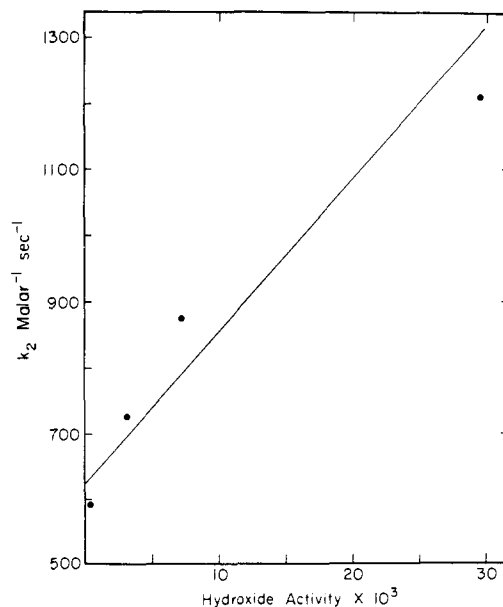
power of the amine concentration. If a reaction of this type occurs it would be expected to be significant at very high amine concentrations where the carbamate–carbonate ratio will be large because of the reaction first order in amine and, therefore, difficult to determine accurately. A slightly different analysis was required for determining k_{amine}' with aniline since the low solubility of this compound prevented studies in which the amine concentration was varied at constant pH except at low pH (10.90). Determination of k_{amine}' for this compound was accomplished by varying the pH from 10.90 to 12.06 at constant (0.2 M) amine concentration and values of k_{amine} and k_{amine}' were determined from the intercept and slope of a linear plot of (carbamate), (carbonate) $\times$ k_{OH} $\times$ (OH) vs. the hydroxide activity. The amount of carbamate formed via the hydroxide-catalyzed pathway is small but the occurrence of this reaction is strongly suggested. For example, from the value of k_{amine} calculated from results obtained at pH 10.90 the calculated yields of carbamate at pH 11.64 and 12.01 are 13.16 and 6.96%, respectively. The values observed at pH 11.64 ± 0.04 in five reactions is 15.1 (standard deviation 1.0) and 8.55% (standard deviation 0.01) at pH 12.01 ± 0.05 in a similar number of experiments.

A Brønsted plot of the rate constants for uncatalyzed aminolysis is given in Figure 3. Separate lines of slope 0.43 and 0.48 have been drawn for the reaction of primary and secondary amines and for the reaction of amines which, because of the α effect, have been found to react as a separate class of compounds in several acyl transfer reactions.[9a] Chipperfield has observed a Brønsted β of 0.262 for reactions of amino acids with carbon dioxide.[9b]

The rate constants given in Figure 3 for imidazole and aziridine are considered to be upper limits for these reactions. The rate constant for the former compound was obtained from studies of the reaction of 1.0 M

(9) (a) T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *J. Am. Chem. Soc.*, **89**, 2106 (1967); (b) J. R. Chipperfield, *Proc. Roy. Soc.* (London), B164, 401 (1966).

Table I. Rates of Reaction of Amines with Carbon Dioxide at 10° [a]

Nucleophile	pH	No. of reactions	Concn, M	k_{amine} (obsd), $M^{-1} sec^{-1}$	k_{amine},[b] $M^{-1} sec^{-1}$	k_{amine},[b] $M^{-2} sec^{-1}$	pK_a[c]
Acetohydrazide[d]	11.10	6	0.02–0.10	22			
	12.00	8	0.10–0.50	64			
	12.40	6	0.10–0.50	128			
					16	15,000	3.24[e]
Aniline	10.90	10	0.018–0.165	2.2			
					2.3[f]	388[f]	4.58[g]
Aziridine	11.10	12	0.1–1.0	2.4	2.4		8.16[f]
Benzylamine	11.02	6	0.0002–0.002	594			
	12.01	6	0.002–0.02	728			
	12.38	6	0.004–0.04	875			
	13.00	8	0.02–0.20	1,210			
					645	20,800	9.37[g]
n-Butylamine	12.01	13	0.0019–0.028	1,290			
	12.81	9	0.01–0.10	1,980			
	13.82	9	0.05–0.50	2,720			
					1,670	5,300	10.61[g]
Glycine	11.02	9	0.0001–0.001	1,120			
	12.54	10	0.005–0.08	1,370			
	12.77	8	0.01–0.15	1,290			
					1,260		9.78[h]
Hydrazine	11.10	3	0.0001–0.0006	2,140			
	12.18	8	0.0015–0.015	3,500			
	12.20	6	0.0003–0.003	3,700			
	12.53	6	0.005–0.03	3,080			
	12.78	6	0.01–0.06	2,940			
	13.70	6	0.02–0.20	5,570			
					3,080[i]		8.20[j]
Hydroxylamine[k]	11.00	12	0.001–0.01	87			
	12.02	17	0.02–0.20	151			
	12.35	12	0.02–0.20	181			
					104	13,000	5.97[l]
Methoxyamine	11.01	15	0.001–0.03	50			
	11.60	14	0.05–0.15	55			
	12.10	6	0.05–0.50	55.4			
					53		4.60[m]
Morpholine	11.06	21	0.00015–0.0025	690			
	11.80	13	0.001–0.015	1,010			
	13.40	10	0.015–0.045	3,110			
	13.64	6	0.05–0.40	3,430			
					800	24,000	8.39[n]
Piperidine	11.88	6	0.0005–0.005	2,080			
	12.05	5	0.0005–0.005	3,500			
	12.97	14	0.0025–0.025	5,820			
	13.01	10	0.003–0.030	8,250			
	13.27	10	0.003–0.030	6,550			
	13.38	6	0.005–0.050	6,360			
	13.72	10	0.025–0.250	10,300			
					3,200	57,000	11.22[o]
Semicarbazide	10.81	9	0.005–0.25	11			
	11.24	15	0.04–0.70	11			
	11.80	9	0.05–0.90	16			
					13		3.65[p]
Thiosemicarbazide	10.43	6	0.005–0.02	7.6	7.6		1.75[f]
Trifluoroethylamine	11.02	13	0.01–0.10	18			
	12.01	8	0.1–1.0	31			
	12.53	6	0.15–1.5	38.9			
					20	22,000	5.63[i,q]
Pyrrolidine	12.01	10	0.00025–0.002	13,100			
	13.00	6	0.003–0.02	15,900			
					12,700		11.27[o]

