sion method, 6–8.4 insulin units/ml. Since the theoretical amount of insulin formed, based on the amount of B chain S-sulfonate used, is 20 units/ml (0.8 mg of insulin/ml), the combination yield in these experiments ranges from 30 to 42%. This half-synthetic human insulin and, furthermore, the all-synthetic human insulin were isolated in highly purified form as the hydrochloride salts and found to possess by the mouse convulsion method 22–24 IU/mg. The experimental details of this part of the work will be reported in a later communication.[25]

Combination of Natural Porcine (Human) A Chain and Natural Bovine B Chain. Porcine insulin was sulfitolyzed and its A and B chains were isolated in the S-sulfonated form using the conditions employed for the preparations of the bovine insulin chains.[19] Combination of the A chain S-sulfonate (20 mg) thus obtained with natural bovine B chain S-sulfonate (5 mg) was carried out as described previously. The solution of the recombination mixture (total volume approximately 10 ml) upon assay by the mouse convulsion method was shown to possess 7–10 insulin units/ml. On the basis of the amount of the B chain S-sulfonate used, the recombination yield for the natural chains is 35–50% of the theoretically expected value.

Acknowledgments. The authors wish to express their appreciation to Miss Karin Scheibe for the amino acid analyses and enzymatic analyses and to Miss Roberta Klimaski for the biological assays. They wish also to thank Eli Lilly and Co. for generous gifts of crystalline bovine and porcine insulins.

(25) P. G. Katsoyannis, A. Trakatellis, C. Zalut, S. Johnson, A. Tometsko, G. Schwartz, and J. Ginos, *Biochemistry*, in press.

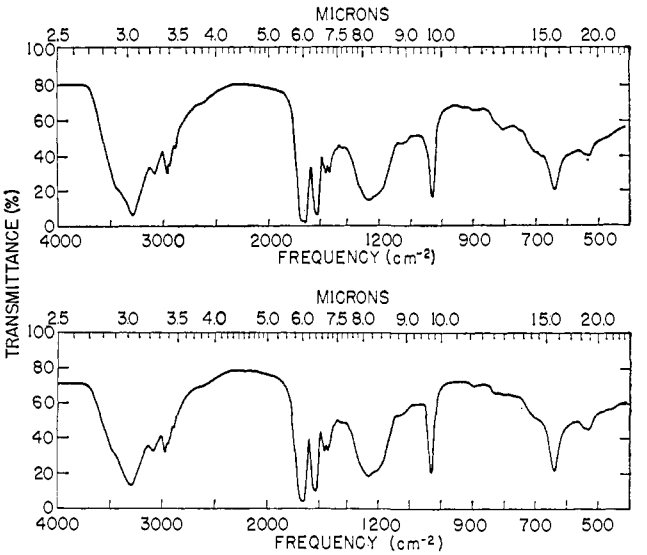

Figure 13. Infrared spectrum of the S-sulfonates of synthetic human A chain (top) and natural porcine A chain (bottom).

Studies on the Mechanism of Biotin Catalysis. II[1,2]

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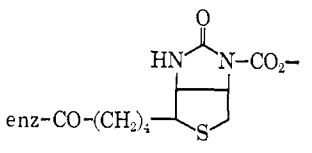
Contribution from the Department of Biochemistry, Yale University, New Haven, Connecticut 06510. *Received March 22, 1967*

Abstract: A kinetic study of the decarboxylation of a carboxybiotin analog, N-carboxy-2-imidazolidone, has been carried out. The rate law for decarboxylation is: rate = $k_1(S) + k_2(H_3O^+)(S) + k_3(BH)(S)$, where (S) and (BH) are the concentrations of the carboxyimidazolidone anion and acidic form of the buffer. At 25°, k_1 is 2.5×10^{-3} min^{-1}, k_2 is 3.5×10^5 M^{-1} min^{-1}, and k_3 is proportional to the acid dissociation constant of the buffer. At 6° the Brønsted α value, determined from a study with nine acidic catalysts, is 0.9. Metal ions prevent decarboxylation of carboxyimidazolidone; the dissociation constants for the metal complexes with Cu(II) and Mn(II) are 5.5×10^{-5} M and 3.5×10^{-3} M, respectively. The refractoriness of the carboxyimidazolidone anion is attributed to the poor leaving ability of the imidazolidone anion, and the insensitivity of this compound to general and specific acid catalysis is ascribed to the low basicity of the ureido system of the carbamic acid.

Carbon dioxide transfer reactions mediated by biotin–enzymes have been demonstrated to proceed by a mechanism in which an N-carboxybiotin intermediate I is formed in an initial reaction with ATP and bicarbonate.[3] Although this pathway is well documented, relatively little is known about the kinetic properties of N-carboxybiotin or the susceptibility of this compound to different types of catalytic interactions. We report here a detailed study of the kinetics of the decarboxylation of a similar compound, N-carboxy-2-imidazolidone, and the reaction of this compound with metal ions, hydronium ion, and general acids.



I

Experimental Section

Materials. Phenyl chloroformate containing ^{14}C in the carbonyl group was synthesized by the dropwise addition of 4.1 ml of a freshly prepared 2.44 M aqueous sodium phenolate solution to an ice-cold solution containing 1 mcurie (0.6 mcurie/mmole) of phosgene-^{14}C dissolved in 10 ml of a 12.5% solution of phosgene in benzene. After the addition of phenolate was complete, 4 ml of redistilled nonradioactive phenyl chloroformate was added.

(1) Supported by a grant from the National Institutes of Health (GM 11820).

(2) For the first paper in this series see M. Caplow, *J. Am. Chem. Soc.*, **87**, 5774 (1965).

(3) (a) S. Ochoa and Y. Kaziro, *Comp. Biochem.*, **16**, 210 (1966); (b) F. Lynen, J. Knappe, and E. Lorch, *Proc. Intern. Congr. Biochem., 5th, Moscow, 1961*, **4**, 225 (1963); (c) H. G. Wood and M. F. Utter, *Essays Biochem.*, **1**, 1 (1965).

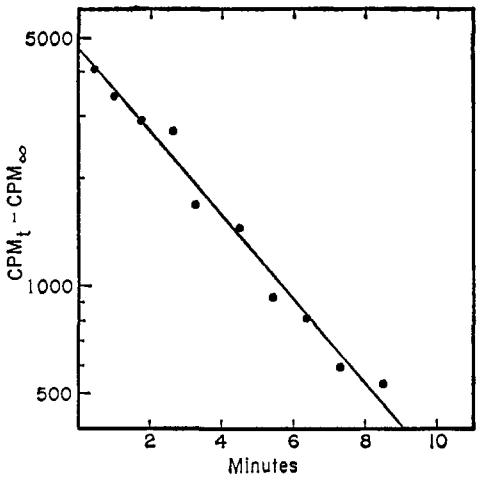


Figure 1. Decarboxylation of carboxyimidazolidone in the presence of 0.03 *M* cupric chloride at 25°. Ionic strength maintained at 0.3 with potassium nitrate, 1.25% acetone, 3.25×10^{-4} *M* substrate.

The benzene layer was immediately removed, and the aqueous layer was extracted several times with ethyl ether. The ether and benzene were combined, and after removal of the solvents by distillation at atmospheric pressure, 2.4 ml of product boiling at 53–54° (2.3 mm) was obtained. Vapor phase chromatography revealed that this material contained approximately 5% phenol; the infrared spectrum showed almost no absorption in the 3.1–3.2-μ region and a single carbonyl band at 5.61 μ.

