that above pH 5 C-protonation was rate limiting. This step was sensitive to general acid catalysis with $\alpha = 0.50$. On considering the results obtained for ECC, it is clear that the rate-determining step at high buffer concentration, where the observed rate constant increased with increasing hydrogen ion concentration from pH 7 to pH 4 and had a solvent isotope effect consistent with specific acid catalysis, must be hydration of the imine.

The rate-determining step at zero buffer concentration is subject to general acid–base catalysis, as is shown by its sensitivity to buffer concentration, and the solvent isotope effect. Both C-protonation of the enamine and loss of amine from the carbinolamine are subject to general acid catalysis. However, the process at zero buffer concentration is still decreasing in rate with decreasing hydrogen ion concentration at pH 6, so that the uncatalyzed rate is less than 0.003 min^{-1}; the equilibrium constant for enamine formation at pH 6 is 0.94 M^{-1}, so that if this process were loss of amine from the carbinolamine, the rate constant for uncatalyzed formation of the carbinolamine would have to be less than 0.003 M^{-1} min^{-1}; this is contrary to what is found in related systems,[16] where carbinolamine formation is fast as long as the amine is free to react. Therefore the rate-determining step at zero buffer concentration at pH 5 and 6 cannot be loss of amine from the carbinolamine and must be C-protonation of the enamine. The decrease in rate constant at pH 4, though small, is outside experimental error; this may represent a change to partly rate limiting loss of amine from 3. Loss of amine from the carbinolamine is expected[14,15] to become rate limiting at a pH around 4.[21]

In a recent study[6] of the hydrolysis of a variety of conjugated enamines, Bruice and Coward proposed that C-protonation of the enamine was rate determining in all cases which they studied, for pH >2. One of the compounds included in their study was ethyl β-anilinocrotonate which is closely similar to ECC. To confirm the generality of our observations, the effect of buffer concentration on the rate of hydrolysis of ethyl anilinocrotonate was investigated. The results (Tables I and II) clearly show that for this compound as well there is a nonlinear dependence of k_{obsd} on buffer concentration. By analogy with ECC we propose that the rate-determining step at high buffer concentration is hydration of the imine tautomer, and that C-protonation of the enamine becomes rate limiting at low buffer concentration. For ethyl anilinocrotonate, at least, our conclusions differ from those of Bruice and Coward.[22]

Acknowledgment. We gratefully acknowledge critical discussions, advice, and encouragement provided by Professor F. H. Westheimer, who originally suggested this problem.

(21) Analysis of the kinetics of the AAN-catalyzed decarboxylation of acetoacetate[5] led to rate constants for imine formation, and for the imine–enamine tautomerization. Imine formation was catalyzed by H^+ but insensitive to buffer concentration; imine–enamine tautomerization showed a large buffer catalysis term.

(22) Bruice and Coward observed only modest catalysis by buffer for any of the compounds they studied, which were mostly conjugated cyano enamines. This is quite different from the behavior observed for C-protonation of the enamines ECC or ethyl anilinocrotonate, as well as simpler enamines,[11,20] but similar to the behavior expected for rate limiting imine hydration.

Amine-Catalyzed Decarboxylation of Acetoacetic Acid. The Rate Constant for Decarboxylation of a β-Imino Acid[1]

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Abstract: Aminoacetonitrile (AAN) is the most powerful catalyst for the decarboxylation of acetoacetic acid out of a series of primary amines of widely varying pK_a. The rate constants for AAN-catalyzed decarboxylation are closely similar to those for imine formation from AAN and ethyl acetoacetate. Spectrophotometric study of AAN-catalyzed decarboxylation revealed three successive kinetic processes characterized by three pseudo-first-order rate constants. The imine from AAN and acetoacetate could be trapped by cyanoborohydride. Analysis of the kinetics led to rate constants for imine formation and hydrolysis and for interconversion of imine and enamine, as well as for the decarboxylation of the imine. This latter rate constant, 10 sec^{-1}, is 300,000 times faster than that for the decarboxylation of acetoacetic acid itself.