[a] Reactions carried out in 0.05 M triethylamine, ionic strength 1.0 with KCl. [b] Second- and third-order rate constants defined in the rate law given in eq 2. [c] Dissociation constants are for 25°. [d] Yields of carbamate were unaffected by incubation of stock solutions of amine at high pH indicating that hydrazide hydrolysis is not significant under the reaction conditions. [e] C. R. Lindegren and C. Niemann, *J. Amer. Chem. Soc.*, **71**, 1504 (1949). [f] See text for procedure used for determination of this value. [g] "Handbook of Chemistry and Physics," 44th ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1962. [h] J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958. [i] Estimated by averaging values obtained at pH 11.10–12.78. [j] T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *J. Am. Chem. Soc.*, **89**, 2106 (1967). [k] Reactions run in the presence of 10^{-4} M EDTA. [l] W. P. Jencks, M. Caplow, M. Gilchrist, and R. G. Kallen, *Biochemistry*, **2**, 1313 (1963). [m] T. C. Bissot, R. W. Parry, and D. H. Campbell, *J. Am. Chem. Soc.*, **79**, 796 (1957). [n] A. R. Ingram and W. F. Luder, *ibid.*, **64**, 3043 (1942). [o] S. Searles, M. Tamres, F. Block, and L. A. Quarterman, *ibid.*, **78**, 4917 (1956). [p] W. F. Hall, *ibid.*, **52**, 5115 (1930). [q] Measured at 30°.
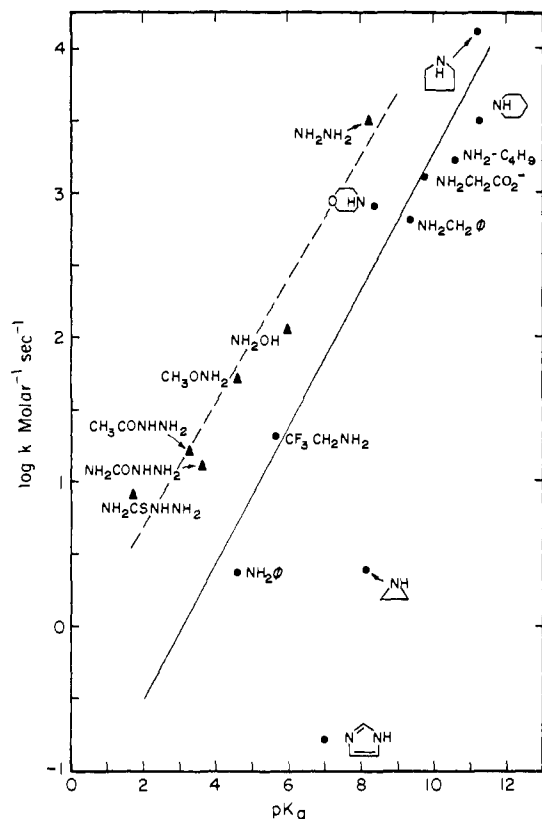
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Figure 3. Brønsted plot for the uncatalyzed reaction of amines with carbon dioxide at 10°.
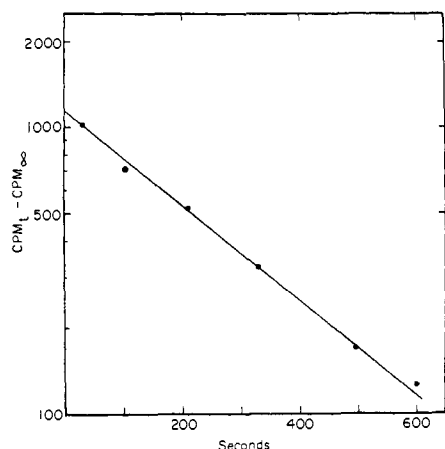

Figure 4. Decarboxylation of morpholine carbamate at pH 10.72.

amine at pH 12.09 where only 1.85% of 80,000 cpm, and at pH 13.43 where 2.65% of 15,000 cpm were found to be barium hydroxide soluble. The rate constants calculated from these results are 0.16 and 5.0 M^{-1} sec^{-1}, respectively, and the former value was included in the Brønsted plot. The rate constant obtained with aziridine is approximately two orders of magnitude below that predicted from the Brønsted relationship and this constant could easily contain a major contribution from the reaction of a primary or secondary amine of comparable basicity present as a 1 or 2% impurity.

Decarboxylation of Carbamates. A reaction scheme for decomposition of carbamates is given in eq 3.[10]

(10) Loss of carbon dioxide is assumed to proceed exclusively via hydration rather than escape to the atmosphere. Two pathways are
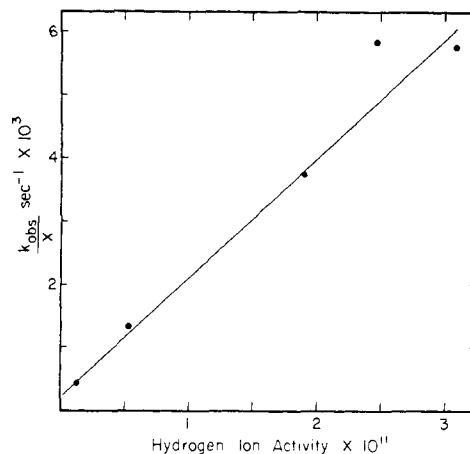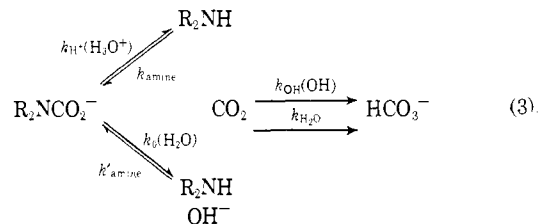

Figure 5. Depencence of the corrected first-order rate constant for decarboxylation of morpholine carbamate on the hydrogen ion activity.