N-Phenoxycarbonyl-2-imidazolidone containing ^{14}C in the exocyclic carbonyl group was synthesized by refluxing 18.6 mmoles of the acyl chloride described above with 16.3 mmoles of imidazolidone in 15 ml of chloroform for 42 hr. The reaction mixture was cooled, filtered, and dried under vacuum to give 2.9 g of product melting at 185–186°.

N-Methyl-2-imidazolidone was synthesized by the dropwise addition of 61.5 ml of a 12.5% solution of phosgene in benzene to 5 ml (0.065 mole) of N-methylethylenediamine (prepared by the method of O'Gee and Woodburn[4]) dissolved in 120 ml of acetone and 20.2 ml of dry triethylamine at 0°. The reaction mixture was filtered, and after nitrogen was passed through the supernatant for 1 hr, this material was taken to dryness under vacuum. The yellow oil thus obtained solidified when triturated with ethyl ether and was recrystallized from acetone and/or benzene. The former solvent gave a white solid with mp 107.5–110° and sublimation gave a material with mp 110–112° (lit. 115–116°[5] and 68°[6]). Synthesis of N-phenoxycarbonyl-N'-methyl-2-imidazolidone was accomplished by refluxing 246 mg of N-methyl-2-imidazolidone and 0.36 ml of radioactive phenyl chloroformate in 2.5 ml of chloroform for 40 hr. The product obtained after removal of the solvent under vacuum was recrystallized from benzene to constant specific activity and melted at 141–142°. *Anal.* Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.69; H, 5.64; N, 12.60.

Radioactive N-carboxy-2-imidazolidone and N-carboxy-N'-methyl-2-imidazolidone were prepared within 1 hr of use by mixing 0.3 ml of an acetone solution of the ester with 2.1 ml of 0.1 *M* sodium hydroxide. Saponification is complete in about 10 min, and the material was used after this time. Dimethyl phosphate was prepared immediately before use by saponification of dimethylphosphoryl chloride. This material was synthesized by the reaction of dimethyl phosphite (1 mole) and N-chlorosuccinimide (1 mole) in dry benzene and was isolated by vacuum distillation. Deuterium exchange of compounds that were used for deuterium isotope studies was carried out by the method described previously.[2] All other materials were reagent grade and were recrystallized or distilled; water and deuterium oxide were glass distilled.

Methods. Rates of decarboxylation were measured by quenching aliquots of the reaction mixture with an equal volume or two-fold excess of barium hydroxide which serves to precipitate the carbonate and carbon dioxide in solution without affecting the carbamate. The carbamate was separated from the barium carbonate by passing the quenched reaction mixture through a Metricel VM-6 filter installed in a Swinny attachment on a hypodermic syringe. Heavy precipitates formed in the reaction of buffer ions with barium were removed by centrifugation prior to filtering. The barium hydroxide solution used for quenching was 0.5 *M* for most reactions and in all cases the resulting solution was sufficiently alkaline so that decarboxylation was terminated; it is important to avoid excess alkali since this can give rise to spurious results in the scintillation counting. A 0.3-ml aliquot of the filtrate was then mixed with 10 ml of counting fluid made by mixing 100 g of naphthalene, 5 g of 2,5-diphenyloxazole, 0.125 g of 1,4-bis-2-(5-phenyloxazolyl)-1-benzene, and 500 ml of dioxane. It was found that reproducible results could only be obtained if approximately 0.3 g of Packard thixotropic gel powder was included in each vial containing the counting solution, and this material was therefore used throughout. The gel powder evidently affects the manner in which the radioactive carbamate and accompanying salts are precipitated in the counting fluid since addition of the thixotropic gel powder after addition of the radioactive solution was found to be less effective. Furthermore, in the absence of gel powder the counting efficiency could be improved by stirring the counting fluid with a magnetic stirrer while the radioactive solution was added.

Rates of decarboxylation of N-carboxyimidazolidone in the presence of metal ions were studied at constant pH using a Radiometer SBR2/SBU1/TTA31 titration assembly. Metal ions were added to the reaction mixture after the excess alkali used to saponify the phenoxycarbonylimidazolidone was neutralized to avoid precipitation of the metal hydroxides. The pH was then adjusted to the desired value and the reaction was started; assay of aliquots of the reaction mixture was carried out as described above.

Acid dissociation constants of buffer compounds were determined under the conditions used for rate measurements and the titration data was analyzed by the method of Reed and Berkson.[7] Values for the p*K* for 0.5 *M* phosphate determined by the method of partial neutralization were found to be dependent upon the per cent ionization of the monoanion and ranged from 6.32 at 8% ionization to 6.59 at 68% ionization (6°). Titration of 0.5 *M* dipotassium phosphate at ionic strength 1.5, 6° (final phosphate 0.40 *M*, final ionic strength 1.2), showed good agreement with a theoretical titration curve with a p*K* of 7.12 through approximately pH 6.7, below which the observed curve deviated from theory by approximately 25%. The results obtained with 0.10 *M* phosphate under identical conditions showed no deviation from theory and gave a p*K* of 6.62.

Results

An assay utilizing ^{14}C-labeled carboxyimidazolidone has been developed for the study of the kinetics of the decomposition of this compound, and the results obtained in a typical experiment are shown in Figure 1. The assay is similar to the one used by Faurholt;[8] however, the use of an isotopic substrate increases the sensitivity of the assay and permits the use of very low substrate concentrations. This method is probably the only one applicable in a study of metal ion effects on decarboxylation of this substrate since the precipitation of metal carbonates precludes manometric or spectrophotometric analysis.

Decarboxylation of Carboxyimidazolidone in the Presence of Metal Ions. Metal ions have a profound effect on the rate of decarboxylation of N-carboxyimidazolidone. Decarboxylation is acid catalyzed and is too fast to measure at 25° at pH's very much below 6, while, as seen in Figure 1, the half-life for decarboxylation is 2.7 min at pH 3.52 in the presence of 0.03 *M* cupric ion. The decarboxylation of carboxyimidazoli-

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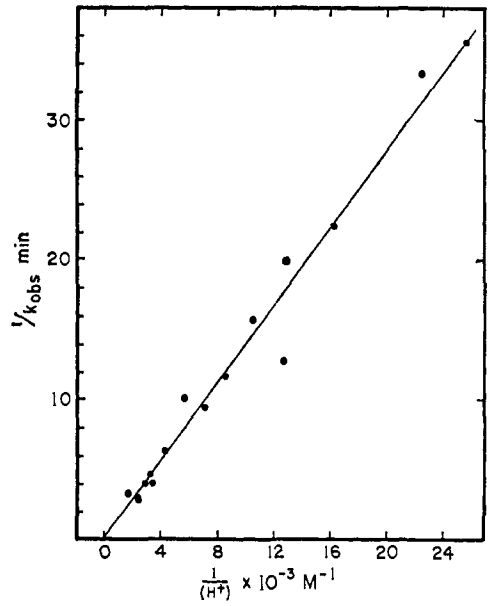

Figure 2. Reciprocal plot of results obtained in a study of the decarboxylation of carboxyimidazolidone in 0.03 *M* cupric chloride. Conditions are as given in the legend to Figure 1.

