shown that the conformation of the enzyme at pH 10.5 is greatly different from that at neutrality.[32] The protonated isoleucine amino group is thought to form a salt linkage with aspartic acid-194,[33] thereby influencing the configuration of the active site. Thus, interactions between charged groups have an important influence upon conformation. Unfavorable charge interactions or necessity for movement of the acyl group toward the solvent in the present case could move the acyl group away from histidine-57, and explain the relatively slow first-order rates, the pH independence of the reaction, and the lack of effect of urea.

Conclusions

It is clear that the acyl chymotrypsin derivatives prepared from I and II differ qualitatively in their hydrolytic behavior, and that both show striking mechanistic differences in comparison with normal ester acyl enzymes. The nucleophilic attack by a functional group in the protein, releasing *p*-nitrophenol and giving an inhibited enzyme, that most likely takes place with the initial acyl enzyme formed from bis(4-nitrophenyl) carbonate, cannot be detected in hydrolysis of the acyl enzyme derived from *o*-(4-nitrophenylene) carbonate even though both compounds are reactive nitrophenyl esters. With the acyl enzyme from II, the pH-independent release of 4-nitrocatechol to give an active enyzme must then result from the presence of a neighboring phenoxide ion. Thus, in both cases, the mechanism of the deacylation reaction is dependent on the chemical nature of the acyl enzyme compound.

(32) J. McConn, G. D. Fasman, and G. P. Hess, *J. Mol. Biol.*, **39**, 551 (1969).
(33) P. B. Sigler, D. M. Blow, B. W. Matthews, and R. Henderson, *ibid.*, **35**, 143 (1968).

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Kinetics and Mechanism of Decarboxylation of *N*-Arylcarbamates. Evidence for Kinetically Important Zwitterionic Carbamic Acid Species of Short Lifetime

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Abstract: The rates of decarboxylation of a series of substituted *N*-arylcarbamates are examined as a function of pH and buffer concentration. Specific and general acid catalysis, a water reaction, and an alkaline inhibition, which is not accountable for in terms of the ionic state of the reactants, are found. The latter reaction is explainable only in terms of an unstable zwitterionic form of the carbamic acid as a reaction intermediate. The lifetime of this intermediate is estimated to be in the range of 10^{-8}–10^{-10} sec. The high pH inhibition of decarboxylation shows N–CO_2 cleavage to be rate limiting for carbamates derived from weakly basic amines and proton transfer to the nitrogen atom to be rate limiting for carbamates derived from strongly basic amines. The transition state for decarboxylation has a large amount of unimolecular character with little or no participation of nucleophilic agents. The pK_a of *p*-nitrophenylcarbamic acid is 4.2 and the pK_a for nitrogen protonation of carbamates to form the zwitterionic species is estimated to be about −4.

Only recently has the mechanism of CO_2 transfer reactions been studied with the view of understanding the nature of the transition state(s) involved. Early studies of Faurholt and coworkers[1] have demonstrated the phenomenon of carbamate formation from CO_2 and amines, and over a limited pH range the rate of approach to equilibrium in aqueous solutions containing amines, CO_2, and carbonate has been measured. More recent interest in CO_2 transfer reactions has centered around the nature of the enzyme-bound *N*-carboxybiotin I, which is involved in CO_2 transfer from carbonate to appropriate substrates such as acetylCoA, pyruvate, or urea.[2] The investigations of Caplow[3,4] and of Caplow and Yager[5] have established that carbamates undergo acid-catalyzed decomposition and that carbamates derived from amines with pK_a values less than 5 are dependent upon the pK_a of the parent amine with a β value of +0.77. Carbamates derived from more strongly basic amines decarboxylate at nearly the same rate with a rate constant of $\sim 10^8$ M^{-1} sec^{-1}. In addition, general acid catalysis was found for the decarboxylation of the biotin model *N*-carboxyimidazolidone (α = 0.9) and a striking stabilization of this carbamate was observed with metal ions such as cupric and manganous ions.

In the present investigation we more fully investigate the pH dependence of carbamate decomposition over a very wide pH range, and establish free-energy relationships with a series of compounds more closely allied to each other in structure than those used by Caplow.[4] For this purpose *N*-arylcarbamates were used. Be-

(1) C. Faurholt, *J. Chim. Phys. Physicochim. Biol.*, 22, 1 (1925), and subsequent papers.
(2) For a recent review of the pertinent literature see J. Knappe, *Annu. Rev. Biochem.*, **39**, 757 (1970).
(3) M. Caplow, *J. Amer. Chem. Soc.*, **87**, 5774 (1965).
(4) M. Caplow, *ibid.*, **90**, 6795 (1968).
(5) M. Caplow and M. Yager, *ibid.*, **89**, 4513 (1967).

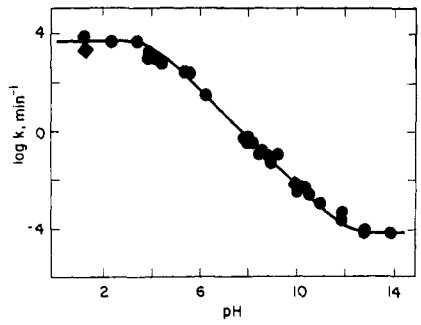


Figure 1. Effect of pH on the rate of decarboxylation of *N*-(*p*-nitrophenyl)carbamate in water at 25°: O, light water; ◊, heavy water.

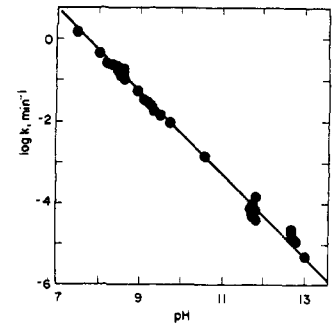


Figure 2. Effect of pH on the rate of decarboxylation of *N*,*N*-diphenylcarbamate in water at 25°.

cause these compounds undergo changes in absorbance in the ultraviolet region during decarboxylation, very small amounts of substrate could be used in the kinetic determinations, making back reactions of negligible importance. Of biological importance is the question of direct CO_2 transfer reactions to and from the carbon atom in carbamates. The possibility of nucleophilic reactions at the carbon atom of the carbamates used here was examined. Also of biological importance is the role of metal ions in mediating the reactions of biotin-containing enzymes. The effects of the metal ions which are effective in stabilizing *N*-carboxyimidazolidone[5] are studied for the *N*-arylcarbamates, and the effects of electrophilic catalysts which are effective in bicarbonate[6] and phosphoramidate[7] decomposition are investigated here.

Experimental Section

Materials. Carbamates derived from primary arylamines were prepared by treating the corresponding Aldrich aromatic isocyanates (all liquids except for *p*-nitrophenyl isocyanate) dissolved in acetonitrile or dioxane with sodium hydroxide solution. The solid which precipitates from these solutions was dried and stored in the freezer until used. Cyclohexylammonium *N*-cyclohexylcarbamate from Fisher Scientific was recrystallized from benzene before use. Sodium *N*,*N*-diphenylcarbamate was prepared by stirring 19 *M* NaOH into a concentrated dioxane solution of *N*,*N*-diphenylcarbamoyl chloride (Fisher Scientific). The precipitate was washed with dilute NaOH and dried. The infrared spectrum of this solid shows the complete absence of the carbonyl stretching frequency of the diphenylcarbamoyl chloride starting material at 1720 cm^{-1}. The carbamate has a low intensity stretching frequency at 1666 cm^{-1}. During the hydrolysis of the aromatic carbamates, the λ_{max} of the carbamate was replaced by λ_{max} in the uv region characteristic of the aromatic amine. In the pH range 10–13 the following spectral changes were observed: *p*-methoxyphenylcarbamate, a band at 237 nm was replaced by a band at 292 nm; *o*-ethoxyphenylcarbamate, 237 nm replaced by 281 nm; phenylcarbamate, 238 nm replaced by 275 nm; *p*-chlorophenylcarbamate, 244 nm replaced by 290 nm; *o*-chlorophenylcarbamate, 248 nm replaced by 280 nm; *p*-nitrophenylcarbamate, 349 nm replaced by 381 nm; *m*-nitrophenylcarbamate, 278 nm replaced by decreased absorbance; diphenylcarbamate, 245 nm replaced by 279 nm. Only decrease in end absorption in the 230-nm region was observed for *N*-cyclohexylcarbamate.

All buffer components are commercial products of reagent grade, with the exception of imidazole, which was recrystallized before use, and *N*-methylimidazole from K and K Laboratories. Deuterium oxide, 99.7%, is from General Dynamics Corporation, Liquid Carbonic Division.

Kinetic Determination. Stock solutions of the *N*-arylcarbamates were prepared in 0.01 *M* NaOH or methanolic 0.01 *M* NaOH before a kinetic experiment. A measured quantity of the stock solution, usually 10 μl, is added with rapid manual mixing to 3 ml of the desired buffer in a cuvette which has been temperature equilibrated.

Absorbance readings were taken at a constant wavelength in the 250–280-nm region with a Beckman DU-2 spectrophotometer which is thermostated at 25.0 ± 0.01°. For *p*-nitrophenylcarbamate, readings are taken at 380–400 nm, and, for *N*-cyclohexylcarbamate, readings are taken at 200–230 nm. For the latter compound and some of the arylcarbamates, a Cary-16 spectrophotometer thermostated at 25.0 ± 0.03 was used to measure the absorbance changes because of the greater sensitivity of this instrument to small changes in absorbance. Plots are made of log $(A_t - A_\infty)$ or log $(A_\infty - A_t)$, where A_∞ and A_t are the infinity time absorbance and the absorbance at time t. A linear relationship is obtained to greater than 90% reaction; the rate constant is the slope × 2.303. Very slow reaction rates of *p*-nitrophenylcarbamate and diphenylcarbamate in NaOH solutions were obtained by placing a portion of the reacting solution in the Cary 16 spectrophotometer for three or more days without disturbing the cell or its contents during the time readings are taken. The end point is obtained by adding a known volume of an appropriate concentration of acetic acid to a known volume of a separate portion of the reacting mixture, and measuring the absorbance. Due to a side reaction of diphenylamine in highly basic solutions it was not possible to follow reactions of diphenylcarbamate in these media. The determination of very large rate constants was accomplished with the aid of a Durrum-Gibson stopped flow spectrophotometer.