Decarboxylation proceeds via acid-catalyzed and uncatalyzed pathways, which correspond, respectively, to the reverse of the uncatalyzed and hydroxide ion catalyzed reactions observed in carbamate synthesis. Both

pathways for decarboxylation have been observed with carboxyimidazolidone.[2] Assuming a steady-state concentration for carbon dioxide, the observed rate constant for decarboxylation is

$$k_{\text{obsd}} = X[k_{\text{H}^+}(\text{H}_3\text{O}^+) + k_0] \tag{4}$$

where X is equal to $[k_{\text{OH}}(\text{OH}) + k_{\text{H}_2\text{O}}]/[k_{\text{OH}}(\text{OH}) + k_{\text{H}_2\text{O}} + k_{\text{amine}}(\text{R}_2\text{NH}) + k_{\text{amine}}'(\text{R}_2\text{NH})(\text{OH})]$. The value for X, which corrects for return of carbon dioxide to carbamate, was maintained near unity by working at the lowest possible amine concentration. For the rates reported X was almost always at least 0.80 but was generally in the order of 0.90–0.95. The validity of this correction was confirmed in studies with morpholine carbamate at pH 10.50 with amine concentrations from 3.44×10^{-5} to 1.16×10^{-3} M. Verification was also accomplished under conditions where the neutral hydration reaction predominates using 1.25×10^{-4} to 2.44×10^{-3} M semicarbazide at pH 8.10. In these studies k_{obsd}/X was invariant for each set of reactions, although an approximately fivefold fluctuation in the measured first-order rate constant for decarboxylation was observed. Typical rate data obtained in a study with morpholine carbamate are shown in Figure 4 and the corrected first-order rate constants obtained with this compound are shown to follow eq 4 in Figure 5. Plots

available for hydrolysis, k_{OH} and $k_{\text{H}_2\text{O}}$, each predominating at high and low pH, respectively. A value of $k_{\text{H}_2\text{O}}$ equal to 8.0 and 10^{-3} sec^{-1} at 10° was computed from the energy of activation, which is 19,200 cal mol^{-1} [B. H. Gibbons and J. T. Edsall, *J. Biol. Chem.*, **238**, 3502 (1963)], and a value of $k_{\text{H}_2\text{O}}$ equal to 2.9 × 10^{-3} sec^{-1} measured at 0° [J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. 1, Academic Press, New York, N. Y., 1958, Chapter 10].

of the corrected first-order rate constants vs. hydrogen ion activity generally gave nonzero intercepts, but experimental scatter prevented estimation of k_0 in eq 4. Reproducible rate data could not be obtained in studies of the decomposition of the product of the reaction of hydroxylamine with carbon dioxide despite the inclusion of EDTA in reaction mixtures. Second-order rate constants for hydrogen ion catalysis of decarboxylation are summarized in Table II and a Brønsted plot for this reaction is given in Figure 6. In a study at 25° the rate constant for morpholine carbamate decomposition was found to be increased to 3.73×10^8 M^{-1} sec^{-1}. From this result and those obtained at 10° the calculated values for ΔH^* and ΔS^* are 6.46 kcal/mol and 2.4 eu at 25°.

Table II. Rates of Decarboxylation of Carbamates at 10° [a]

Amine	pK[b]	No. of points run	pH range	k_2, M^{-1} sec^{-1} [c]
Acetohydrazide[d]	3.24	12	7.98–9.11	1.5×10^6
Aniline	4.58	6	10.25–11.18	2.9×10^7
Benzylamine	9.37	5	10.03–11.10	4.2×10^7
n-Butylamine	10.61	7	10.00–11.17	7.9×10^7
Glycine	9.78	6	10.31–11.25	9.0×10^7
Hydrazine	8.20	20	10.25–12.06	1.4×10^8
Imidazolidone[e]	−1.05			1.3×10^3
Methoxyamine	4.60	5	9.98–11.02	5.6×10^7
Morpholine	8.39	8	10.48–11.85	2.0×10^8
Piperidine	11.22	10	11.82–12.94	1.7×10^9
Semicarbazide[d]	3.65	8	8.07–9.70	8.0×10^5
Thiosemicarbazide[f]	1.75	8	6.80–7.90	1.2×10^5
Trifluoroethylamine[d]	5.65	7	8.54–10.50	2.5×10^6
Pyrrolidine	11.27	6	10.18–11.25	7.8×10^7

[a] Ionic strength maintained at 1.0 with KCl, 0.05 M triethylamine buffer unless stated otherwise. [b] See Table I for source of pK data. [c] Second-order rate constant for hydrogen ion catalysis with hydrogen ion determined from the pH and expressed in terms of activity. [d] Reaction carried out in 0.05 M Tris buffer. [e] Calculated from results obtained at 6 and 25°; rates extrapolated to zero buffer concentration.[2] [f] pH maintained with 0.05 M imidazole buffer.