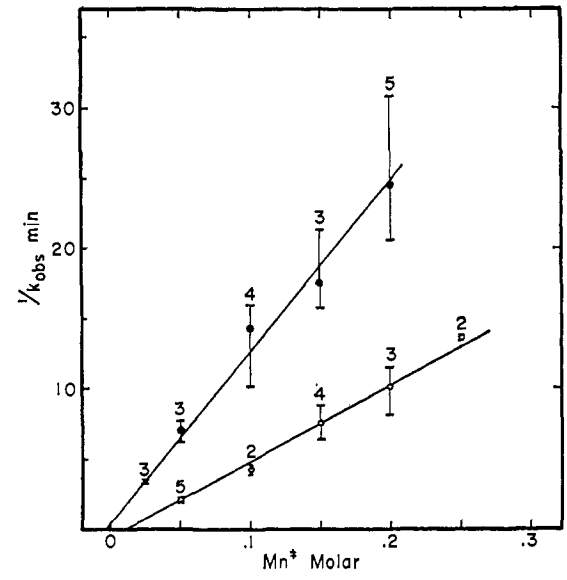

Figure 3. Effect of manganous chloride on the rate of decarboxylation of carboxyimidazolidone at 25°: ●, averages of values obtained at pH 5.24; ○, pH 4.78; , the range of values observed; the number of experiments is indicated above the vertical bars. The ionic strength was maintained at 0.9 with potassium nitrate, 1.25% acetone, 3.25×10^{-4} *M* carboxyimidazolidone.

done in the presence of metal ions has been analyzed according to the scheme given in eq 1 which involves

$$\begin{array}{c} S \underset{K_1}{\overset{H^+}{\rightleftharpoons}} SH \xrightarrow{k_1} P \\ M^{2+}\Big\downarrow\Big\uparrow K_2 \\ SM \end{array} \qquad (1)$$

the formation of a catalytically inactive chelate and a catalytically active protonated compound. According to this scheme when $(M^{2+}) \gg (S)$

$$k_{obsd} = \frac{k_1K_2(H^+)}{K_1K_2 + K_1(M^{2+}) + K_2(H^+)} \qquad (2)$$

in which K_1 and K_2 are the respective constants for dissociation of a proton and metal ion from carboxyimidazolidone. Equation 2 predicts that plots of $1/k_{obsd}$ *vs.* $1/(H^+)$ will be linear with slope $(K_1/k_1)[1 + (M^{2+})/K_2]$ and intercept $1/k_1$. The results obtained in the reaction with 0.03 *M* cupric ion are plotted this way in Figure 2 and give k_1 equal to 10 min^{-1}. Using a value of k_1/K_1 equal to 3.49×10^5 M^{-1} min^{-1} obtained from plots of k_{obsd} *vs.* (H^+) from reactions under conditions where $K_1 \gg (H^+)$ and $(M^{2+}) = 0$ (ionic strength 0.3), values of K_1 and K_2 were calculated to be 2.87×10^{-5} *M* (p*K* = 4.54) and 6.2×10^{-5} *M*, respectively. Identical analysis of data obtained with 0.1 *M* cupric ion gave k_1 equal to 10 min^{-1}, and 4.8×10^{-5} *M* for K_2. Values of K_2 which are obtained from the slopes of the plots described above are, of course, considerably more accurate than those of K_1 and k_1 obtained from the extrapolated intercept. The latter values are considered lower limits for these constants.

The dissociation constant for the manganous–carboxyimidazolidone complex was determined from rate measurements at constant pH with varying manganous concentration. Equation 2 predicts that plots of $1/k_{obsd}$ *vs.* the metal ion concentration will be linear (Figure 3) and using the slope of these plots (slope = $K_1/k_1K_2H^+$) along with the value for k_1/K_1 described above, values of the dissociation constant of 4.1×10^{-3} *M* and 3.1×10^{-3} *M* were calculated from data obtained at pH 5.24 and 4.78, respectively. We are unable to account for the failure of the curves to intercept at the left of the origin as would be expected.

Decarboxylation of Carboxyimidazolidone. We have previously reported[2] that the decarboxylation of carboxyimidazolidone is acid catalyzed and have now found that in addition to this pathway there is a slower reaction involving the anionic compound. The rate constant for decarboxylation of the anion at ionic strength 1.5 is 2.5×10^{-3} min^{-1} in 0.1 *M* sodium hydroxide at 25°, 5.85×10^{-2} min^{-1} at 50° in 0.094 *M* base, and 5.90×10^{-2} min^{-1} in 99% deuterium oxide containing 0.10 *M* potassium deuterioxide (50°). The activation parameters calculated from these results are 23.3 kcal for $\Delta H^{\pm}$ and 0 eu for $\Delta S^{\pm}$ at 25°. Barium ions decrease the rate of decarboxylation of the anion, and the rate of decarboxylation is decreased to 1.3×10^{-2} min^{-1} in the presence of 0.5 *M* barium chloride (μ = 1.5, 50°).

Buffer ions have a significant effect on the rate of decarboxylation. This is illustrated in Figure 4 which shows the dependence of the rate on the total phosphate concentration at constant pH. The rate law for reaction at low pH's where the anion reaction can be neglected is

$$\text{rate} = k_{H_3O^+}(H_3O^+)(S) + k_b(B)(S) \qquad (3)$$

where (S) is the carboxyimidazolidone anion concentration. Accordingly, plots of $k_{obsd}/(H_3O^+)$ *vs.* the buffer concentration give $k_{H_3O^+}$ as the intercept and $k_b/(H_3O^+)$ as the slope. The data were plotted this way so as to correct for the small pH variations at different buffer concentrations and while the k_b values thus obtained are subject to some uncertainty because the hydronium concentration was often not identical at all buffer concentrations, or in different runs at a given buffer concentration, this method does rule out

Table I. Effect of Buffers on the Rate of Decarboxylation of Carboxyimidazolidone[a]