To detect buffer catalysis, dilutions were made from a stock buffer solution and KCl was added to maintain a constant ionic strength of 0.6 *M* in most cases. The pH of these buffers was measured with a Radiometer TTT-1 pH meter equipped with a Radiometer PHA-630T scale expander. Values of pH higher than 13 in NaOH solutions were estimated using Harned and Heckers' activity data for NaOH.[8] These values agreed within 0.02 pH unit of the measured pH values using a Radiometer combined electrode GK 2301 B, standardized against Fisher pH 11 buffer and corrected for sodium ion error with the aid of a nomograph supplied with the electrode. The pD values of heavy water solutions were calculated by adding 0.40 to the pH meter readings. A plot of k_{obsd} *vs.* total buffer concentration gives, in general, a straight line, the slope of which is k_{cat} and the intercept is k_{int}. Division of k_{cat} by the fraction of the buffer present in the acid form gives k_{BH}. To correct for pH drift in the buffer dilutions $k_{obsd}/[H^+]$ was plotted against the buffer concentration. The intercept of this plot is k_H and the slope is $k_{cat}/[H^+]$. Multiplication of the slope by the average $[H^+]$ and division by the fraction of the buffer in acid form gives k_{BH}.

Results

The decarboxylations of all the *N*-arylcarbamates examined here involve buffer catalysis. With the exception of certain amine buffers the catalysis is of the general acid type. Extensive buffer studies were carried out for *N*,*N*-diphenylcarbamate and *N*-(*p*-nitrophenyl)-carbamate, the results for which are shown in Tables I and II. Ionic strength effects and possible cosolvent effects of buffer components were checked by adding KCl and dioxane to Tris buffers containing the car-

(6) M. M. Sharma and P. V. Dankwerts, *Trans. Faraday Soc.*, **59**, 386 (1965).

(7) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **86**, 1410 (1964).

(8) H. S. Harned and J. C. Hecker, *ibid.*, **55**, 4838 (1933).

Table I. Kinetic Parameters for the Decomposition of *N*-(*p*-Nitrophenyl)carbamate in Water at 25°

Buffer	pK_a[a]	μ, *M*	Stock buffer composition in *M* basic form/*M* basic form[b]	pH range[i]	k_{cat}, M^{-1} min^{-1}	k_{BH}, M^{-1} min^{-1}
Borate	9.24[k]	0.6	0.10/0.10	9.15-9.08	0.128	0.257
Carbonate	10.33[n]	0.6	0.040/0.40	8.84-8.91	0.190	0.209
Phosphate	7.20[q]	0.6	0.16/0.016	7.52[c]		87
Phosphate	7.20	0.6	0.20/0.005	8.33-8.22	2.52	106
Imidazole	6.99[o]	0.1	1.00/0.10	8.12-8.17	0.500	5.55
Imidazole	6.99	0.6	1.00/0.10	8.27-8.23	0.473	5.17
Triethanolamine	7.76[p]	0.6	0.80/0.40	8.31-8.35	−0.077[d]	−0.23[d]
Tris(hydroxymethyl)-aminomethane	8.07[s]	0.6	0.70/0.30	8.69-8.63	0 ± 0.001	0 ± 0.003
Tris(hydroxymethyl)-aminomethane	8.07[s]	0.6	1.2/0.06	8.68-8.74	0 ± 0.001	0 ± 0.004
Tris(hydroxymethyl)-aminomethane in D_2O		0.6	0.10/0.10	9.97-9.93[j]	0.055	0.11
Dithiothreitol	9.10[e]	0.6	0.05/0.50	8.20-8.18	−0.246[d]	−0.261[d]
Glycylglycine	8.25[r]	0.6	0.60/0.60	8.54-8.41	0.673	1.35
Glycylglycine		0.6	0.40/0.20	8.78-8.72	0.396	1.19
Glycylglycine		0.6	0.50/1.10	9.12-9.04	0.181	1.09
Glycylglycine		0.6	0.56/0.07	9.42-9.32	0.0900	0.810
Butylamine	10.61[u]	0.6	0.06/0.60	9.60-9.70	−0.0066[d]	−0.0073[d]
Butylamine	10.61	0.6	0.16/0.60	10.01-10.08	−0.0019[d]	−0.0024[d]
Acetylacetone	8.95[m]	0.6	0.60/0.60	9.12-9.45	0.145	0.29
Acetylacetone	8.95[m]	0.6	0.30/0.90	8.60-8.43	0.25	0.33
Copper(II) acetylacetone		0.6	0.05 *M* $CuSO_4$ in 0.1 *M* acetylacetone buffer	8.9	0 ± 0.02	0.33
Arsenite	9.22[l]	1.0	1 *M* sodium arsenite	9.86	0.033	0.16
Arsenite			1 *M* sodium arsenite	8.94	0.080	0.15
Copper(II) glycylglycine	9.2[t]	0.6	0.05 *M* $CuSO_4$ in 0.1 *M* glycylglycine buffer	8.21	0 ± 0.02	
Bromine			0.1 *M* Br_2 in 0.15 *M* Na_2CO_3–0.15 *M* $NaHCO_3$	8.91	0 ± 0.02	
Formaldehyde	13.29[f]	0.4	0.1 *M* in 0.05 *M* Na_2CO_3–0.25 *M* $NaHCO_3$	9.04	0 ± 0.02	
Isobutyraldehyde	13.6[g]	0.6	0.11 *M* isobutyraldehyde in 0.15 *M* Na_2CO_3, 0.15 *M* $NaHCO_3$	9.47	−0.13	
Carbonic anhydrase[h]			Veronal, 0.03 *M*	9.12		
Phosphite	6.58[l]		0.1 *M* sodium phosphite	8.62	1.5	160
Chloral	10.04[f]	0.25	0.05 *M* chloral in 0.15 *M* Na_2CO_3–0.15 *M* $NaHCO_3$	9.11-9.53	−0.008 ± 0.003	

[a] pK_a values are for zero ionic strength and 25° unless otherwise noted. [b] The stock buffer and at least four dilutions are used for the determination of the catalytic coefficients. [c] One point only. [d] Linear buffer inhibition. [e] Estimated by potentiometric titration. [f] Aldehyde hydrate ionization: R. P. Bell and D. P. Onwood, *Trans. Faraday Soc.*, **58**, 1557 (1962). [g] Estimated from pK_a of acetaldehyde hydrate ionization; footnote *f*. [h] Worthington carbonic anhydrase (1 mg) in 100 ml of 0.03 *M* veronal buffer. [i] The pH values are listed according to the stock and the most dilute buffer, usually 0.1 or 0.2 dilution. [j] pD = pH meter reading + 0.4. [k] B. B. Owen, *J. Amer. Chem. Soc.*, **56**, 1695 (1934). [l] J. Bjerrum, G. Schwarzenbach, and L. G. Sillen, "Stability Constants of Metal-Ion Complexes, Part II, Inorganic Ligands," Chemical Society, London, 1958. [m] R. G. Pearson and R. L. Dillon, *J. Amer. Chem. Soc.*, **75**, 2439 (1953). [n] J. T. Edsall and J. Wyman, "Biophysical Chemistry," Academic Press, New York, N. Y., 1958. [o] S. P. Datta and A. K. Grzyzbowski, *J. Chem. Soc.*, 136 (1966). [p] R. G. Bates and G. F. Allen, *J. Res. Nat. Bur. Stand., Sect. A*, **64**, 343 (1960). [q] J. J. Christensen and R. M. Izatt, *J. Phys. Chem.*, **66**, 1030 (1962). [r] E. R. B. Smith and P. K. Smith, *J. Biol. Chem.*, **146**, 187 (1942). [s] S. P. Datta, A. K. Grzyzbowski, and B. A. Weston, *J. Chem. Soc.*, 792 (1963). [t] E. Breslow in "The Biochemistry of Copper," Academic Press, New York, N. Y., 1965, p 149. [u] H. C. Brown, D. H. McDaniel, and O. Haffinger in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955.

bamates. For diphenylcarbamate in a 0.2 *M* Tris buffer at pH 8.2 an increase in the ionic strength from 0.1 to 1.75 *M* with KCl results in a linear rate decrease with a slope of -0.6 M^{-1} min^{-1}. The effect of the addition of 1 *M* dioxane in the decarboxylation of *p*-nitrophenylcarbamate in a Tris buffer is to increase the rate by 16%. These results are shown in Table III. Tris buffer was chosen to demonstrate the solvent effects because the Tris buffer components themselves show very little catalytic activity. The pH drift in the buffers due to the added components is corrected for in these buffers by computing the quantity $k/[H^+]$ which in this case represents the catalytic coefficient for the hydronium ion.