Discussion

Carbamate Formation. Comparison with earlier results[11] for the several compounds which have been studied previously is complicated by the difference in temperature at which the reactions were carried out. We can, nevertheless, ignore this factor and compare the yield of carbamate obtained in earlier experiments with that predicted from the rate data summarized in Table I. For example, Jensen records a 49% yield of carbamate in the reaction of 0.1M n-butylamine in 0.19 M hydroxide at 18°.[11c] The yield calculated by substitution into eq 2 is 37%. Comparable agreement has been observed in comparisons with piperidine, glycine, benzylamine, and aniline. The agreement is not nearly as good in comparisons of the second-order rate constants reported for several of these reactions. Aside from the temperature factor and the differing values assigned to k_{OH}, the lack of agreement is related to the failure of earlier workers to study the reactions over a wide enough pH range to detect the contribution of specific base catalysis for the aminolysis reaction. Such

Figure 6. Dependence of the second-order rate constant for hydrogen ion catalyzed decarboxylation of carbamates on the pK_a of the parent amine. The slope for curves A and B are 0.60 and 0.45, respectively; the slope of the solid line is 0.77 and 0.

catalysis has been demonstrated previously in acyl transfer reactions,[9,12] and has been reported in studies of the reaction of Tris with carbon dioxide.[13] For the latter reaction it was suggested, on the basis of the hydroxide ion dependence of the rate, that the product is an alkyl carbonate. The results reported here indicate that this need not be the case. The involvement of a specific base catalyzed aminolysis reaction is further suggested from studies with carboxyimidazolidone. This substance decarboxylates in an uncatalyzed or solvent-catalyzed reaction involving the anion, as well as by an acid-catalyzed pathway, the former reaction predominating at pH's greater than 9.[2] The reverse of the uncatalyzed reaction, which has not yet been observed, must proceed via an identical pathway involving an anionic transition state with hydroxide functioning as a catalyst in some phase of the reaction.

Hydroxide-catalyzed aminolysis reactions may be described in terms of two kinetically indistinguishable pathways. In one, hydroxide functions as a general base catalyst facilitating proton loss in the rate-limiting transition state. The other involves formation of an amine anion prior to the reaction with carbon dioxide. The respective rate laws for these alternate pathways are

$$\text{rate} = k'(\text{R}_2\text{NH})(\text{OH}^-)(\text{CO}_2) \text{ or } k''(\text{R}_2\text{N}^-)(\text{CO}_2) \quad (5)$$

where k'' equals $k'K_w/K_a$, with K_w and K_a equal to the ion product of water and the amine dissociation con-

(11) (a) A. Jensen, J. B. Jensen, and C. Faurholt, *Acta Chem. Scand.*, **6**, 395 (1956), and references therein; (b) A. Jensen, M. B. Jensen, and C. Faurholt, *ibid.*, **6**, 1073 (1956), and references therein; (c) M. B. Jensen, *ibid.*, **11**, 499 (1957), and references therein.
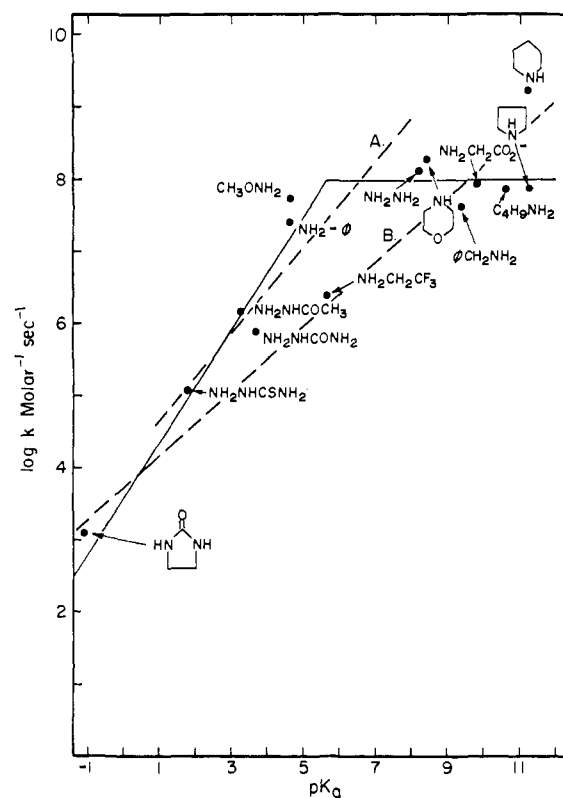
(12) (a) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 675 (1960); (b) J. F. Kirsch and W. P. Jencks, *ibid.*, **86**, 833 (1964).

(13) M. Grønvald, M. B. Jensen, and V. S. Andersen, *Acta Chem. Scand.*, **17**, 2461 (1963).
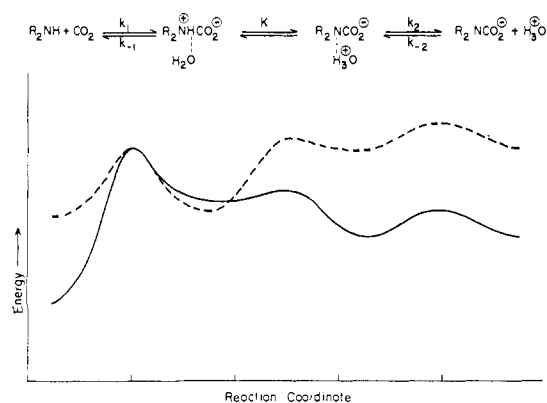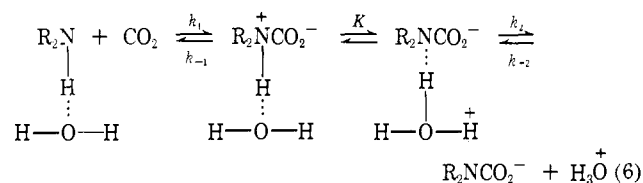
Figure 7. Free-energy diagrams for carbamate formation and breakdown. The solid line is for the case where $k_{-1}K < k_2$, the dotted line for $k_{-1}K > k_2$.

Figure 8. Equilibrium constant for carbamate formation as a function of amine basicity.

stant, respectively. An estimate of the latter for ammonia is 10^{-35}.[14] Using values of the third-order rate constants for hydroxide-catalyzed aminolysis given in Table I (k_{amine}' in this table), along with an estimate of K_a equal to 10^{-25}, gives values for k'' that exceed that of a diffusion-controlled reaction. These calculations indicate that the predominant pathway for hydroxide catalysis involves proton transfer in the rate-limiting step.