Catalyst	Concn, M	No. of reactions	pH range	(Av k_{obsd}/(H^+)) × 10^{-4} M^{-1} min^{-1}	Stand dev of the mean	P^b	pK_a	k_{cat}, M^{-1} min^{-1} [c]	pK_a, cor	k_{cat}, M^{-1} min^{-1} cor
Dimethyl-phosphate[d]	0.03	2	5.89 ±0.00	4.70	0.10					
	0.25	3	5.90 ± 0.01	5.74	0.37					
	0.50	3	5.88 ± 0.02	7.39	0.59					
							1.29[e]	2.98×10^3	1.29	2.98×10^3
Diethylamino-acetonitrile	0.03	2	5.69 ± 0.00	5.17	0.00					
	0.25	2	5.68 ± 0.01	5.07	0.00					
	0.50	1	5.72	5.24	...					
							5.18	8.9×10^{-2}[f]	5.18	8.9×10^{-2}
Pyridine	0.03	6	5.54 ± 0.09	5.41	0.97	...				
	0.25	5	5.56 ± 0.08	5.81	0.46	0.80				
	0.50	5	5.52 ± 0.11	6.31	0.70	0.95				
							5.75	9.10×10^{-2}	5.75	9.10×10^{-2}
Triethylene-diamine	0.05	6	5.39 ± 0.04	4.30	0.62	...				
	0.25	4	5.39 ± 0.04	5.20	0.10	>0.99				
	0.50	5	5.39 ± 0.04	5.99	0.63	>0.99				
							3.61	9.22	3.91	4.61
Trimethyl-amine N-oxide	0.03	4	6.30 ± 0.03	5.13	1.12					
	0.25	4	6.27 ± 0.01	7.29	1.11	0.98				
	0.50	4	6.29 ± 0.01	9.65	1.36	>0.99				
							5.08	8.42×10^{-1}	5.08	8.42×10^{-1}
Trimethyl-amine N-oxide	0.03	6	5.32 ± 0.04	5.49	0.86					
	0.25	5	5.33 ± 0.08	6.86	1.04	0.98				
	0.50	5	5.36 ± 0.07	8.48	0.93	>0.99				
							5.08	8.27×10^{-1}	5.08	8.27×10^{-1}
Acetic acid	0.053	1	5.08	6.35						
	0.267	1	5.05	8.18						
	0.428	1	5.08	10.0						
							4.73	2.64	4.46	2.64
Acetic acid[g]	0.00	2	6.47 ± 0.02	5.08	0.08					
	0.25	2	6.50 ± 0.03	9.10	0.00					
	0.50	2	6.58 ± 0.00	14.6	1.20					
							4.73	3.48	4.46	3.48
Phosphate	0.03	5	7.11 ± 0.02	11.3	4.0					
	0.25	3	7.14 ± 0.01	29.8	1.6					
	0.50	3	7.16 ± 0.02	53.9	7.0					
							6.62	7.14×10^{-2}[h]	6.45	
Phosphate	0.03	1	5.84	6.39						
	0.20	1	5.84	11.7						
	0.40	1	5.80	16.7						
							6.62	4.72×10^{-1}[h]	6.45	
Phosphate[i]	0.00	4	5.08 ± 0.02	5.26	0.32					
	0.20	4	5.06 ± 0.03	10.78	2.21					
	0.40	3	5.06 ± 0.03	13.91	1.79					
							6.62	1.95[h]	6.45	
Phosphate (25°)[j]	0.00	2	7.90 ± 0.02	43.8	2.2					
	0.25	2	8.01 ± 0.01	213.0	19.0					
	0.50	2	7.97 ± 0.02	355.0	12.0					
							6.44	6.58×10^{-2}[h]		
Phosphate (25°)	0.03	3	6.96 ± 0.03	39.8	1.8					
	0.25	2	6.97 ± 0.05	134.0	8.0					
	0.50	4	7.07 ± 0.04	301.0	20.0					
							6.44	4.90×10^{-1}[h]		
Phosphate (25°)	0.03	2	6.13 ± 0.02	35.7	1.0					
	0.25	2	6.12 ± 0.01	81.1	0.0					
	0.50	2	6.12 ± 0.02	158.0	16.0					
								1.80[h]		
Phosphate (25°)	0.03	2	6.11 ± 0.02	33.8	1.5					
	0.25	2	6.09 ± 0.01	87.7	2.8					
	0.50	2	6.08 ± 0.01	134.0	21.0					
								1.98[h]		
Phosphate (25°) in D_2O	0.03	3	6.63 ± 0.01	49.4	3.1					
	0.25	3	6.54 ± 0.01	149.0	23.0					
	0.50	2	6.59 ± 0.01	249.0	20.0					
								1.18[h]		
Imidazole (25°)	0.01	3	6.47 ± 0.03	32.2	3.4					
	0.25	2	6.43 ± 0.02	30.0	0.8					
	0.50	2	6.39 ± 0.05	29.5	2.1					
							7.23	1.38×10^{-2}[f]		
Carbonate[j] (25°)	0.00	3	7.93 ± 0.03	26.7	1.2					
	0.17	3	8.01 ± 0.02	33.8	2.0					
	0.33	3	8.04 ± 0.03	43.9	2.6					
							10.33[k]	5.2×10^{-3}[h]		

Table I (*Continued*)

Catalyst	Concn, *M*	No. of reactions	pH range	(Av k_{obsd}/(H^+)) × 10^{-4} M^{-1} min^{-1}	Stand dev of the mean	*P*[b]	pK_a	k_{cat}, M^{-1} min^{-1} [c]	pK_a, cor	k_{cat}, M^{-1} min^{-1} cor
Tris (25°)	0.05	2	7.46 ± 0.05	35.9						
	0.50	2	7.46 ± 0.03	30.5						
	1.00	2	7.41 ± 0.02	25.0						
							8.1[k]			

[a] Reactions at 6° unless stated otherwise, ionic strength 1.5 with KCl, 3.7×10^{-4} *M* substrate, 1.7% acetone. Most reactions contained 1.0×10^{-3} *M* EDTA and in several cases the results were not found to be substantially changed by omission of this material. [b] Confidence level calculated from a one-tail t-test (A. Goldstein, "Biostatistics," The Macmillan Co., New York, N. Y., 1965, p 50) that the second-order rate constant is larger than the one observed at the lowest buffer concentration. [c] For the reaction of the conjugate acid of the buffer. [d] Reactions carried out in 0.05 *M* pyridine buffer. [e] Determined at 25° by W. D. Kumler and J. J. Eiler, *J. Am. Chem. Soc.*, **65**, 2355 (1943). [f] Maximum value calculated on the basis that a rate increase of 5% with increasing buffer concentration would not have been detected. [g] In the presence of 0.1 *M* imidazole. [h] Second-order rate constant uncorrected for buffer ionizations. [i] In the presence of 0.05 *M* acetate. [j] In 0.05 *M* Tris buffer. [k] J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press Inc., New York, N. Y., 1958.

the mistaken observation of catalysis arising from pH variations. This is especially critical since the hydronium ion catalyzed reaction prevents study of acidic catalysts under conditions where these compounds are predominantly in the acidic form and the catalysis observed was, therefore, frequently fairly meager. The results obtained with a number of buffers are summarized in Table I and a Bronsted plot using the appropriate statistical corrections made according to the method of Benson[9] is given in Figure 5. Statistical corrections for alkyl groups that do not affect the results and for hydronium ion were not made. Catalysis by acetic acid and trimethylamine N-oxide was studied

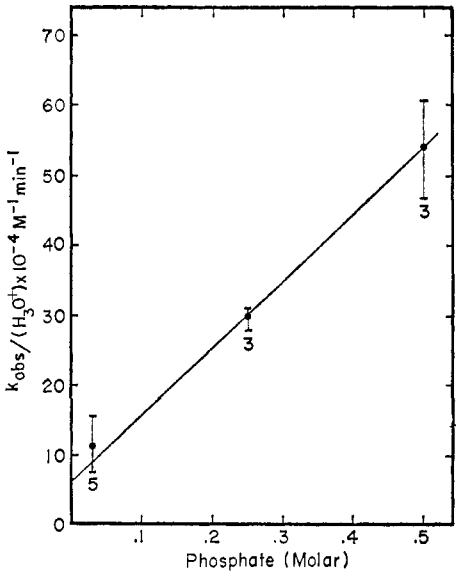

Figure 4. Effect of phosphate concentration on the rate of decarboxylation of carboxyimidazolidone at pH 7.13 ± 0.05, 6°. The circles are the averages, the number of experiments is indicated by the numbers, and the vertical bars represent the range of values recorded.

as a function of pH (Table I) and although there is some uncertainty in the analysis of the results obtained with acetic acid, this study indicates that catalysis is effected by the acidic form of the buffers. Catalysis by phosphate was studied as a function of pH and is shown in Figure 6. The results are only compatible with the assumption that the phosphate monoanion and the neutral phosphoric acid molecule are the catalytically active species. Curve C is a theoretical curve for the rate law

$$\text{rate} = k_1(H_2PO_4^-)(S) + k_2(H_3PO_4)(S) \qquad (4)$$

with values of k_1 and k_2 equal to 0.289 M^{-1} min^{-1} and 3.06×10^3 M^{-1} min^{-1}, respectively. The rapid rates at 25° prevent observation of catalysis by neutral phosphoric acid; however, the deviations observed in a