Logarithmic plots of the buffer-independent decarboxylation rate constants *vs.* pH shown in Figures 1 and 2 indicate that only a hydrogen ion dependent term is important in the pH range 8–13 for diphenylcarbamate and from 5 to 12 for *p*-nitrophenylcarbamate. The decarboxylation rate of the latter carbamate which was measured below pH 7 becomes pH independent below pH 4 and above pH 13. The rate constant fits the general equation

$$k_{obsd} = \frac{k_0[H^+]}{[H^+] + K_c} + k_w \qquad (1)$$

where k_w is the high pH-independent rate constant, k_0 is the low pH-independent rate constant, and K_c is an apparent ionization constant. The rate levels off with decreasing pH by conversion of the carbamate to a reactive acid form, the apparent K_c of which is $10^{-4.16}$ *M*. The solid line in Figure 2 is calculated from eq 2; the solid line in Figure 1 is calculated from eq 3. On the

$$k_{obsd} = 10^{7.65}[H^+] \text{ min}^{-1} \qquad (2)$$

$$k_{obsd} = \frac{10^{3.66}[H^+]}{[H^-] + 10^{-4.16}} - 10^{-4.12} \text{ min}^{-1} \qquad (3)$$

Table II. Kinetic Parameters for the Decarboxylation of *N,N*-Diphenylcarbamate in Water at 25°

Buffer	pK_a	μ, *M*	Stock buffer composition,[b] *M* basic form/*M* acidic form	pH range	k_{cat}, M^{-1} min^{-1}	k_{BH}, M^{-1} min^{-1}
Ethylenediamine	9.91[c]	0.59	0.59/0.59 (half neutralized 1.28 *M* ethylenediamine)	8.72–8.90	0.0326	0.0652
Glycine	9.78[j]	0.60	0.10/0.90	8.85–8.84	0.0937	0.104
Glycine	9.78	0.60	0.20/0.80	9.20–9.21	0.0514	0.0643
Glycine	9.78	0.60	0.30/0.70	9.41–9.46	0.0358	0.0512
N-Methylimidazole	6.95[i]	0.60	1.70/0.170	8.18–8.28	3.1 ± 0.09	34 ± 10
Butylamine	10.61[k]	0.60	0.40/0.60	10.45–10.60	0.00156 ± 0.0001	0.0026 ± 0.0002
Butylamine	10.61[k]	0.60	0.065/0.60	9.71–9.76	0 ± 0.00003	0.0000 ± 0.0003
Tris(hydroxymethyl)-aminomethane	8.07[d]	0.60	0.219/0.281	8.13–8.23	0.080	0.14
Tris(hydroxymethyl)-aminomethane	8.07[d]	0.60	0.451/0.0482	9.34–9.31	0.012	0.12
Tris(hydroxymethyl)-aminomethane	8.07[d]	0.60	0.67/0.34	8.53–8.57	0.0057	0.017
Tris(hydroxymethyl)-aminomethane	8.07[d]	0.08	0.72/0.08	9.17–9.16	0.024	0.24
Tris(hydroxymethyl)-aminomethane	8.07[d]	0.20	0.40/0.20	8.66–8.51	−0.067	−0.20
Tris(hydroxymethyl)-aminomethane	8.07[d]	0.32	0.32/0.32	8.27–8.31	0 ± 0.001	0.00 ± 0.001
Tris(hydroxymethyl)-aminomethane	8.07[d]	0.14	0.70/0.14	8.97–8.92	0.022	0.13
Triethanolamine	7.76[e]	0.60	0.40/0.20	8.42–8.46	0 ± 0.02	0 ± 0.03
Ammonia	9.25[e]	0.20	0.50/1.50	9.46–9.57	0.059	0.079
Carbonate	10.33[f]	0.975	0.12/0.60	9.26–9.37	0.026	0.079
Carbonate	10.33[f]	0.488	0.375/0.375	9.18–9.28	0.035	0.074
Carbonate	10.33[f]	0.975	0.075/0.75	9.06–9.22	0.0087	0.096
Phosphate	7.20[g]	3.1	1.00 Na_2HPO_4/0.010 NaH_2PO_4	7.45–7.42	2.56	28.3
Imidazole	6.99[h]	0.08	1.00/0.100	8.174–8.145	1.9	21
Hydroxylamine	5.97[l]	0.30	*m*	8.3–7.7	0.00 ± 0.001	0.00 ± 0.001

[a] pK_a values are for zero ionic strength and 25° unless otherwise noted. [b] The stock buffer and at least four dilutions are used for the determination of the catalytic coefficients. [c] J. A. Partridge, J. J. Christensen, and R. M. Izatt, *J. Amer. Chem. Soc.*, **88**, 1649 (1966). [d] Table I, footnote *s*. [e] Table I, footnote *p*. [f] Table I, footnote *n*. [g] Table I, footnote *q*. [h] Table I, footnote *o*. [i] D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solutions," Butterworths, London, 1965. [j] E. J. King, *J. Amer. Chem. Soc.*, **73**, 155 (1951). [k] H. C. Brown, D. H. McDaniel, and O. Haffinger in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955, p 507. [l] H. K. Hall, *J. Amer. Chem. Soc.*, **79**, 5441 (1957). [m] 0.2 *M* hydroxylamine added to Tris buffers.

Table III. Ionic Strength and Solvent Effects in the Decarboxylation of *N*-(*p*-Nitrophenyl)carbamate and *N,N*-Diphenylcarbamate at 25°

Buffer	μ, *M*[a]	pH	k, min^{-1}	$k/[H^+] \times 10^{-7}$, M^{-1} min^{-1}
	p-Nitrophenylcarbamate			
0.2 *M* Tris	0.10	8.143	0.502	6.98
0.2 *M* Tris	0.50	8.188	0.389	6.00
0.2 *M* Tris	0.75	8.224	0.352	5.89
0.2 *M* Tris	1.0	8.310	0.333	6.80
	Diphenylcarbamate			
0.2 *M* Tris	0.10	8.141	0.315	4.36
0.2 *M* Tris	0.50	8.286	0.219	4.23
0.2 *M* Tris	0.75	8.278	0.204	3.87
0.2 *M* Tris	1.0	8.287	0.189	3.66
0.2 *M* Tris	1.5	8.329	0.156	3.32
0.2 *M* Tris	1.75	8.350	0.148	3.32
0.2 *M* Tris	1.75	8.370	0.140	3.29
0.88 *M* Tris	0.08	9.221	0.0467	7.77
0.88 *M* Tris	0.08	9.209	0.0429	6.94
0.88 *M* Tris 0.512 *M* dioxane	0.08	9.217	0.0495	8.19
0.88 *M* Tris 0.512 *M* dioxane	0.08	9.222	0.0517	8.61
0.88 *M* Tris 0.512 *M* dioxane	0.08	9.208	0.0569	9.19

[a] Adjusted with KCl.

hydronium ion catalyzed portion of the pH–rate profile for *p*-nitrophenylcarbamate the solvent deuterium isotope effect $k_H^{H_2O}/k_D^{D_2O}$ is 1.01; on the low pH-independent portion of the pH–rate profile the solvent deuterium isotope effect for k_0 is 3.3. k_H refers to the hydronium ion catalytic coefficient which, according to eq 1, is given by k_0/K_c. The ratio of solvent deuterium isotope effects for k_0/k_H gives the isotope effect for K_c which is 3.3.

Buffer Catalysis. Diverse types of buffers were used with diphenylcarbamate and *p*-nitrophenylcarbamate, including Tris, phosphate, carbonate, acetylacetone, glycylglycine, glycine, dithiothreitol, imidazole, *N*-methylimidazole, triethanolamine, ammonia, and butylamine. In the Brønsted plots of this data in Figure 3 it can be seen that a good correlation is obtained with all the acid catalysts including hydronium ion, with the exception of primary ammonium ions and dithiothreitol (Table I) which exhibit large negative deviations or are inhibitory. In contrast to the results of Caplow and Yager[5] with *N*-carboxyimidazolidone, imidazolium ion is an effective catalyst, as is also *N*-methylimidazolium ion. Brønsted α values of 0.74, 0.76, and 0.78 are obtained for diphenylcarbamate, phenylcarbamate, and *p*-nitrophenylcarbamate, respectively, as shown in Figure 3. Certain catalysts which are very effective in CO_2 hydration–dehydration were also tested. These include arsenite, phosphite, bromine, carbonic anhydrase, copper(II) glycylglycine,[9a] and several alde-

(9) (a) E. Breslow in "Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N. Y., 1965, p

Table IV. Rate of Decarboxylation of Ring-Substituted *N*-Phenylcarbamates and of Cyclohexylamine Carbamate in Water at 25°

Carbamate	pK_a of leaving amine[a]	Buffer[d]	k_{BH}, M^{-1} min^{-1}	$10^{-10}k_H$, M^{-1} min^{-1}	α^b	α^c
Diphenyl-	0.9[i]	*h*	*h*	0.0045	0.76	
p-Nitrophenyl-	1.2	*g*	*g*	0.0078	0.71	
m-Nitrophenyl-	2.45	0.05 *M* $NaHCO_3$/0.025 *M* Na_2CO_3	0.643	0.0396	0.72	
o-Chlorophenyl-	3.32	0.10 *M* Na_3PO_4/0.05 *M* Na_2HPO_4	0.085			
m-Chlorophenyl-	3.32	0.10 *M* $NaHCO_3$/0.02 *M* Na_2CO_3	0.785	0.117	0.76	
p-Chlorophenyl-	3.81	0.10 *M* $NaHCO_3$/0.02 *M* Na_2CO_3	1.09	0.175	0.76	
o-Ethoxyphenyl-	4.47	0.19 *M* Na_2CO_3/0.019 *M* $NaHCO_3$	4.83	0.93	0.76	0.77
o-Methoxyphenyl-	4.49	0.05 *M* Na_3PO_4/0.05 *M* Na_2HPO_4	0.088	2.65		0.85
Phenyl-	4.58	*f*		0.36	0.73	
p-Methoxyphenyl-	5.29	0.05 *M* Na_3PO_4/0.05 *M* Na_2HPO_4	0.131	0.735		0.80
Cyclohexylamine	10.64	NaOH buffers[e]		1.2 ± 0.2		
		0.20 *M* Na_3PO_4/0.15 *M* Na_2HPO_4 μ = 1.8	−0.0015 ± 0.0003	1.2		
		0.60 *M* Na_2CO_3/0.06 *M* $NaHCO_3$ μ = 3.7	0 ± 0.005	0.92		>0.9