The Rate-Limiting Step. A mechanism for carbamate formation and breakdown is outlined in eq 6.

$$\text{R}_2\text{N} + \text{CO}_2 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{R}_2\overset{+}{\text{N}}\text{CO}_2^- \overset{K}{\rightleftarrows} \text{R}_2\text{NCO}_2^- \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}}$$

$$\text{R}_2\text{NCO}_2^- + \text{H}_3\overset{+}{\text{O}} \quad (6)$$

In the direction of synthesis, following carbon–nitrogen bond formation the amine proton is transferred to a water molecule to give charged products which dissociate at a diffusion-limited rate. Proton transfer occurs within a hydrogen-bonded complex and is exceedingly rapid in the direction in which the equilibrium is favorable. Rate laws for reactions under conditions where the reverse reaction is negligible are given in eq 7 and 8, where $K = (\text{R}_2\text{NH}^+\text{CO}_2^- \cdot \cdot \text{H}_2\text{O})/(\text{R}_2\text{NCO}_2^- \cdot \cdot \text{H}_3\text{O}^+)$.

formation

rate = $k_{amine}(\text{R}_2\text{NH})(\text{CO}_2)$;

$$k_{amine} = k_1 k_2/(k_{-1}K + k_2) \quad (7)$$

breakdown

rate = $k_{\text{H}^+}(\text{R}_2\text{NCO}_2^-)(\text{H}^+)$;

$$k_{\text{H}^+} = k_{-2}k_{-1}K/(k_{-1}K + k_2) \quad (8)$$

Free energy profiles for the reaction, given in Figure 7, illustrate that the nature of the rate-limiting step is determined by the relative size of k_2 and $k_{-1}K$. That is, if dissociation of the hydrogen-bonded complex to carbamate and hydronium ion is faster than loss of carbon dioxide ($k_2 > k_{-1}K$) the highest point in the energy profile, i.e., the rate-limiting step, will involve carbon–nitrogen bond formation or cleavage depending on the direction in which the reaction is considered. If the re-

(14) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 88.
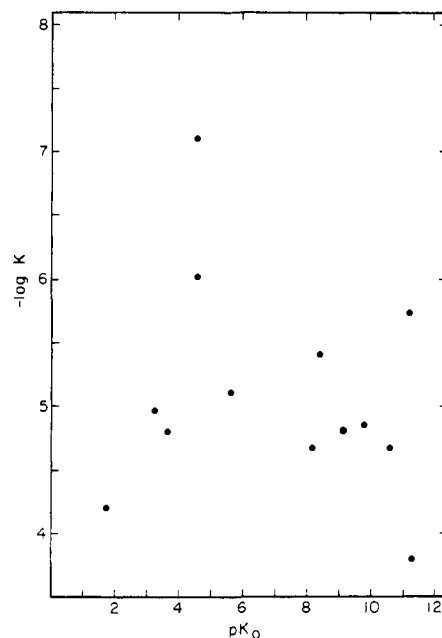
verse case holds and carbon dioxide expulsion is faster than proton loss ($k_2 < k_{-1}K$), the rate-determining step will be loss and addition of a proton in the forward and reverse reactions, respectively. Evidence bearing on this question comes from the dependence of the rate of decarboxylation on amine basicity. The establishment of a Brønsted relationship for this reaction is subject to several sources of uncertainty, which are outlined below, and perfect adherence to this relationship is not expected. First, since the carbamate pK's are unknown it is necessary to assume that the amine and carbamate pK's are proportionally related. Also, in order to encompass an adequate range of basicity it has been necessary to use amines of widely varying structure so that precise correlation of rates with basicity is only expected within a class of related compounds. Finally, an attempted correlation of the amine pK and the equilibrium constant for reaction 9 gives a nonlinear

$$\text{R}_2\text{NH} + \text{CO}_2 \underset{k_{\text{H}^+}}{\overset{k_{amine}}{\rightleftarrows}} \text{R}_2\text{NCO}_2^- + \text{H}^+ \quad (9)$$
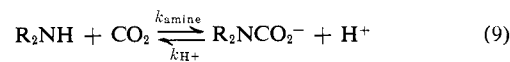
relationship (Figure 8) which suggests that factors other than basicity play a role in the synthesis and breakdown reactions.[15]

The Brønsted plot for hydronium ion catalyzed decarboxylation reveals a complex pattern which may be fitted to two straight lines of slope 0.45 and 0.60, respectively, for reaction of carbamates formed from ordinary amines and amines of the hydroxylamine–hydrazine class. The fit to these lines is poor, especially for ordinary amines where approximately fivefold deviations are observed for four of the nine amines studied. These deviations are very much greater than experimental er-

(15) The equilibrium constant for eq 9, which may be calculated from the ratio of rate constants, reflects the tendency for an amine proton to be replaced by an alternate electrophile. As is the case for replacement of an amine proton by a hydroxymethyl group (R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, **241**, 5864 (1966)) the equilibrium is insensitive to basicity and varies by a factor of only 2060 over a pK range of more than 10 units.

ror. Rates for weakly basic amines ($pK = -1.05-5$) clearly show a greater dependence on basicity than basic amines which suggests an alternate plot, shown in Figure 6, in which the slope is 0.77 for amines of $pK = -1.05-5.6$ and zero for more basic compounds. The results are better correlated with this more complicated relationship, the implication of which are discussed below.

Weakly Basic Amines. Rates of reaction of weakly basic carbamates are clearly dependent on the amine pK. For these reactions the k_2 term predominates in the denominator of eq 7 and 8 so that $k_{H^+} = k_{-2}k_{-1}K/k_2$, and $k_{\text{amine}} = k_1$. Effects of basicity on the breakdown reaction are expected for k_{-1} and K and these effects will be in the opposite direction; increases in basicity will increase K and decrease k_{-1}. The positive slope of the Brønsted plot for decarboxylation indicates that the equilibrium (K) is more sensitive to amine basicity than the breakdown of the zwitterion (k_{-1}). In the synthesis reaction the attack step is rate limiting and, as expected, the reaction is increased by electron donation (Figure 3).