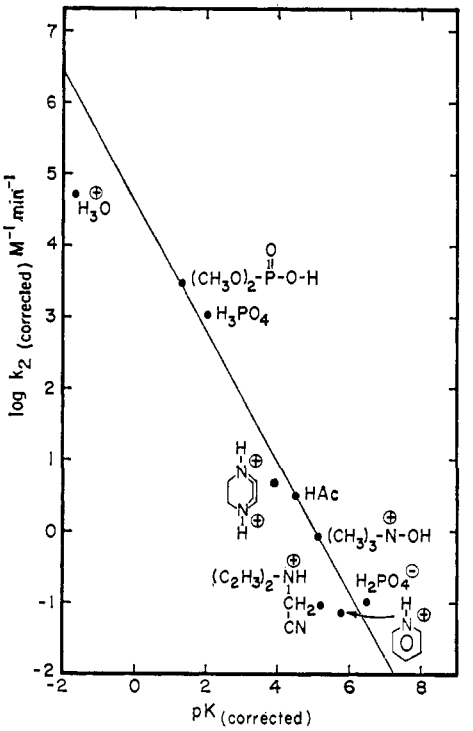

Figure 5. Brønsted plot for general acid catalysis of the decarboxylation of carboxyimidazolidone at 6°. The slope at the line, α, is 0.9.

theoretical curve (curve A, Figure 6) drawn with the assumption that only the phosphate monoanion is catalytically active suggest that this pathway is also operative under these conditions. The value for k_{cat} for the phosphate monoanion obtained from the results at the two most alkaline pH's is 2.25 M^{-1} at 25°.

(9) S. W. Benson, *J. Am. Chem. Soc.*, 80, 5151 (1958).

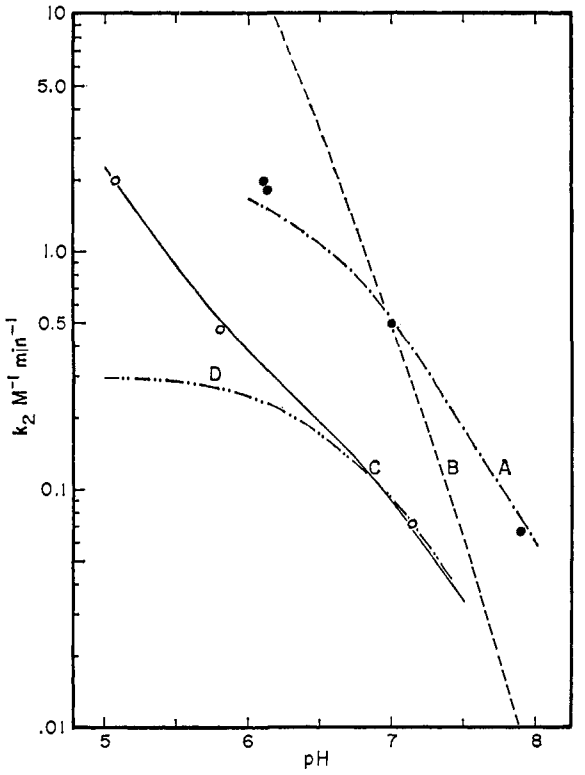

Figure 6. Effect of pH on phosphate catalysis of decarboxylation of carboxyimidazolidone. The lines are theoretical curves for the rate laws: (A) rate (25°) = 2.25 M^{-1} min^{-1} ($H_2PO_4^-$)(S), (B) rate (25°) = 3.44 × 10^5 M^{-1} min^{-1} (H_3PO_4)(S), (C) rate (6°) = 0.289 M^{-1} min^{-1} ($H_2PO_4^-$)(S) + 3.06 × 10^3 M^{-1} min^{-1} (H_3PO_4)(S), (D) rate (6°) = 0.302 M^{-1} min^{-1} (H_2PO_4); ● and ○ represent the results obtained at 25 and 6°, respectively.

A study of the deuterium isotope effect for phosphate catalysis was carried out, and the results (Table I) are to be compared to those obtained in water at pH 6.09 since both reactions were carried out under conditions where the phosphate was 70% in the form of the monoanion. The k_{H_2O}/k_{D_2O} ratio is 1.75; this ratio may not accurately reflect the effect of deuterium substitution on the catalytic effectiveness of the phosphate monoanion since the rate in deuterium oxide may contain a significant contribution from the phosphoric acid catalyzed reaction. Furthermore, the complex reactions associated with the deviations from theory observed in a study of phosphate ionization (*vida supra*) may be different in deuterium oxide and water.

The buffer catalysis appears to be reasonably specific and cationic amines consistently show negative deviations in the Brønsted plot. The largest deviation is observed with diethylaminoacetonitrile and this is undoubtedly at least partly caused by steric hindrance by the bulky alkyl groups. The catalytic specificity is best illustrated by comparing the results obtained at 25° where carbonate and phosphate are catalytically active and no catalysis can be observed with either imidazole or tris(hydroxymethyl)aminomethane. The initial results obtained in studies with phosphate, carbonate, acetate, pyridine, and imidazole suggested that the catalytic specificity might be related to complexation of the catalysts with carboxyimidazolidone by hydrogen bonding to the ureido carbonyl group and the N-3 proton. Catalysts that might conceivably coordinate in this manner were found to be more effective than those that could not, *i.e.*, pyridine and imidazole. This possibility was, however, ruled out since phosphate was found to be an effective catalyst for the decarboxylation of N-carboxy-N′-methyl-2-imidazolidone (k_{obsd}/total phosphate is 1.06 M^{-1} min^{-1} at pH 7.04, 25°). The rate constant for hydronium ion catalysis of decarboxylation of this compound, determined at 25° in imidazole buffer, is 5.1 × 10^3 M min^{-1}. The hypothesis was also rejected from results subsequently obtained with the unsubstituted compound using dimethyl phosphate and trimethylamine N-oxide as catalysts. The near identity of the rates of breakdown of the products formed from saponification of phenoxycarbonyl-2-imidazolidone and the methyl-substituted compound indicates that cleavage of the unsubstituted ester does not proceed by the elimination pathway observed by Sayigh and co-workers with allophanoyl chlorides.[10]

Since high concentrations of buffers are necessary to clearly demonstrate catalysis it was imperative to be certain that the catalytic effects are indeed specific. Clearly, the regularity of the catalysis as illustrated by the absence of any remarkable deviations in the Brønsted plot speaks against this possibility. Furthermore, a study was made of the effect of several nonacidic agents which might produce solvent conditions similar to those in concentrated buffer solutions. It was found that the rate is not measurably changed by the addition of 5 or 10% dioxane (in 0.03 M pyridine buffer at pH 5.5) which would be expected to produce an environment similar to that found with unprotonated amine buffers such as pyridine. Also, the salt used to maintain the ionic strength was changed from potassium to tetramethylammonium chloride (0.5 M, with a final ionic strength of 1.5 with potassium chloride, pH 5.3, in 0.05 M acetic buffer, 6°) and the average value of $k_{obsd}/(H_3O^+)$ was 5.64 × 10^4 M^{-1} min^{-1} (three reactions, standard deviation of the mean 0.24) as compared to 5.34 × 10^4 M^{-1} min^{-1} (four reactions, standard deviation of the mean 0.68) in the absence of tetramethylammonium ions. The rate increase with tetramethylammonium is almost 6%, and this effect is probably larger than that produced in any of the buffer studies, since the concentration of cations replacing the potassium ions used to maintain the ionic strength was not this high in any reaction studied.