[a] From H. A. Sober, Ed., "Chemical Rubber Handbook of Biochemistry," Chemical Rubber Publishing Co., Cleveland, Ohio, 1968. [b] Calculated from the bicarbonate and hydronium ion terms. [c] Calculated from the dianionic phosphate and hydronium ion terms. [d] Unless otherwise noted, the ionic strength is 0.6 *M*. The stock buffer listed here and four dilutions were used to determine the kinetic parameters. [e] NaOH, 0.02–0.5 *M* was used. The pH before and after reaction was determined and the pH drift (less than 0.2 unit) was taken into account in calculating k_H. [f] See Table V. [g] See Table I. [h] See Table II. [i] N. F. Hall, *J. Amer. Chem. Soc.*, **52**, 5115 (1930).

hydes.[8] No extraordinary catalysis with these agents was found. Phosphite and arsenite fit the Brønsted line for acids of their pK_a values. Acetylacetone is plotted both as its carbon acid (pK_a 8.92) and as corrected for its enol form (pK 6.25).[9b]

Amine Buffers. Of the monofunctional amine buffers, Tris and butylamine were studied in detail. With diphenylcarbamate and *p*-nitrophenylcarbamate the catalytic coefficient increases with increasing percentage of the basic form of the buffer, and at low pH the buffers have either no catalytic effect or are inhibitory. Rate constants obtained in highly alkaline solutions containing the amine rule out a significant amine reaction. Plots of the rate data according to eq 4, where

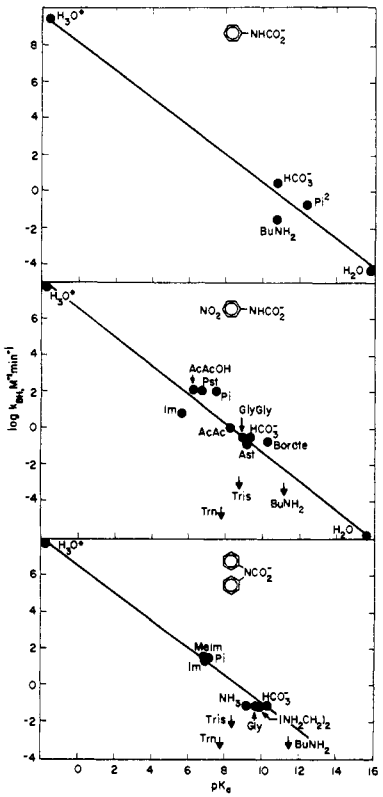


Figure 3. Brønsted plots for the general acid catalytic coefficients for the decarboxylation of *N*-phenylcarbamate, *N*-(*p*-nitrophenyl)-carbamate, and *N*,*N*-diphenylcarbamate in water at 25°: Pst, phosphite; Pi, phosphate; Im, imidazole; AcAc, acetylacetone; AcAcOH, enol form of acetylacetone; Ast, arsenite; Gly, glycine; GlyGly, glycylglycine; Trn, triethanolamine.

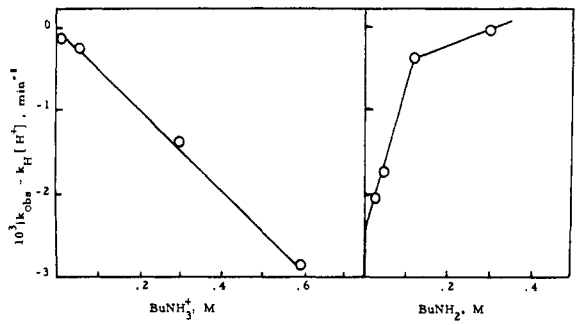


Figure 4. Effect of butylamine and butylammonium ion on the rate of decarboxylation of *N*-(*p*-nitrophenyl)carbamate in water at 25°, μ = 0.6 *M* (KCl): (a) butylamine held constant at 0.06 *M* and butylammonium ion varied; (b) butylammonium ion held constant at 0.03 *M* and butylamine varied.

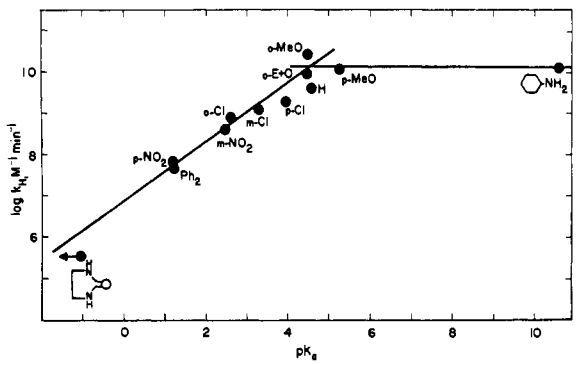


Figure 5. Effect of substituents on the rate of hydronium ior catalyzed decarboxylation of *N*-arylcarbamates in water at 25° Log k_H *vs.* pK_a of leaving amine group.

149; (b) M. Eigen and W. Kruse quoted in *Progr. React. Kinet.*, **2**, 303 (1964).

Table V. Rate of Decarboxylation of *N*-Phenyl- and *N*-*o*-Ethoxyphenylcarbamate at 25°

Buffer[b]	pH[d]	$10^3 k_{int}$, min^{-1}	k_{BH}, M^{-1} min^{-1}
	N-*o*-Ethoxyphenylcarbamate		
0.01 *M* NaOH	11.88	10.5	
0.04 *M* NaOH	12.46	4.47	
0.07 *M* NaOH	12.68	3.30	
0.2 *M* NaOH	13.03	2.42	
0.3 *M* NaOH	13.16	2.02	
0.5 *M* NaOH	13.28	1.41	
0.7 *M* NaOH	13.33	1.10	
1.0 *M* NaOH	13.39	1.01	
2.5 *M* NaOH	14.25[a]	0.227	
0.05 *M* Na_3PO_4/0.05 *M* Na_2HPO_4	11.43	33	0 ± 0.001
0.19 *M* Na_2CO_3/0.019 *M* $NaHCO_3$	10.84	105	4.83
	N-Phenylcarbamate		
0.002 *M* NaOH	11.30	17.3	
0.0040 *M* NaOH	11.81	10.7	
0.0080 *M* NaOH	11.89	6.83	
0.010 *M* NaOH	11.95	5.46	
0.030 *M* NaOH	12.23	2.26	
0.060 *M* NaOH	12.63	1.59	
0.090 *M* NaOH	12.82	1.13	
0.10 *M* NaOH[e]	12.91	1.33	
0.20 *M* NaOH[e]	13.3	1.05	
0.30 *M* NaOH[e]	13.4	0.746	
0.40 *M* NaOH[e]	13.4	0.900	
0.50 *M* NaOH[e]	13.5	0.614	
0.60 *M* NaOH[e]	13.5	0.550	
0.70 *M* NaOH[e]	13.6	0.492	
0.80 *M* NaOH[e]	13.6	0.444	
0.90 *M* NaOH[e]	13.7	0.436	
0.40 *M*/0.04 *M* Na_2CO_3/$NaHCO_3$ μ = 1.2	10.47	226	1.02
0.10 *M*/0.05 *M* Na_3PO_4/$NaHPO_4$ μ = 0.75	11.53	8.4	0.108 ± 0.007
0.408 *M*/0.60 *M* $BuNH_2$/$BuNH_3^+$	10.61	9.75	0.035 ± 0.002
0.18 *M*/0.018 *M* Na_2CO_3/$NaHCO_3$	10.72	60.8	3.69 ± 0.01
0.15 *M*/0.03 *M* Na_2CO_3/$NaHCO_3$	10.53	106	2.45 ± 0.2
0.15 *M*/0.15 *M* Na_2CO_3/$NaHCO_3$ in D_2O	10.39	137	2.42 ± 0.15
0.15 *M*/0.075 *M* Na_2CO_3/$NaHCO_3$ in D_2O	10.67	71.1	1.76 ± 0.08
0.15 *M*/0.05 *M* Na_2CO_3/$NaHCO_3$ in D_2O	10.95	40.6	1.42 ± 0.12
0.15 *M*/0.05 *M* Na_2CO_3/$NaHCO_3$ in D_2O	11.09	27.0	1.42 ± 0.12
0.01 *M* NaOD	12.82	0.781	
0.03 *M* NaOD	13.27	0.462	
0.05 *M* NaOD	13.48	0.386	
0.07 *M* NaOD	13.62	0.355	
0.09 *M* NaOD	13.72	0.345	
0.1 *M* NaOD	13.75	0.315	
0.3 *M* NaOD	14.20	0.238	
0.6 *M* NaOD	14.48	0.207	
0.8 *M* NaOD	14.59	0.175	
0.8 *M* NaOD	14.59	0.217	
1.0 *M* NaOD	14.69	0.116	
0.10 *M* NaOH	12.79	1.20	
0.10 *M* NaOH and 0.20 *M* KCl	12.81	1.20	
0.10 *M* NaOH and 0.40 *M* KCl	12.80	1.18	
0.10 *M* NaOH and 0.60 *M* KCl	12.81	1.20	

[a] Values for pH >13 were calculated from Harned and Heckers' activity data for NaOH,[8] and directly measured as explained in the Experimental Section. [b] Ionic strength 0.6 *M* (KCl) unless otherwise designated. The buffer composition and four dilutions were used to determine k_{BH} and k_{int}. [c] In unbuffered systems, k_{int} refers to k_{obsd}. In buffered systems, k_{int} refers to the intercept values of plots of k_{obsd} *vs.* buffer concentration. [d] Values of pH in D_2O refer to the pH meter reading +0.40. [e] No ionic strength control.

$$k_{obsd} - k_H[H^+] = k_B[B] + k_{BH}[BH] + k_{BHB}[B][BH] \quad (4)$$

either the amine or the ammonium ion of the buffer is kept constant while the other component varies from 0 to 0.7 *M*, give consistent results for dihenylcarbamate decomposition in butylamine buffers, where k_B, k_{BH}, and k_{BHB} are 1.2 × 10^{-3} M^{-1} min^{-1}, 1.3 × 10^{-3} M^{-1} min^{-1}, and 2.8–3.0 × 10^{-3} M^{-2} min^{-1}. The value of k_{BH} is negative for *p*-nitrophenylcarbamate in butylamine buffers and the k_B value depends upon the buffer composition as shown in Figure 4. In Tris buffers the k_B and k_{BH} values are negligible.