Results obtained with the weakly basic carboxybiotin analog, carboxyimidazolidone, are interpretable in terms of a similar scheme. Decarboxylation of this compound is in general acid catalyzed with a Brønsted α value of 0.9.[2] The dependence of the catalysis on the acid strength of the catalyzing acid is related to the proportionality of the acid dissociation constant and the equilibrium constant for formation of the hydrogen-bonded complex of the conjugate base of the catalyst and the zwitterionic carbamate (eq 6). The observed α for catalysis is close to a theoretical α of 1.0 for equilibrium proton exchange. An alternate scheme for this reaction, as well as those of other carbamates, involves concerted proton transfer and carbon–nitrogen bond formation or cleavage. This may be expressed in terms of the energy diagrams in Figure 7 simply by omission of the energy minima so that the reaction is represented by one or another of two skewed transition states. The problem that we have addressed ourselves to remains the same—does the rate-limiting step principally reflect carbon–nitrogen bond cleavage and formation or proton transfer. There is no conclusive evidence implicating either a stepwise or concerted pathway, and we have presented the former for reasons of clarity. One line of evidence which strongly suggests a transition state or intermediate in which the proton has not been lost at the time the carbon–nitrogen bond has been formed comes from the α value for decarboxylation of carboxyimidazolidone. From the relationship $\beta = 1 - \alpha$, a Brønsted β value equal to 0.1 may be calculated for the general base catalyzed reaction of imidazolidone with carbon dioxide. This constant may be considered to be roughly proportional to the degree of proton loss from the amine in the transition state and the small value for β is, therefore, consistent with a zwitterionic structure. Looking at this another way, the small value for β reflects the fact that the amine proton had been made sufficiently acidic that catalysis directed toward removal of the proton is virtually independent of the catalyst basicity. The high acidity of the proton reflects the extensive carbon–nitrogen bond formation in the transition state. The conclusion for imidazolidone will certainly be valid for more basic amines for which general base

catalysis should be of decreasing significance so that β values will be smaller.

Basic Amines. Results obtained with basic carbamates are difficult to interpret with certainty. A leveling out of the Brønsted plot is suggested from the results and is predicted when rates are equal to the rate of encounter of hydronium ion and carbamate. In terms of eq 8, when increases in basicity increase the equilibrium constant K so that the $k_{-1}K$ term predominates in the denominator, $k_{H^+} = k_2$. The constant k_{-2} is for encounter of hydronium ion and carbamate, and this reaction is expected to be independent of basicity. The principal objections to the assignment $k_{-1}K > k_2$ are that maximal rates are only approximately $10^8\ M^{-1}\ \text{sec}^{-1}$ at $10°$ and the ΔH^* for hydronium ion catalyzed decarboxylation of morpholine carbamate is 6.46 kcal/mol. This is to be compared with the diffusion-limited reaction of hydronium ion with amines which is independent of basicity with rates equal to approximately $10^{10}\ M^{-1}\ \text{sec}^{-1}$ at $25°$.[16] Values for ΔH^* for diffusion-limited proton exchange are 2–4 kcal/mol.[17] There are several factors which may account for these differences. First, we have previously found that hydronium ion is 20 times less reactive than predicted from the Brønsted relationship in the general acid catalyzed decarboxylation of carboxyimidazolidone.[2] Hydronium ion may for some reason react relatively slowly with carbamates. Also, rate constants for proton exchange between ammonium ion and methyl-substituted ammonium ions and their conjugate bases, and phosphate mono- and dianion, which are presumably diffusion-limited processes, are in the range observed for carbamate breakdown.[18] Finally, because of electrostatic attraction and the fact that the carboxyl group is undoubtedly the basic locus of carbamates the initial site of protonation by hydronium ion may be on the carboxyl function (eq 10). If generation of the catalytically active species

$$\tag{10}$$
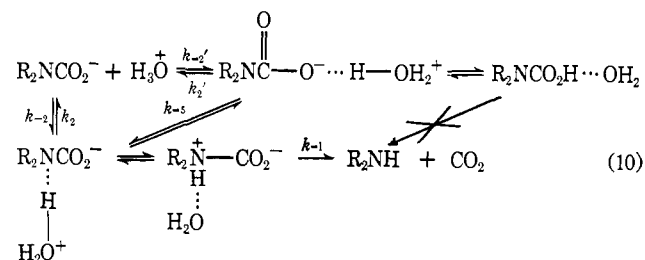
having the proton on the nitrogen atom *via* the k_{-5} path is not faster than dissociation of the hydrogen-bonded complex (k_2'), a significant fraction of encounters between hydronium ion and carbamate may be unsuccessful in giving rise to products.[19a,b] Proton

(16) (a) M. Eigen, *Angew. Chem. Intern. Ed. Engl.*, **3**, 1 (1964); (b) M. Eigen, W. Kruse, G. Maass, and L. De Maeyer, *Progr. Reaction Kinetics*, **2**, 287 (1963).

(17) (a) A. Lowenstein and A. Szöke, *J. Am. Chem. Soc.*, **84**, 1151 (1962); (b) Z. Luz and S. Meiboom, *ibid.*, **86**, 4768 (1964).

(18) (a) E. Grunwald and A. Y. Ku, *ibid.*, **90**, 29 (1968); (b) Z. Luz and S. Meiboom, *ibid.*, **86**, 4764 (1964).