Discussion

Metal Ion Studies. The catalytic role of metal ions in enzymatic transcarboxylation was first postulated by Stiles[11] on the basis of evidence obtained in studies of nonenzymatic carboxylation. This hypothesis was recently confirmed by Utter and co-workers[12] who have found that manganese is part of the active site for transcarboxylation in chicken liver pyruvate carboxylase. The postulated role of the metal ion in enzymatic transcarboxylation involves chelation with the N-carboxybiotin intermediate,[2,11] and although this intermediate would presumably be stable, there is little evidence with similar compounds to support this contention. The results described above indicate that

(10) A. A. Sayigh, J. N. Tilley, and H. Ulrich, *J. Org. Chem.*, **29**, 3344 (1964).
(11) M. Stiles, *Ann. N. Y. Acad. Sci.*, **88**, 332 (1960).
(12) M. C. Scrutton, M. F. Utter, and A. S. Mildvan, *J. Biol. Chem.*, **241**, 3480 (1966).

carboxyimidazolidone chelates effectively with manganese and cupric ions; further work is required to determine if this is the case with the biotin carbamic acid. Chelation of carboxyimidazolidone prevents unimolecular decarboxylation but may possibly facilitate bimolecular carbon dioxide transfer. The latter reaction has not yet been detected. The reaction of carboxyimidazolidone with metal ions appears to be similar to the stabilization of pyridinecarboxylic acids by bivalent metal ions[13] and the inhibition of the decarboxylation of nitroacetate by metals and hydronium ion.[14] Doering has observed that methylethyl-α-pyridylacetic acid, which decarboxylates readily in neutral solutions, is stable to prolonged boiling in concentrated hydrochloric acid.[15] Electrophiles apparently function in these reactions by preventing carbon–oxygen double bond formation and expulsion of the leaving group. It is somewhat surprising that metal ions do not affect the rates of decarboxylation of the monoester of dimethyloxaloacetic acid[16] or acetoacetic acid,[17] especially since metals act as catalysts for the decarboxylation of the similar compound, acetonedicarboxylic acid.[17] Prue has demonstrated that chelation with the ground state of acetonedicarboxylic acid is meager,[17] and the results cited above suggest that this is also the case with acetoacetate and the oxaloacetate ester. The lesser tendency of these compounds to form stable chelates may be related to the lower electron density on the ketone carbonyl group as compared with the ureido carbonyl in carboxyimidazolidone.

It is not possible to estimate the stability of a chelate formed by an enzyme-bound N-carboxybiotin intermediate since, at least with chicken liver pyruvate carboxylase,[12] the metal ion is tightly bound to the enzyme, and the protein ligands undoubtedly affect the properties of the metal. Utter and co-workers have interpreted the failure to observe a change in the proton relaxation rate of the water molecules associated with the manganese of pyruvate carboxylase following carboxylation of the biotin with ATP and bicarbonate as evidence against chelate formation in the carboxybiotin–enzyme intermediate.[18] In the light of the evidence on the stability of the chelated carboxyimidazolidone, the lability of isolated carboxybiotin–enzymes at neutral pH[3a] suggests that chelation does not occur with other biotin enzymes. Perhaps chelation occurs when the nucleophilic acceptor for the carbon dioxide is present.

Decarboxylation of the Carboxyimidazolidone Anion. The anion of carboxyimidazolidone is extremely stable, having a half-life of approximately 4 hr at 25°. The stability apparently results from the poor leaving tendency of the imidazolidone anion, which is apparent from the near identity of the rates of breakdown of this compound and bicarbonate[19] where the leaving group is hydroxide. Decarboxylation of the anion occurs at least 4000 times slower than of the neutral compound. This compares with the 72,400-fold difference in the rates of decarboxylation of carbonic acid and bicarbonate ion,[19] the 53-fold difference with acetoacetic acid and the corresponding anion,[20] and the apparently exclusive reaction of the anion in decarboxylation of trihalogenoacetic acids.[21] The factors underlying the susceptibility of carboxylic acid substrates to acid catalysis will be discussed below. The decarboxylation of the anion, which is kinetically first order, may actually involve general acid catalysis by solvent water. If this is the case, it might be expected that this reaction would be subject to a sizeable deuterium isotope effect as a result of the approximately fivefold lower protonating ability of deuterium oxide as compared with water.[22] Our failure to observe a deuterium isotope effect for this reaction might be explained by assuming that proton transfer is virtually complete in the activated complex, an assumption supported by the large α for general acid catalysis of this reaction. A similar explanation has been advanced by Wiberg[23] and by Jencks[24] to account for the failure to observe an isotope effect in reactions involving rate-limiting proton transfer. Westheimer has provided theoretical justification for the prediction that the isotope effect is maximal when the proton being transferred is symmetrically located between the donor and acceptor in the activated complex.[25] The results obtained with carboxyimidazolidone appear to be unambiguous since it is not necessary to correct for any isotope effects on equilibria, and the pH independence of the rate makes it unnecessary to be concerned with the small changes in solvent acidity caused by substitution of deuterium oxide for water. The explanation given above is, however, weakened by our observation of a deuterium isotope effect for phosphate catalysis of decarboxylation. An obvious alternate interpretation of our result is that water does not function as a general acid catalyst in this reaction. The rate constant for general acid catalysis by water, calculated from the Bronsted equation using values of the pK_a, α, and water concentration equal to 16.47, 0.9, and 55 M, respectively, is $3.5 \times 10^{-9}\ M^{-1}\ \text{min}^{-1}$ at 10°. The discrepancy between this value and the rate constant calculated from the activation parameters, equal to $5.1 \times 10^{-5}\ M^{-1}\ \text{min}^{-1}$, suggests that the predominant reaction is an uncatalyzed one.

Acid Catalysis for Decarboxylation. Proton transfer in hydronium ion catalyzed decarboxylation may proceed in a preequilibrium or as part of the rate-limiting step. A preequilibrium mechanism has been proposed in pyridineacetic acid decarboxylation[15] since high reactivity is observed with the α- and γ-substituted compounds. Although these results exclude a mechanism involving intramolecular proton transfer from the carboxyl group they are also consistent with a mechanism in which hydronium ion functions as a general acid catalyst. The situation is unresolved with acetoacetic acid where preequilibrium[26]

(13) P. Haake and J. Mantecón, *J. Am. Chem. Soc.*, **86**, 5230 (1964).
(14) (a) H. L. Finkbeiner and M. Stiles, *ibid.*, **85**, 616 (1963); (b) K. J. Pedersen, *Acta Chem. Scand.*, **3**, 676 (1949).
(15) W. von E. Doering, *J. Am. Chem. Soc.*, **72**, 143 (1950).
(16) R. Steinberger and F. H. Westheimer, *ibid.*, **71**, 4158 (1949).
(17) J. E. Prue, *J. Chem. Soc.*, 2331 (1952).
(18) A. S. Mildvan, M. C. Scrutton, and M. F. Utter, *J. Biol. Chem.*, **241**, 3488 (1966).
(19) B. H. Gibbons and J. T. Edsall, *ibid.*, **238**, 3502 (1963).
(20) K. J. Pedersen, *J. Am. Chem. Soc.*, **58**, 240 (1936).
(21) B. R. Brown, *Quart. Rev.* (London), **5**, 131 (1951).
(22) (a) Y. Pocker, *Chem. Ind.* (London), 599, 1383 (1959); (b) T. Riley and F. A. Long, *J. Am. Chem. Soc.*, **84**, 522 (1962).
(23) K. B. Wiberg, *ibid.*, **77**, 5987 (1955).
(24) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).
(25) F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).
(26) K. J. Pedersen, *J. Phys. Chem.*, **38**, 559 (1934).

and rate-limiting proton transfer mechanisms[27] have been proposed. The general acid catalyzed decarboxylation of carboxyimidazolidone involves proton transfer in the rate-limiting step, and the reasonably good adherence of the hydronium ion catalysis to the Brønsted relationship for general acid catalysis strongly suggests that this reaction occurs by a similar mechanism. The acid catalysis in this reaction is undoubtedly directed toward the ureido system rather than the more basic carboxyl group since in the latter case the carboxyimidazolidone would be stabilized. The protonation of the weakly basic ureido system in the reaction is consistent with the postulate of Reimann and Jencks[28] that in general acid catalyzed reactions the catalysis will be directed toward the atom(s) becoming more basic as the reaction proceeds.