Substituent Effects. The effect of substituents in the aromatic ring portion of *N*-arylcarbamates on the value of k_H is shown in Figure 5, where log k_H *vs.* the pK_a of the leaving arylamine is plotted. A linear relation is found for the arylamines with a β value of 0.71. The

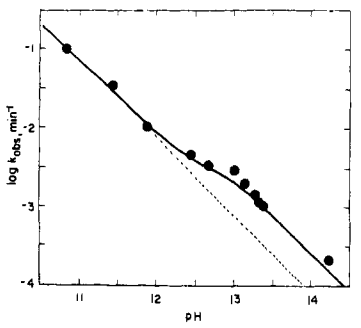


Figure 6. Effect of pH on the rate of decarboxylation of *N*-(*o*-ethoxyphenyl)carbamate as a function of pH at 25° in water. Solid line calculated from eq 5 with $k_H = 0.82 \times 10^{10}$ M^{-1} min^{-1}, $k_w = 1.8 \times 10^{-3}$ min^{-1}, and $K_i = 6 \times 10^{-14}$ M. The dotted line is the rate expected if only the k_H term is present.

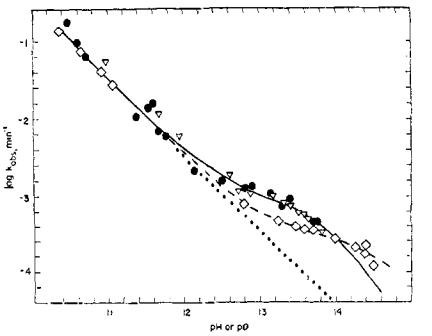


Figure 7. Effect of pH on the rate of decarboxylation of *N*-phenylcarbamate at 25° in water: ○, light water; ◊, heavy water; ▽, data of Christienson; ●, calculated using only k_H in light water. The solid line (H_2O) was calculated from eq 5 with $k_H = 0.36 \times 10^{10}$ M^{-1} min^{-1}, $k_w = 1.0 \times 10^{-3}$ min^{-1}, and $K = 3.2 \times 10^{-14}$ M. The dashed line (D_2O) was calculated from eq 5 with $k_D = 0.35 \times 10^{10}$ M^{-1} min^{-1}, $k_{D_2O} = 0.25 \times 10^{-3}$ min^{-1}, and $K_i = 2 \times 10^{-15}$ M.

k_H value for *N*-carboxyimidazolidone from the data of Caplow and Yager[5] and the k_H value for cyclohexylamine carbamate are included in Figure 5 for comparison. Actually, the pK_a value used for imidazolidone of −0.97 is only the upper limit of the pK_a of the nitrogen atom of the imidazolidone molecule because the compound behaves as an O acid rather than as a N acid.[10] All of the arylcarbamates studied are subject to buffer-catalyzed decarboxylation. The buffer catalysis is fairly constant as the basicity of the leaving amine increases, as shown in Table IV. For example, using the α value calculated from k_H and the bicarbonate catalytic coefficient, α is 0.71, 0.72, 0.76, 0.76, 0.73 in the series *p*-nitro, *m*-nitro, *p*-chloro, *m*-chloro, and H. Similarly α calculated from k_H and the biphosphate catalytic coefficient increases in the order 0.77, 0.81, 0.85, >0.9 for the series *o*-ethoxyl-, *p*-methoxyl-, *o*-methoxyl-, and cyclohexylamine. The large value of α for cyclohexylamine carbamate may not be real because of the difficulty of examining catalysis in the highly basic solutions used for this substrate.

Inhibition of Decarboxylation at High pH. The rate of decarboxylation of phenylcarbamate and *o*-ethoxyphenylcarbamate was studied in the high pH region and the results are presented in Table V and in Figures 6 and 7. At pH values greater than 13 a second hydrogen ion dependent region in the pH–rate curve is apparent. Salt effects were ruled out as the source of the high pH inhibition for phenylcarbamate because of the lack of effect of added KCl up to 0.6 M in 0.10 M NaOH. In the case of phenylcarbamate the alkaline inhibition was previously measured by Christienson.[11] The data in Figures 7 and 8 can be treated according to eq 5 where k_H is the hydronium ion term observed over most of the pH range, k_w is the water term, and K_i is the apparent alkaline inhibition constant. The kinetic parameters which best fit eq 5 are given in Table VI. For phenylcarbamate, a solvent deuterium isotope effect of 1.0 is observed for the k_H term and a value of 4 is observed for k_w. A very large isotope effect of *ca.* 15 is apparent for K_i, but these results are only approximate.

$$k_{obsd} = \frac{k_H[H^+] + k_w}{1 + K_i/[H^+]} \quad (5)$$

(10) G. A. Olah and A. M. White, *J. Amer. Chem. Soc.*, 90, 6087 (1968).

(11) I. Christienson, *Acta Chem. Scand.*, 18, 904 (1964).

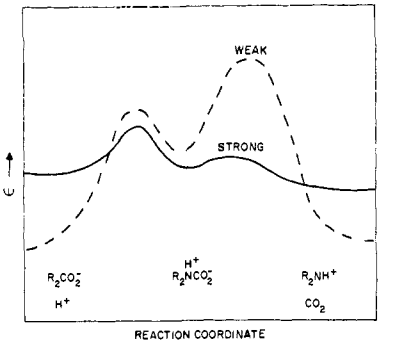


Figure 8. Energy-reaction coordinate for the carbamate + $H^+ \rightleftarrows CO_2$ + amine system: dotted line, weakly basic amine; solid line, strongly basic amine.

Metal Ion and Special Buffer Effects. Cupric ion, 0.01 M at pH 5.7 and 0.1 M at pH 4.2, has a negligible effect on the stability of *p*-nitrophenylcarbamate. This pH range was chosen to maximize the effect of metal ions in complexing with the carbamate in competition with the hydronium ion (pK_a = 4.2). In addition, 0.1 M cupric ion from pH 0 to 3.6 has no effect on the rate of decomposition of *p*-nitrophenylcarbamate. Manganous ion has a similarly negligible effect from pH 1 to 4. Barium and calcium ions, 0.1–0.8 M in Tris buffers, have a small stabilizing effect on both *p*-nitrophenylcarbamate and on diphenylcarbamate which can be accounted for by the increased ionic strength of such solutions. The behavior of *p*-nitrophenylcarbamate and diphenylcarbamate was investigated in the presence of strong carbonyl group nucleophiles. Hydroxylamine, dithiothreitol, hypochlorite, hypobromite, and hydroperoxide (Tables I and II) have little effect on the decarboxylation rate. With the possibility in mind that metal ions might increase the rate of nucleophilic interaction, Ba^{2+}, 0.1–0.8 M, was added to Tris buffers containing diphenylcarbamate and 0.2 M hydroxylamine. Only a decrease in the rate of decarboxylation, attributable to ionic strength effects, was found. Similarly, cupric ions added to buffer solutions of acetylacetone or glycylglycine (Table II) fail to show a rate effect.

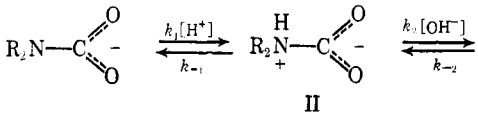
Table VI. Kinetic Parameters for Decarboxylation of Selected Carbamates at 25° in Water

Carbamate	pK_a of leaving amine	pK_c[c]	k_0, min^{-1}	$10^{10}k_H$, M^{-1} min^{-1}	k_w, min^{-1}	K_i
Imidazoline[a]	−0.97			3.5×10^{-5}	2.5×10^{-3}	
Diphenyl-	0.9			0.45×10^{-2}	$<0.56 \times 10^{-5}$	
p-Nitrophenyl-	1.2	4.2	4.6×10^{3}	0.78×10^{-2}	0.31×10^{-5}	$<2 \times 10^{-15}$
Phenyl-	4.62			0.36	1.0×10^{-3}	3.2×10^{-14}
Phenyl- in D_2O				0.35	0.25×10^{-3}	2×10^{-15}
o-Ethoxyphenyl-	4.47			0.82	1.8×10^{-3}	6.0×10^{-14}
Glycylglycine[d]	8.13	5.33	1.32×10^{4}	0.28		
Ammonia[d]	9.25	5.25	3.36×10^{4}	0.60		
Glycine[d]	9.6	5.18	4.98×10^{4}	0.75		
N,N-Diethyldithiocarbamate[e]	10.93	3.37[f]	6.0	7.76×10^{-6} [h]		
Pyrrolidinedithiocarbamate[e]	11.27	3.25[g]	0.021	6.6×10^{-6} [e]		

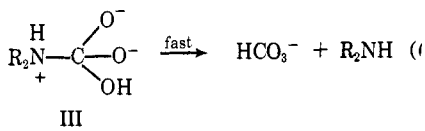
[a] M. Caplow and M. Yager, *J. Amer. Chem. Soc.*, **89**, 4513 (1967). [b] pK_a for O protonation. The p*K* for N protonation would be more negative: see M. Caplow, *Biochemistry*, **8**, 2656 (1969). [c] Kinetically determined p*K* values of the carbamic acid. [d] At 5°; from F. J. W. Roughton and L. Rossi-Bernardi in "CO_2: Chemical, Biochemical and Physiological Aspects," NASA SP-188 Symposium, Haverford, Pa., 1968, p 41. [e] K. I. Aspila, V. S. Sastri, and C. I. Chakrabarti, *Talanta*, **16**, 1099 (1969). [f] K. I. Aspila, S. J. Joris, and C. L. Chakrabarti, *J. Phys. Chem.*, **74**, 3625 (1970). [g] S. J. Joris, K. I. Aspila, and C. L. Chakrabarti, *Anal. Chem.*, **41**, 1441 (1969). [h] Deuterium ion catalysis at 28°, ref 35.