(19) (a) Intramolecular proton transfer to nitrogen without solvent participation has not been included in this scheme. There is no reason to believe that this process will be faster than the solvent-mediated path since direct transfer can be expected to require loss of the water molecule hydrogen bonded to oxygen. Loss of water from a solvent-separated acid–base pair is rate limiting in the nonsolvent-mediated proton transfer between ammonium ions and their conjugate bases (ref 18a). (b) According to this scheme it may be possible, using special techniques, to measure a pK_a for protonation of the carboxyl group of a carbamic acid formed from a basic amine.

transfer to the nitrogen atom in a neutral carbamic acid to give the zwitterion is thermodynamically unfavorable and may, therefore, be relatively slow. In a related reaction, in which the equilibrium constant is 1, the rate constant for proton transfer between oxygen atoms in acetic acid mediated by two solvent water molecules is only 4.8×10^7 M^{-1} at 25.[19c]

Determination of the rate-determining step in carbamate decomposition permits a similar analysis of the synthesis reaction. The reaction of weakly basic amines has been described above. For basic amines the assignment $k_{-1}K > k_2$ predicts an eventual leveling of the Brønsted plot for carbamate formation since an approximate cancellation of effects of basicity is expected in the k_1/k_{-1} and K terms in the reduced form of eq 7, $k_{amine} = k_1 k_2 / k_{-1} K$. A Brønsted β can be obtained for k_1 from the reaction of nonbasic amines with carbon dioxide since for these reactions $k_{amine} = k_1$. The β for K is 1.0 by definition and a β for k_{-1} may be calculated from studies of carbamate decomposition with nonbasic amines for which $k_{H^+} = k_{-1} K k_{-2}/k_2$. It is expected that k_{-2}/k_2 is independent of basicity. Uncertainty in assigning values to β in different portions of the Brønsted plots in Figures 3 and 6 prevents a quantitative estimate of the slope of the Brønsted plot in the region in which a leveling is predicted. The Brønsted plot shown in Figure 3 shows no clear indication of a leveling, although the position of the points for aniline, trifluoroethylamine, and morpholine, and perhaps imidazole and aziridine, suggest that the reactions of weakly basic amines have a greater dependence on basicity than more basic compounds.

The assignment $k_{-1}K > k_2$ requires that in the reaction of basic amines with carbon dioxide the rate-limiting step is loss of a proton from the zwitterionic intermediate. This is not altogether unexpected in the light of results obtained in studies of the reaction of N-methylhydroxylamine with p-chlorobenzaldehyde.[20] In this reaction it has been calculated that loss of a proton from a zwitterionic carbinolamine intermediate would not be adequately fast to account for the observed rates were it not for the intervention of a special pathway in which there is an intramolecular proton transfer to the alkoxide function. A similar mechanism apparently holds in the hydration of acetaldehyde.[21] The reaction of amines with carbon dioxide differs from these reactions in that the formation of a basic center does not accompany amine attack and as a result, loss of carbon dioxide from the zwitterionic carbamate may be faster than deprotonation. This has precedent in the reaction of thiol anions with acetaldehyde where breakdown of a hemithioacetal anion to starting materials is faster than protonation of the intermediate by solvent.[22] Finally, a direct comparison may be made between the rates of proton and carbon dioxide loss from an identical base (hydroxide ion) by comparing the rates of ionization of water and decarboxylation of bicarbonate ion. The observed rate constant for the former reaction, which is a complex constant reflecting an unfavorable equilibrium prior to the rate-determining step, is 2.5×10^{-5} sec^{-1}.[16b] The rate constant for bicarbonate decomposition is 1.9×10^{-4} sec^{-1}.[23]

Hydrogen ion catalyzed decarboxylation of carbamates is kinetically indistinguishable from a mechanism in which proton addition occurs in a rapid equilibrium with water functioning as the proton donor. The pH

$$R_2NCO_2^- + H_2O \overset{\text{fast}}{\rightleftharpoons} R_2NH^+CO_2^- +$$

$$OH^- \underset{\text{limiting}}{\overset{\text{rate}}{\rightleftharpoons}} R_2NH + CO_2 \quad (11)$$

dependence of this process is similar to that for hydronium-catalyzed reaction since the position of the equilibrium for protonation is displaced to the right with decreasing pH and the concomitant increase in the concentration of the component involved in the rate-determining step will be reflected in the rate. This mechanism is ruled out for carboxyimidazolidone since the reaction is general acid catalyzed.[2] The correlation of the value of k_{H^+} obtained with this compound to the Brønsted relationship for hydronium ion catalyzed decarboxylation suggests a similar mechanism for other carbamates.

The mechanism described in eq 11 is not consistent with a biphasic Brønsted plot for carbamic decarboxylation. The linear dependence of the rates on the hydrogen ion activity rules out an interpretation in which it is assumed that basic carbamates are fully protonated and, therefore, decompose at nearly identical rates.

Structure–Reactivity Effects. Several significant deviations are observed in the Brønsted plot for carbamate formation. Although these deviations may be associated with curvature in the Brønsted plot, other factors may be involved. The reactivity of aziridine is low, which is surprising since this substance is unusually reactive in displacement reactions with esters.[6] Low reactivity is also observed with aniline and imidazole and this is reflected in the markedly low equilibrium constant for aniline carbamate formation (Figure 8). The low reactivity of aniline may be caused by electron withdrawal by the carboxyl function preventing resonance involving the nitrogen atom's unshared electron pair and the benzene ring. This effect may not be compensated for by amide resonance with the carboxyl group; a similar factor may account for the instability of anilides as compared with amides.[24] The destabilization of aniline carbamate relative to aniline should also be reflected in the breakdown reaction since this factor will be of importance, although not necessarily to the same extent, for the reaction in both directions. Although the reactivity of aniline carbamate does not appear to be remarkable from inspection of the Brønsted plot, it is reasonable to expect that as in the synthesis reaction separate Brønsted relationships will describe the reactions of ordinary amines and amines of the hydroxylamine–hydrazine class. From the position of the point for trifluoroethylamine, which is the only other nonbasic ordinary amine studied, it appears that aniline carbamate is considerably more reactive than predicted from its basicity. Similar factors to those described for aniline may possibly account for the low re-
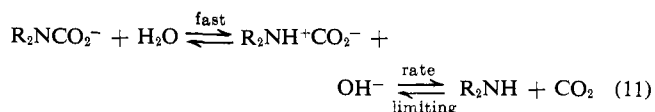
(19) (c) S. Luz and S. Meiboom J. Am. Chem. Soc., **85**, 3923 (1963).
(20) J. E. Reimann and W. P. Jencks, ibid., **88**, 3993 (1966).
(21) M. Eigen, Discussions Faraday Soc., **39**, 7 (1965).
(22) R. Barnett and W. P. Jencks, J. Am. Chem. Soc., **89**, 5963 (1967).