The insensitivity of carboxyimidazolidone and also presumably carboxybiotin to specific acid catalysis is probably the most remarkable feature of these compounds and apparently accounts for the fact that carboxybiotin–enzyme intermediates are isolable. Susceptibility to acid catalysis can be correlated with the stability of the conjugate acid of the leaving group, and this criterion appears to account for the relative rates of the uncatalyzed and acid-catalyzed decarboxylation of carboxyl compounds described above. It might be expected that acid catalysis would be observed with all carboxyl compounds since the basicity of the leaving group increases enormously as the reaction proceeds and acid catalysis directed toward this group should facilitate the reaction. It is evidently important for the proton transfer to be concerted with carbon–oxygen double bond formation, and for this to occur with the trihalogenoacetic acids the catalysis would have to be directed to the carbon–carbon bond. Except for some recent work in which ketone halogenation has been demonstrated to occur prior to enolization[29] there does not appear to be any precedent for this type of reaction. An especially dramatic demonstration of the potential reactivity of a carboxyl substrate in which the leaving group is highly basic has come from some recent work with morpholine carbamate. Previous studies of carbamate decomposition did not separate the contributions of the acid-catalyzed and uncatalyzed reactions, and when this is done with morpholine carbamate the rate constant for hydronium ion catalysis is found to be approximately 1.3×10^{10} M^{-1} min^{-1} at 10°.[30] Comparison of this carbamate with carboxyimidazolidone suggest that the lesser reactivity of the latter compound arises from the low basicity of the urea nitrogen atom.

Enzymatic Transcarboxylation. Turnover numbers for biotin enzymes have been found to be in the range $0.1–8.5 \times 10^3$ moles/min/mole of biotin[3a] which must be compared with the rate constant 2.5×10^{-3} min^{-1} for the decarboxylation of the carboxyimidazolidone anion. The rate-limiting step in enzymatic transcarboxylation is not known and, therefore, the rates of cleavage of the C–N bond in an N-carboxybiotin–enzyme intermediate are equal to or greater than the rates cited above. The far greater rate of C–N bond cleavage in the enzymatic carbon dioxide transfer reaction might originate from there being a greater driving force for displacement of the biotin anion by nucleophilic attack of the acyl coenzyme A as compared with the unimolecular decarboxylation. Although the magnitude of this effect is difficult to evaluate, comparison of the rate constant for expulsion of the acetone anion by decarboxylation of acetoacetate ($k = 4.5 \times 10^{-4}$ min^{-1})[20] with the rate constant for the hydroxide-catalyzed dealdolization of diacetone alcohol (2.2×10^{-1} M^{-1} min^{-1})[31] suggests that the decomposition of an anionic tetrahedral intermediate might be expected to provide a greater driving force than decarboxylation. Greater reactivity would also be expected if the reaction proceeds without a tetrahedral intermediate but rather involves a direct attack by the anion of an acyl coenzyme A. A limitation of this proposal is that the rate difference with acetoacetate and diacetone alcohol disappears at neutral pH where ionization of the alcohol function is repressed. A similar limitation would be expected to hold with the enzymatic transcarboxylation where at neutral pH an anionic tetrahedral intermediate or an anion of acyl CoA would only be formed at very low concentrations. Results obtained in studies of nonenzymatic hydrolyses of anilides,[32] Schiff bases of aliphatic amines,[33] and an iminolactone[34] have been interpreted in terms of a mechanism in which proton transfer from the hydroxyl group of a tetrahedral intermediate is required for C–N bond cleavage. With this limitation in mind, we would like to propose a mechanism for enzymatic transcarboxylation in which proton transfer from the hydroxyl group of a tetrahedral intermediate is general base catalyzed and is concerted with general acid catalysis directed toward the ureido nitrogen atom. This mechanism is favored since it avoids the formation of unstable intermediates and receives support from recent studies of iminolactone[35] and anilide[36] hydrolysis in which the enormous catalytic advantage of concerted acid–base catalysis for decomposition of a tetrahedral intermediate has been demonstrated. Formation of the tetrahedral intermediate is envisioned as involving metal ion or general base catalysis for the attack of the acyl coenzyme A as described by Utter and co-workers.[18] The special property of biotin that we would like to call attention to is the insensitivity of the carbamic acid of this compound to both specific and general acid catalysis, which assures the existence of the carboxybiotin intermediate in the presence of solvent hydronium ions and the general acid catalysts associated with the enzyme.

Equilibrium Constant for Decarboxylation. Wood and co-workers[37] have carried out equilibrium measurements of a series of reactions which have enabled them to estimate a value of −4.74 kcal for the change in standard free energy for decarboxylation of enzyme-bound carboxybiotin at pH 7 (eq 5). If it is assumed

(27) F. H. Westheimer and W. A. Jones, *J. Am. Chem. Soc.*, **63**, 3283 (1941).
(28) J. E. Reimann and W. P. Jencks, *ibid.*, **88**, 3973 (1966).
(29) (a) C. Rappe, *Acta Chem. Scand.*, **20**, 1721 (1966); (b) R. P. Bell and K. Yates, *J. Chem. Soc.*, 1927 (1962).
(30) Unpublished work in this laboratory.
(31) V. K. La Mer and M. L. Miller, *J. Am. Chem. Soc.*, **57**, 2674 (1935).
(32) M. L. Bender and R. J. Thomas, *ibid.*, **83**, 4183 (1961).
(33) E. H. Cordes and W. P. Jencks, *ibid.*, **85**, 2843 (1963).
(34) G. L. Schmir and B. A. Cunningham, *ibid.*, **87**, 5692 (1965).
(35) B. A. Cunningham and G. L. Schmir, *ibid.*, **88**, 551 (1966).
(36) S. O. Eriksson and C. Holst, *Acta Chem. Scand.*, **20**, 1892 (1966).
(37) H. G. Wood, H. Lochmüller, C. Riepertinger, and F. Lynen, *Biochem. Z.*, **337**, 247 (1963).

$$\text{enz–biotin–CO}_2 + \text{H}_3\text{O}^+ \rightleftharpoons \text{enz–biotin} + \text{CO}_2 \quad (5)$$

that a similar constant describes the equilibrium for decarboxylation of carboxyimidazolidone,[38] it is possible to calculate the rate constant for the back reaction, k_r, in eq 6 from the equilibrium constant and the rate constant for hydronium ion catalysis ($k_f = k_{H_3O}/$

$$\text{imidazolidone–CO}_2 + \text{H}_3\text{O}^+ \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{imidazolidone} + \text{CO}_2 \quad (6)$$