Discussion

pH–Rate Profiles. The inhibition of decarboxylation in pH >13 buffers in the case of phenyl- and *o*-ethoxyphenyl-, but not *p*-nitrophenylcarbamate, is clearly not due to ionization at the NH bond, for the order of the apparent K_i values would be reversed and *p*-nitrophenylcarbamate would be most likely to show the alkaline inhibition. Therefore, the negative deviation of the pH–rate profile is due to a change in mechanism of decarboxylation as a function of pH. The various schemes involving tetrahedral intermediates at the CO_2 level of oxidation, though explaining the observed pH–rate profile, suffer from the fact that the specific rate constants derived from those which we have measured are much too large to account for the formation of tetrahedral intermediates when these rate constants are compared with known rate constants in model reactions, as, for example, the scheme in eq 6, which involves a



II



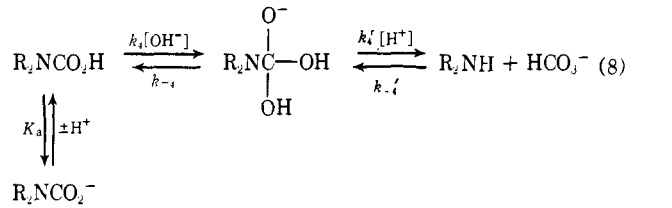
III

hydroxide ion addition to free carbamic acid to form a tetrahedral intermediate III with one negative charge, followed by decarboxylation. According to this scheme the observed rate constant is given by eq 7. The water

$$k_{obsd} = \frac{k_1 k_2 K_w}{k_{-1} + k_2[OH^-]} \quad (7)$$

term is equal to $k_1K_wk_2/k_{-1}$ or $K_wk_2/K_{a\pm}$ where $K_{a\pm}$ is the acid dissociation constant of the zwitterionic form of the carbamic acid and is equal to $[I][H^+]/[II]$ or k_{-1}/k_1. The high pH hydronium ion term is given by k_1, which according to eq 6 represents the specific rate constant for protonation of the carbamate ion on its nitrogen atom. The value of k_1 according to our data is $\sim 10^8$ M^{-1} sec^{-1}, a reasonable value. Using a conservative estimate of 10 for $K_{a\pm}$ the value of k_2 according to our data is 6×10^9 M^{-1} sec^{-1} for *o*-ethoxyphenylcarbamate. This value is clearly too large when compared to the specific rate coefficient of addition of hydroxide ion to *p*-nitrophenyl *N,N*-dimethylcarbamate,[12] 3×10^{-4} M^{-1} sec^{-1}, or *N*-*p*-nitrophenoxycarbonyl-2-imidazolidone,[3] 0.45 M^{-1} sec^{-1}, or to an extreme case of addition to CO_2,[13] 8.5×10^4 M^{-1} sec^{-1}.

Another example is eq 8, in which the acidic form of



the carbamic acid reacts with hydroxide ion to give the monoanionic tetrahedral intermediate which undergoes an elimination of amine from bicarbonate. The observed rate constant for this process is given by eq 9

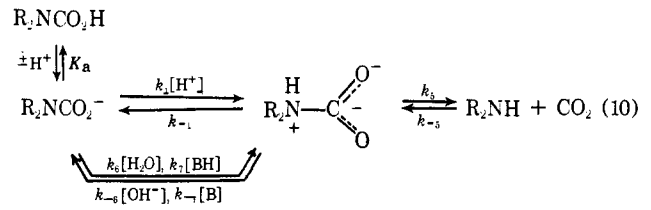
$$k_{obsd} = \frac{k_4 K_w}{K_a k_{-4}/(k_4'[H^+] + 1)} \quad (9)$$

in which the water term is equal to k_4K_w. For *o*-ethoxyphenylcarbamate the calculated value of k_4 is 6×10^9 M^{-1} sec^{-1} which represents the specific rate constant for hydroxide addition to the carbonyl bond. This value is clearly too large when compared to the much smaller rate constant of 3×10^{-1} M^{-1} sec^{-1} for the hydroxide reaction with *p*-nitrophenyl *N,N*-dimethylcarbamate or similar reactions discussed above.

The value of k_w for phenylcarbamate, 1.6×10^{-5} sec^{-1}, is too small for mechanisms involving oxygen protonation of the carbamate anion (p$K \sim 4$) by water (pK 15.8) for which a value of $\sim 10°$ sec^{-1} would be expected.[14]

(12) L. W. Dittert and T. Higuchi, *J. Pharm. Sci.*, 52, 852 (1963).
(13) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958, Chapter 10.
(14) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, 3, 1 (1964).

Equation 10 represents a mechanism which fits all the known facts of carbamate formation and decomposition, and has been previously presented to account for carbamate formation and decomposition.[4] Ac-



cording to this scheme the anionic carbamate is protonated by hydronium ion, water, and general acids to form the zwitterionic form of the carbamic acid, which decomposes to CO_2 and amine. The nature of the rate-limiting step now centers around whether the zwitterionic carbamic acid can lose its proton or its CO_2 group more readily. The observed rate constant is given by eq 11. When making and breaking of the

$$k_{obsd} = \frac{(k_1[H^+] + k_6 + k_7[BH])k_5}{k_{-1} + k_5 + k_{-6}[OH^-] + k_{-7}[B]} \quad (11)$$

N–C bond occurs more readily than making and breaking of the N–H bond, then k_5 will dominate the denominator and at pH values above the acid dissociation constant of the carbamic acid the observed rate constant is given by eq 12, where k_1 represents the value of

$$k_{obsd} = k_1[H^+] + k_6 + k_7[BH] \quad (12)$$

k_H, k_6 represents k_w, and the k_7 terms represent the general acid terms. At very high pH it is possible for the $k_{-6}[OH^-]$ term for the specific base catalyzed removal of a proton from NH_2 to become more important than the k_5 term which represents the spontaneous N–C bond cleavage. Ignoring for the moment the k_7 and k_{-7} buffer terms, the observed rate constant at very high pH is given by eq 13. The rate-determining step has

$$k_{obsd} = \frac{k_5k_6}{k_{-6}[OH^-]} = \frac{k_5[H^+]}{K_{a^\pm}} \quad (13)$$

changed from one of rate-limiting protonation of the carbamate nitrogen at lower pH to a rate-limiting N–C cleavage process at high pH. Expected by this scheme is a rate inhibition at high buffer concentrations and at high pH values where the k_w term dominates. It is possible that the phosphate inhibition of cyclohexylamine carbamate decomposition represents this phenomenon (Table IV).

The values of the equilibrium constant of carbamate formation and the rate constant for the hydroxide ion catalyzed interaction of aniline with CO_2, determined by Caplow at 10°,[4] predict the value of k_w which should be observed for the decarboxylation of phenylcarbamate at 10°. According to eq 10 the rate constant for the hydroxide ion catalyzed interaction of aniline with CO_2 is given by $k_{-5}k_{-6}/k_5$. The equilibrium constant for carbamate formation as defined as [carbamate][H+]/[amine][CO_2] is 0.793×10^{-7} M. Therefore the equilibrium constant as defined as [carbamate]/[OH^-][CO_2]·[amine] is given by $0.793 \times 10^{-7}/K_w$[10] $= 0.27 \times 10^8$ M^{-2} and is equal to $k_{-5}k_{-6}/k_5k_6$. With the value of $k_{-5}k_{-6}/k_5$, which is the k_{OHRNH_2} value given by Caplow, the value of k_6 or of k_w is calculated to be 0.86×10^{-3} min^{-1} at 10°, which is to be compared with the value measured here at 25° of 1.26×10^{-3} min^{-1}. If one assumes an activation energy of 20 kcal/mol[15] for the k_w term, a value of 4.9×10^{-3} min^{-1} is predicted for k_w at 25°. Taking into consideration that the energy of activation may be smaller and the difficulties encountered in obtaining the rate coefficients for the hydroxide-catalyzed amidation of CO_2, the agreement obtained here can be considered to be fair.

Inhibition of decarboxylation at high pH is not observed for diphenylcarbamate and for *p*-nitrophenylcarbamate. This is because the leaving amine group is very weakly basic (p$K \sim 1$) and in this case N–H bond cleavage takes place more readily than N–C bond cleavage, *i.e.*, the zwitterionic form of the carbamate has greater kinetic affinity for CO_2 than for H^+, giving rise to the condition $k_{-1} > k_5$. The k_H value for carbamates derived from very weakly basic amines is given by $k_5/K_{a^\pm}$ and the water term is given by k_5k_6/k_{-1}. At very high pH no inhibition by hydroxide ion can be seen because when the $k_{-6}[OH^-]$ term dominates the denominator the observed rate constant is given by $k_5k_6/k_{-6}[OH^-]$ which is equal to $[H^+]k_5/K_{a^\pm}$ and is identical with the k_H term. It is not possible to observe such a term at a high pH where $k_w > k_H[H^+]$.

The observations here thus confirm the hypothesis of Caplow[4] that the decomposition of carbamates derived from weakly basic amines involves rate-limiting proton transfer while the decomposition of carbamates derived from strongly basic amines involves rate-limiting decarboxylation.