(23) See citation to B. H. Gibbons and J. T. Edsall in ref 10.
(24) (a) I. Öney and M. Caplow, J. Am. Chem. Soc., **89**, 6972 (1967); (b) ref l in Table I.

activity of imidazole. The transition state for imidazole carbamate formation can be expected to be destabilized by loss of the aromatic resonance involving the nitrogen atom's unshared electron pair.

The Acid-Catalyzed Hydrolysis of Acyl Phosphates

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Contribution from the Department of Biochemistry, University of Southern California, Los Angeles, California 90033. Received March 29, 1968

Abstract: The rates of acid-catalyzed hydrolysis of a series of aliphatic acyl phosphates with varying steric bulk in the acyl group have been measured in water at 25°. At constant ionic strength, plots of log k_{obsd} vs. log HCl concentration, with acid concentration increasing to 4.80 M, are linear with slopes deviating slightly from 1.0. At high ionic strength an abnormal order of reactivity is observed, the 3,3-dimethylbutyryl derivative hydrolyzing faster than the other compounds in the series. There is a large positive ionic strength effect with each compound and 3,3-dimethylbutyryl phosphate is affected to the greatest extent. The second-order rate constant for the acid-catalyzed reaction independent of ionic strength effects for 3,3-dimethylbutyryl phosphate is only slightly less than that for acetyl and much larger than that for trimethylacetyl phosphate. Plots of log $(k_{obsd} + H_0)$ vs. log $(C_H + H_0)$ have linear slopes of $+0.31$ for acetyl phosphate and -0.33 for 3,3-dimethylbutyryl phosphate, while trimethylacetyl phosphate yielded a curved line. Values of k_{D_2O}/k_{H_2O} are between 2.2 and 2.6 for all compounds. The ΔS^* for 3,3-dimethylbutyryl phosphate ($+0.6$ eu) is more positive than that for acetyl phosphate (-5.1 eu) or trimethylacetyl phosphate (-9.7 eu). Cleavage of the C–O bond was demonstrated for the 3,3-dimethylbutyryl derivative. The rate constants for acid-catalyzed hydrolysis of substituted benzoyl phosphates in 25% dioxane–H_2O at 25° show only a small sensitivity to electronic effects ($\rho = -0.24$) indicating an A2 mechanism for acyl phosphate hydrolysis. Thus with the aliphatic acyl phosphates, there are very likely differences in the importance of bond breaking in the transition state or differences in ground-state and transition-state hydration.

Water-structuring effects have been postulated to be important in determining protein behavior[2] and could explain in part the efficiency of enzymatic catalysis. The observation of effects due to structured water in hydrolytic reactions in liquid water is, therefore, of considerable importance. Alkyl groups will cause water around a molecule to be more highly ordered.[2] Such effects were previously suggested to explain the pronounced differences in the hydrolysis of N-3,3-dimethylbutyrylimidazolium ion and less highly branched N-acylimidazolium ions in moderately concentrated HCl solutions.[3] It was thought that the acid-catalyzed hydrolysis of highly branched acyl phosphates might prove to be of interest in this regard since compounds of this general type are of great biochemical importance. Therefore, the rates of hydrolysis of a series of acyl phosphates with varying steric bulk in the acyl group have been studied as a function of acid concentration and temperature. Although mechanistic studies have been carried out on acyl phosphate monoanion and dianion reactions[4] very little is known about the acid-catalyzed reaction.

Experimental Section

Materials. Dilithium acetyl phosphate was purchased from Cal Biochem Corp. and was used without further purification. All of the remaining aliphatic acyl phosphates were prepared by the method of Lipmann and Tuttle,[5] and isolated as the disodium salts. Infrared spectra of all the compounds were characteristic of acyl phosphates.[6] Thin layer chromatography using 60% isopropyl alcohol–water as the solvent showed the compounds to contain a trace of inorganic phosphate. The compounds were purified for elemental analysis through isolation as the disilver salts and gave the results in Table I.

Dilithium p-nitrobenzoyl phosphate was also prepared by the method of Lipmann and Tuttle[5] as modified by Ramponi, et al.[7] The remaining benzoyl phosphates were synthesized by the procedure of Avison[8] with the modifications of Di Sabato and Jencks.[4] The barium salts were converted into the disodium salts by stirring in water with an excess of sodium sulfate, and isolation was by precipitation from a water–ethanol–acetone solution. The acyl phosphates were stored in a desiccator at $-4°$, and fresh samples were prepared periodically.

Dioxane was purified by the method of Fieser[9] and was stored frozen. Deuterium oxide (99.8%) was obtained from Bio-Rad Laboratories. The remainder of the chemicals were reagent grade.

Kinetic Measurements. The hydroxamic acid assay was used exclusively for the kinetic runs. The neutral technique as described by Di Sabato and Jencks[10] was used for the aliphatic phosphates.

(1) This study represents part of the work to be submitted by D. R. Phillips in partial fulfillment of the requirements for the Ph.D. degree, University of Southern California, Los Angeles, Calif. 90033.

(2) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, 13, 507 (1945); I. M. Klotz, *Science*, 128, 815 (1958); W. Kauzmann, *Advan. Protein Chem.*, 14, 1 (1959); G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, 36, 3382, 3401 (1962); *J. Phys. Chem.*, 66, 1773 (1962).

(3) J. A. Fee and T. H. Fife, *ibid.*, 70, 3268 (1966).

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(10) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, 83, 4393 (1961).