H_3O^+). The value of k_r obtained from this calculation is 1.0×10^{-5} M^{-1} min^{-1}. We have, as yet, been unable to obtain a rate constant for the reaction of imidazolidone with carbon dioxide; however, we have set an upper limit to this constant equal to 5.3×10^{-2} M^{-1} min^{-1} (25°).[30,39] The value of k_r obtained from the equilibrium constant suggests that the reactivity of 55 M water is 2.3×10^5 times that of 1 M imidazolidone. Although previous efforts to observe nucleophilic reactions of imidazolidone with activated acyl compounds were not successful because of the greater reactivity of water,[2] we are dubious that the difference in nucleophilic reactivity is as large as that calculated here. Studies with acetaldehyde indicate that the pseudo-first-order rate constant for reaction of water[40] is approximately 200 times as large as the second-order rate constant for reaction of urea.[41] The apparently abnormally low reactivity calculated for imidazolidone suggests that the decarboxylation of the carboxybiotin intermediate is more exergonic than carboxyimidazolidone. Possible sources of this greater driving force might be a conformational change accompanying decarboxylation or a lesser dissociation constant for the carboxyl group of the biotin carbamic acid.[42]

(38) Knappe has reported that the rate of decarboxylation of carboxybiotin is 1.6 times that of carboxyimidazolidone supporting the assumption that the chemical properties of carbamates of imidazolidone and biotin are similar: J. Knappe, Abstracts of the 6th International Congress of Biochemistry, Vol. V, New York, N. Y., 1964, p 355.

(39) The rate constant for reaction of imidazolidone with carbon dioxide is calculated at 9.6×10^{-1} M^{-1} min^{-1} at 10° from extrapolation of a Brønsted plot for the reaction of amines of $pK = 3.6$–11.2 with carbon dioxide assuming a pK for imidazolidone of 0 (unpublished results obtained in this laboratory).

(40) R. P. Bell and P. G. Evans, *Proc. Roy. Soc.* (London), **A291**, 297 (1966).

(41) Calculated from the data given in Figure 2 in L. do Amaral, W. A. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **88**, 2225 (1966).

(42) Equation 5, which describes the reaction of a proton with carboxybiotin, is actually the sum of two reactions, one forming the neutral carbamic acid and another producing biotin and carbon dioxide. The composite equilibrium constant, K_1 [K_1 = (enz–biotin–COOH) + (CO_2)(enz–biotin)/(enz–biotin–COO^-)(H_3O^+)], is related to the separate reactions for protonation [K_2 = (enz–biotin–COOH)/(enz–biotin–COO^-)(H_3O^+)] and decarboxylation [K_3 = (enz–biotin)(CO_2)/(enz–biotin–COOH)] by the relationship $K_1 = K_2K_3 + K_2$. Since it is very likely that K_2 is large, a significant portion of the driving force for decarboxylation apparently originates from elimination of the negative charge on the carboxyl function.

Communications to the Editor

The Synthesis of Jervine and Related Alkaloids[1]

Sir:

The title alkaloids are a group of numerous naturally occurring C-nor-D-homosteroid alkaloids,[2] and the structure[2] and configurations[2,3] of all the asymmetric carbons except C-17 and C-20 in jervine (I), a representative member of the group, have been established. We describe herein the conversion of 17-acetyl-5α-etiojerva-12,14,16-trien-3β-ol (VIII) into I.[4] Since compound VIII has been prepared directly[4b] or by degradation[5] of hecogenin, a totally synthesized sapogenin,[6] the present work constitutes, in a formal sense, a total synthesis of jervine. Moreover, in view of the known conversions, this work leads to the related alkaloids, veratramine[7] (II), 11-deoxojervine[7b] (III), and verarine[8] (IV).

N-Chlorination of 3-(*S*)-methylpiperidine[9] (V) with bleaching powder followed by treatment with alkali and then with acetic anhydride[10] afforded a 1:1 mixture of 1-acetyl-3-(*S*)-methyl-Δ^5-piperidine (VI) and its Δ^2 isomer, which could be separated by chromatography. The compound VI,[11] λ_{max}^{EtOH} 235 mμ (ϵ 16,000), ν_{max}^{film} 1670 and 1644 cm^{-1}, nmr ($CDCl_3$) τ 8.93 (Me at C-3), gave, on perbenzoic acid oxidation and pyrolysis, 1-acetyl-3-(*S*)-methyl-5-piperidone (VII), ν_{max}^{film} 1730 and 1634 cm^{-1}, in 9% over-all yield from V.

(1) Part IX of "C-Nor-D-homosteroids and Related Alkaloids"; Part VIII: H. Suginome, N. Sato, and T. Masamune, *Tetrahedron Letters*, 1557 (1967).

(2) C. R. Narayanan, "Progress in the Chemistry of Organic Natural Products," Vol. XX, L. Zechmeister, Ed., Springer-Verlag, Vienna, 1962, p 298.

(3) The configurations of C-17 and C-20 have been assigned solely from the biogenetical ground and those of C-22 and C-23 revised recently as shown in the formula: (a) O. Wintersteiner and M. Moore, *J. Am. Chem. Soc.*, **78**, 6193 (1956); (b) J. W. Scott, L. J. Durham, H. A. P. deJongh, U. Burckhardt, and W. S. Johnson, *Tetrahedron Letters*, in press.

(4) After submission of this communication, Professor Johnson informed us that his group has completed both the synthesis of veratramine II from VIII and the total synthesis of VIII: (a) W. S. Johnson, H. A. P. deJongh, C. E. Coverdale, J. W. Scott, and U. Burckhardt, *J. Am. Chem. Soc.*, **89**, 4523 (1967); (b) W. S. Johnson, J. M. Cox, D. W. Graham, and H. W. Whitlock Jr., *ibid.*, **89**, 4524 (1967). We are grateful to Professor Johnson for making available to us prepublication copies of his manuscripts.

(5) (a) H. Mitsuhashi and K. Shibata, *Tetrahedron Letters*, 2281 (1964); b) W. F. Johns and I. Laos, *J. Org. Chem.*, **30**, 4220 (1965).

(6) Y. Mazur, N. Danieli, and F. Sondheimer, *J. Am. Chem. Soc.*, **82**, 5889 (1960).

(7) (a) T. Masamune, Y. Mori, M. Takasugi, and A. Murai, *Tetrahedron Letters*, 913 (1964); (b) T. Masamune, Y. Mori, M. Takasugi, A. Murai, S. Ohuchi, N. Sato, and N. Kaisui, *Bull. Chem. Soc. Japan*, **38**, 1374 (1965).

(8) T. Masamune, I. Yamazaki, and M. Takasugi, *ibid.*, **39**, 1090 (1966). N-Acetylverarine was converted into IV by saponification.

(9) A. Ladenburg, *Ber.*, 27, 75 (1894). The hydrobromide of its N-methyl derivative had $[\alpha]_{589}$ +0.012°, $[\alpha]_{480}$ 0.0°, and $[\alpha]_{350}$ −1.7° (water): S. Okuda, K. Tsuda, and H. Kataoka, *Chem. Ind.* (London), 512 (1961).

(10) *Cf.* C. Schöpf, A. Komzak, F. Braun, and E. Jacobi, *Ann.*, **559**, 1 (1948).

(11) Satisfactory analyses were obtained for all new compounds described herein.