For carbamates derived from strongly basic amines, the value of k_{obsd} at low pH is given by eq 14, where K_a

$$k_{obsd} = \frac{k_1[H^+]}{(1 + [H^+])/K_a} \quad (14)$$

is equal to [H^+] [I]/[HI], so that the ratio of the observed rate constant at very low pH where the reaction is pH independent, k_0, and k_H is K_a. The values of K_a measured kinetically this way are 5.2, 5.1, and 5.3 for carbamates derived from ammonia, glycine, and glycyglycine, respectively, measured at 5°.[16]

The observed rate constant at low pH for the decomposition of carbamates derived from weakly basic amines is given by eq 15. If the oxygen atom of the

$$k_{obsd} = \frac{k_5}{(1 + K_{a^\pm})/K_a + K_{a^\pm}/[H^+]} \quad (15)$$

carbamate anion is more strongly basic than the nitrogen atom, *i.e.*, if $K_{a^\pm} > K_a$, then the k_0/k_H ratio (K_c in eq 11) is equal to K_a. If the opposite situation holds, $K_a > K_{a^\pm}$, the k_0/k_H ratio is equal to $K_{a^\pm}$. With carbamates derived from weakly basic amines there is, therefore, some uncertainty as to the meaning of the apparent pK values obtained by kinetic means. However, we are convinced that $K_{a^\pm}$ is much larger than K_a by the following arguments: the kinetically obtained pK_a value of 4.2 for *p*-nitrophenylcarbamate is about one unit lower than the corresponding values for carbamates derived from ammonia and aliphatic amines. This suggests that the oxygen atom in the anionic

(15) In analogy with the 23 kcal/mol activation energy for the k_w term of *N*-carboxyimidazolidone, measured by Caplow and Yager.[5]

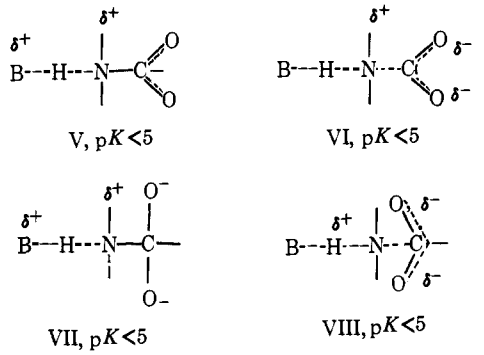
(16) F. J. W. Roughton and L. Rossi-Bernardi in "CO_2: Chemical, Biochemical, and Physiological Aspects," NASA Symposium SP-188, Haverford Pa., 1968, p 41.

carbamate is more strongly basic than the nitrogen atom, because larger substituent effects on the pK_a would be expected if N-protonation occurred. The pK_a difference between *p*-nitroaniline and ammonia is 8 units and a similarly large pK_a difference would be expected in the corresponding carbamates. In fact, the pK_a difference of *p*-nitrophenylcarbamic acid and carbamic acid is identical with that obtained in the corresponding carbon series. *p*-Nitrophenylacetic acid[17] has a pK_a value of 3.85 which is 0.9 pK_a unit smaller than that for acetic acid. Furthermore, the difference in the kinetically obtained pK_a values of carbamic acids and the dithiocarbamic acids of $\sim$2 pK_a units[18] is similar to the difference in the pK_a values obtained for acetic acid *vs.* thioacetic acid (4.8 *vs.* 3.2) or 1.6 units.[19] In comparing the pK_a value of 3.76 for carbonic acid[13] with 5.25 for carbamic acid the amino group replacement for a hydroxyl group increases the basicity of the adjacent oxygen, indicating a significant resonance interaction of the nitrogen atom; inductive effects would predict the opposite result.

From the above data and arguments, as well as from those arguments made by Caplow,[4] the rate-controlling step for the decarboxylation of carbamates derived from amines with pK_a values greater than $\sim$5–6 is nitrogen protonation. Carbamates derived from these more basic amines decarboxylate with the same specific rate constant[1,4] of $\sim 10^8\ M^{-1}\ sec^{-1}$, making it possible to calculate the basicity of the nitrogen atom of these carbamates using the known relationship between the rate of proton transfer and the pK_a difference between the donor and acceptor species.[14] Since diffusion-controlled protonation in the thermodynamically controlled direction occurs with a specific rate constant of $\sim 10^{10}\ M^{-1}\ sec^{-1}$, a specific rate constant of $10^8\ M^{-1}\ sec^{-1}$ for carbamate protonation means that the pK_a of the carbamate is $\sim$2 units smaller than the pK_a of hydronium ion which is -1.8. Therefore, the pK_a of the carbamate nitrogen is estimated to be ~ -4. The pK_a of the nitrogen atom in carboxylic acid amides and carbamic acid esters is less than 0 to -2 and -2 to -3, respectively, which is the pK due to oxygen protonation.[20] The estimated pK of the nitrogen in carbamate ions is more in line with the pK value of ~ -4 reported[21] for nitrogen protonation of *N,N*-diisopropylcarbamic acid.

Substituent Effects. The structural effects on the carbamate stability are shown in Figure 5, where the slope of the line drawn through the arylamine substituents is 0.71. This value is similar to that of 0.77 found by Caplow[4] for amine substituents with a great deal of structure variation, and therefore scatter. The linear dependence on pK_a for bases of pK up to 5 and the leveling off at high pK were found by Caplow[4] and are indicated here by the point for cyclohexylamine in Figure 5. This break is indicative of a change in rate-determining step in the decarboxylation reaction. The energy profile in Figure 8 depicts the deductions made above. The ground-state energy levels in both reactants and products are lowered for weakly basic amines compared with strongly basic amines, and the reverse situation is shown for the zwitterionic intermediate. A great deal of the substituent effect in k_H for weakly basic amines comes from the basicity difference since k_H is equal to $k_5/K_{a^\pm}$, and is reflected in the $K_{a^\pm}$ values, which should parallel in substituent effect the K_a values of the parent amine, giving a β value near unity. For weakly basic amines shown in the dotted line, the right-hand barrier for C–N cleavage has to be the highest barrier in the energy profile. Strongly basic amines will have a somewhat lower energy for the zwitterion form than the weakly basic amine and must have the second barrier for C–N cleavage lower than the left-hand barrier for proton transfer. This means that the substitutent effect for the C–N bond cleavage process is greater than for N-protonation. This behavior is to be expected because CO_2, in analogy with acyl groups which have a β value of 1.6[22] compared with a β value of 1.0 for the proton, should be more electropositive than a proton, and therefore the central N atom of the zwitterionic carbamic acid should be more sensitive to the removal of CO_2 than of the proton.

Buffer Catalysis. The buffer-catalyzed term for carbamates derived from strong bases is explainable in terms of transition-state V in which rate-limiting protonation to form the zwitterionic carbamate takes place. The C–N bond remains intact because of the kinetic demonstration of an intermediate. Buffer catalysis of carbamates derived from weakly basic amines is described by transition state VI in which N–C bond breaking is concerted with the nitrogen protonation.[4] Closely analogous transition states, VII for strongly basic N and VIII for weakly basic N, for the general

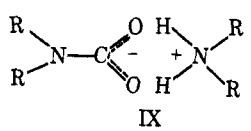


catalyzed decomposition of trifluoro-*N*-methylacetanilides have been deduced from solvent isotope effect and breaks in log k–pK curves.[23]

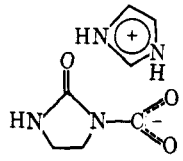
The specificity of the buffer catalysis is a predominant feature of carbamate decomposition. For example, *p*-nitrophenylcarbamate decarboxylation is catalyzed by phosphate, arsenite, acetylacetone (probably in the enol form), glycine, glycylglycine, ethylenediamine, imidazole, and *N*-methylimidazole, but shows negative deviations by butylammonium ion, Tris, triethanolamine, and dithiothreitol. That this phenomenon is

(17) J. F. S. Dippy, *J. Chem. Soc.*, 357 (1938).
(18) Diethyldithiocarbamate has a pK_a of 3.37 at 25° (K. I. Aspila, S. J. Joris, and C. L. Chakrabarti, *J. Phys. Chem.*, 74, 3625 (1970)). The pK_a values of the dithiocarbamates derived from diisopropylamine, pyrrolidine, dimethylamine, dibutylamine, and dipropylamine are 4.2, 3.25 (S. J. Joris, K. I. Aspila, and C. L. Chakrabarti, *Anal. Chem.*, 41, 1441 (1969)); 3.2, 3.9, and 3.6, respectively (K. I. Aspila, V. S. Sastri, and C. L. Chakrabarti, *Talanta*, 16, 1099 (1969)).
(19) R. Barnett and W. P. Jencks, *J. Amer. Chem. Soc.*, 89, 5963 (1967); R. Barnett and W. P. Jencks, *ibid.*, 91, 6758 (1969).
(20) E. M. Arnett, *Progr. Phys. Org. Chem.*, 1, 233 (1963); V. C. Armstrong and R. B. Moodie, *J. Chem. Soc.*, 275 (1968).
(21) V. C. Armstrong, C. W. Farlow, and R. B. Moodie, *Chem. Commun.*, 1362 (1968).
(22) A. R. Fersht and W. P. Jencks, *J. Amer. Chem. Soc.*, 92, 5432 (1970).
(23) L. D. Kershner and R. L. Schowen, *ibid.*, 93, 2014 (1971).

not due to specific interactions of the NH group of the carbamate is shown by the similar behavior with respect to buffer catalysis of diphenylcarbamate. Caplow and Yager[5] have observed specificity with respect to buffer catalysis in the decarboxylation of *N*-carboxyimidazolidone. In this case effective catalysis is observed with oxygen acids and ineffective catalysis is observed with nitrogen acids including imidazole. It is possible that unreactive complexes are produced in which hydrogen bonding between the carboxylate and the ammonium ion is the stabilizing feature as shown in structure IX. Similar structures for *N*-carboxyimidazolidone can be drawn as well as an imidazolium ion complex, X, which



IX



X

utilizes the ureido carbonyl group for hydrogen bonding. The fact that ammonium carbamates are stable in the solid state[24,25] attests to the stability of complexes of ammonium ions and carbamate ions. These complexes are characterized by an intense, very broad N–H stretching band in the 2100–2600-cm^{-1} region of the infrared, indicating the presence of a strong hydrogen bond.[24]

Zwitterionic Carbamic Acid. The lifetime of the zwitterion is very small according to the following estimates of the specific rate constants k_{-1} and k_5; for carbamates derived from strongly basic amines k_5 is greater than k_{-1} because the protonation step (k_1) is rate limiting here. The value of k_{-1} should be of the order of 10^{10} sec^{-1} because this represents a diffusion-limited process in the thermodynamically controlled direction. This is so because of the previously made estimates of the pK_a' of the zwitterion. If the value of k_5 is greater than k_{-1} then it must have a value greater than 10^{10} sec^{-1}, which means that the zwitterion has an exceedingly short lifetime and represents an association complex between a hydronium ion and a carbamate ion. Another estimate of k_5 comes from the observed values of K_i for phenylcarbamate and *o*-ethoxyphenylcarbamate. The value of K_i is given by $K_w k_{-6}/k_5$, reflecting the relative ease of CO_2 expulsion to proton transfer to hydroxide from the zwitterion. The more strongly basic the nitrogen the smaller the k_{-6}/k_5, *i.e.*, the less readily proton transfer to hydroxide ion occurs compared to collapse of the zwitterionic species to CO_2 and amine. Values of k_{-6}/k_5 of 3–6 are obtained from the K_i values in Table VI. Since the value of k_{-6} is diffusion controlled, the value of k_5 is only one-third to one-sixth the value of a diffusion-controlled reaction or 1.3×10^9–3×10^9 sec^{-1}.

Metal Ion and Buffer Effects. The complete lack of stabilization of *p*-nitrophenylcarbamate and diphenylcarbamate by cupric and manganous ions and the lack of effect of the cupric–acetylacetone and cupric–glycylglycine complexes suggest that metal ions are ineffective in stabilizing carbamates derived from primary and secondary amines, as compared to carbamates derived from urea derivatives. Apparently the ureido oxygen plays an important role in complexing with the metal ion. This type of stabilization suggests that metal ions associated with biotin-containing enzymes serve a role in stabilizing *N*-carboxybiotin and increasing its effectiveness as a CO_2 transfer agent.

The small buffer terms in the butylamine buffers corresponding to k_B and k_{BHB}, if not due to special effects, could possibly be due to a direct transfer reaction of the CO_2 group to butylamine with the subsequent more rapid decarboxylation of the butylamine carbamate. The interaction of nucleophiles with carboxylate ions has its analogy in the reaction of the ureido anion with the carboxylate group of *o*-ureidobenzoic acid[26] and in the reverse of the reaction of hydroxide with *p*-nitroacetanilide.[27] However, an unfacilitated reaction of this sort is considered to be highly unlikely in the case of carbamates because such transfer does not occur with the very basic hydroxide ion. The lack of reaction of *p*-nitrophenylcarbamate with the copper complex of acetylacetone and glycylglycine was examined with the expectation that the metal ion could facilitate reaction of the enolate or amine with the carbamate, but such a reaction, if it occurs at all, occurs more slowly than the hydronium ion catalyzed process at pH 8. Similar experiments with hydroxylamine and hydroxylamine in the presence of barium ions lead to the same negative conclusions. The lack of catalysis by electrophilic catalysts which catalyze bicarbonate decarboxylation and phosphoramidate dephosphorylation in the case of carbamates means that leaving group protonation in carbamates competes effectively with alternative modes of leaving group activation.

Isotope Effects. The solvent isotope effect of 1.0 for the k_H terms of *p*-nitrophenylcarbamate and phenylcarbamate decomposition indicates that the hydrogen atoms attached to the transition state have the same zero-point energy compared with those attached to the hydronium ion and carbamate reactants. Solvent isotope effects for the diffusion coefficients of hydronium ions and other ions are of the order of 1.16–1.4[28] making our kinetic isotope effect of the magnitude which might be expected for a diffusion-controlled protonation. It is difficult from this data to come to any more detailed conclusions concerning the transition state. The large isotope effect of ~4 of the water reaction of phenylcarbamate is consistent with a rate-limiting proton transfer from water to the carbamate nitrogen atom in the transition state reflecting the isotope effect of k_6. *N*-Carboxyimidazolidone has an isotope effect of 1.0 for its water term,[5] which is consistent with the composite or concerted nature of the water rate constant of k_5k_6/k_{-1} for carbamates of weakly basic amines, and reflects the change in rate-determining step fom N–H bond formation to N–C bond cleavage in going from carbamates of strongly basis amines to carbamates of weakly basic amines. The equilibrium isotope effect of 3.3 for the dissociation constant of *p*-nitrophenylcarbamic acid is the value expected for an acid with a pK_a value of 4.2.[29] For example, benzoic

(24) S. L. Johnson, unpublished work.
(25) Fr. Fichter and B. Becker, *Chem. Ber.*, **44**, 3481 (1911); M. Frankel and E. Katchakski, *J. Amer. Chem. Soc.*, **65**, 1670 (1943); H. B. Wright and M. B. Moore, *ibid.*, **70**, 3865 (1948).
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(28) G. N. Lewis and T. C. Doody, *ibid.*, **55**, 3504 (1933).
(29) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 189.

acid which has a pK_a of 4.2 has its K_a value lowered by a factor of 3.13 in heavy water.[30]

Conclusions

The unimolecular decarboxylation of the zwitterionic carbamic acid species is the main mode of decomposition of carbamates. Direct transfer of CO_2 from arylamine to an acceptor molecule such as hydroxide ion, amine, or sulfhydryl does not appear to be important. The mode of decomposition is similar to the mode of decomposition of phosphates,[31] phosphoramidates,[32] and sulfates,[33] all of which feature leaving group protonation and loose transition states which can only weakly incorporate a molecule of nucleophile. The positive values of $\Delta S^{\pm}$ for carbamate and dithiocarbamate decomposition are consistent with a transition state with unimolecular character.[34] Enzymes containing biotin are able to transfer CO_2 from *N*-carboxybiotin to appropriate acceptors. The enzyme must therefore increase the specificity of the CO_2 transfer reaction by correct positioning of acceptor and biotin-bound CO_2, or by increasing the susceptibility of *N*-carboxybiotin to nucleophilic attack by the acceptor. It is quite likely that metal ions play an important role in complexing with *N*-carboxybiotin in such a way to fulfill this role.

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(32) W. P. Jencks and M. Gilchrist, *ibid.*, 87, 3199 (1965).
(33) S. J. Benkovic and P. A. Benkovic, *ibid.*, 88, 5504 (1966); S. J. Benkovic, *ibid.*, 88, 5510 (1966).
(34) The $\Delta S^{\pm}$ values for the k_H terms of diethyldithiocarbamate[35] and morpholine carbamate[4] decarboxylation are 23.9 and 2.4 eu, respectively, and the water term for *N*-carboxyimidazolidone decarboxylation[4] has a $\Delta S^{\pm}$ value of 13.9 eu.
(35) S. W. Dale and L. Fishbein, *J. Agr. Food Chem.*, 18, 713 (1970).

Nucleoside *S*-Alkyl Phosphorothioates. V.[1] Synthesis of a Tridecadeoxyribonucleotide

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Abstract: The tridecadeoxyribonucleotide d-pTpTpApApTpCpCpApTpApTpGpC has been chemically synthesized using the *S*-ethyl group for 5′-phosphate protection. The synthetic approach involved sequential addition of oligonucleotides of size 2–4 monomer units to the growing nucleotide chain containing the *S*-ethyl group. The condensations employed mesitylenesulfonyl chloride as the condensing agent, and the yields were in the range of 17–48%. The *S*-ethyl group was stable under the conditions employed in the synthetic scheme, and was retained until the oligonucleotide chain was completed. It was finally removed from the completed tridecamer by treatment with aqueous iodine. The trinucleotide d-pC^{An}pA^{Bz}pT-OAc and the tetranucleotide d-pA^{Bz}pTpG$^{i\text{-}Bu}$pC^{An}-OAc, both of which were required in the synthesis of the tridecamer, were also prepared using the *S*-ethyl method for 5′-phosphate protection. For comparison, the trimer was also prepared using the cyanoethyl group as the phosphate-protecting group, and the overall yields were closely similar.

In previous papers from this laboratory,[1-3] the activation and protection of the phosphate groups of nucleotides *via* their alkythio derivates have been described. The activating feature of the *S*-ethyl group lies in its susceptibility to cleavage by a wide variety of nucleophiles in the presence of mild oxidizing agents; in this way a number of nucleotide derivatives have been prepared by variation of the nucleophilic agent.[2] The protecting feature of this group lies in the relative stability of these compounds under the conditions encountered during oligonucleotide synthesis. Thus, an *S*-ethyl group on the 5′-terminus of a growing nucleotide chain protects this chain against self-condensation until completion of the reaction sequence. It is also stable to the alkaline conditions required for removal of 3′-*O*-acyl groups prior to chain extension, and thus obviates the necessity for reprotection of the 5′-phosphate after each elongation, as is required for the cyanoethyl group. Applications to di- and trinucleotide synthesis have been described.[3] A similar approach to oligonucleotide synthesis involving protection of the 5′-phosphate by phosphoramidate formation has recently been reported.[4] The purpose of this paper is to extend the range of the *S*-ethyl phosphorothioate technique to the synthesis of the tridecanucleotide d-pTpTpApApTpCpCpApTpApTpGpC (**1**), an oligonucleotide of sufficient size to permit its efficient coupling with other oligonucleotides to form larger sequences *via* template-guided joining by the enzyme polynucleotide ligase.[5] The particular sequence chosen for synthesis

(1) Paper IV in this series: A. F. Cook, *J. Amer. Chem. Soc.*, 92, 190 (1970).
(2) A. F. Cook, M. J. Holman, and A. L. Nussbaum, *ibid.*, 91, 1522 (1969).
(3) A. F. Cook, M. J. Holman, and A. L. Nussbaum, *ibid.*, 91, 6479 (1969).
(4) E. Ohtsuka, M. Ubasawa, and M. Ikehara, *ibid.*, 92, 5507 (1970).