The spontaneous thermal decarboxylation of acetoacetic acid and related compounds is a well-understood reaction.[4-10] The amine-catalyzed decarboxylation of acetoacetic acid, though it has been known for a

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long time and studied repeatedly,[5,11,12] is less well understood, in large part because of the multistep nature of the catalytic process.[10] The decarboxylation of acetoacetic acid is also catalyzed by an enzyme, acetoacetate decarboxylase;[13-15] this catalysis has been shown[16-18] to involve imine formation. Thus amine-catalyzed decarboxylation is a simple model for the enzymic reaction, and it is of great interest to establish how much of the enzymic catalysis of decarboxylation is achieved simply by replacing C=O by C=N. The results reported below establish that there is an optimum pK_a for an amine serving as a catalyst. Of the amines studied, aminoacetonitrile (pK_a = 5.34) was the most efficient catalyst. Recent work by O'Leary has shown[19] that the decarboxylation step must be partially rate determining for the amine-catalyzed process.

In attempting to establish the nature of the catalysis by the enzyme, it is obviously very important to know the actual rate of decarboxylation of an imine of acetoacetic acid. We wish to report experiments leading to an analysis of the kinetics of the aminoacetonitrile-catalyzed decarboxylation of acetoacetic acid and to an evaluation of the rate of decarboxylation of the *N*-cyanomethylimine of acetoacetic acid.

Experimental Section

Materials. Aminoacetonitrile sulfate was purified as described previously.[20] Glycinamide bisulfate, *n*-butylamine hydrochloride, aniline hydrochloride, and trifluoroethylamine hydrochloride were recrystallized; purity was checked by melting point or titration. *p*-Aminobenzoic acid was sublimed. Lithium acetoacetate was prepared by the method of Hall.[21] Chemicals for buffers were reagent grade and were used without further purification.

Sodium cyanoborohydride was crystallized (as the dioxanate) from ethanol-dioxane, and then was dried to constant weight at 108°. *N*-Isopropylcyanomethylamine was prepared by treating an aqueous solution of acetone plus aminoacetonitrile sulfate with sodium borohydride; it crystallized as the hydrochloride: mp 165–167° dec; nmr (D_2O) δ 1.40, d, 6 H, J = 7 Hz ($(CH_3)_2CH$–); 3.68, m, 1 H, J = 7 Hz ($(CH_3)_2CH$–); 4.38, s, 2 H, (—ND_2CH_2CN).

Kinetics. Manometric kinetics were carried out using either Warburg manometers or Gilson respirometers; data were treated in the usual way[22,23] to convert readings into micromoles of CO_2 evolved. Rate constants were calculated from pseudo-zero-order graphs of micromoles of CO_2 evolved *vs.* time. Spectrophotometric kinetics were measured at 270 nm. "Slow" reactions with half-times of several seconds or longer were followed using a Cary 15 with a thermostated cell block. For the cyanoborohydride trapping kinetics, it was necessary to have a small Teflon-coated stirring bar in the cuvette to prevent accumulation of bubbles; an air-driven magnetic stirrer was used. Multiple runs were performed to ensure reproducible results.

Stopped-flow kinetics were performed in a Durrum-Gibson instrument. Control experiments showed that the deadtime was less than 3 msec.

Enol Content of Acetoacetate. The iodination procedure of Bell[24] was used. A suitable amount of LAA solution was placed on the flattened tip of a glass rod and added to a cell containing 2 ml of buffer 0.20 M in KI and 4.3×10^{-5} M in I, thermostated at 10°. Extrapolation of the initial pseudo-zero-order change in absorbance to zero time gave the initial absorbance. Zero time was taken at the instant the glass rod passed through the light path as the LAA was added. From the initial absorbance change, the enol content was calculated using an extinction coefficient[24] for the triiodide ion of 2.5×10^4 at 353 nm.

At pH 5.94 the average enol content measured (average of eight values, with acetoacetate concentration ranging from 8×10^{-5} to 1.2×10^{-3} M) was 0.078%. At pH 2.87, the average value (four determinations) was 0.58% enol (lit.[25] 0.73%, at 0°).

Products of Cyanoborohydride Trapping. AAN (0.332 M), LAA (0.228 M), and $NaBH_3CN$ (0.25 M) were allowed to react for 3 hr at pH 5.0 in D_2O. An nmr spectrum of the product showed no signals attributable to acetone or acetoacetate. There were, however, three new doublets at $\delta \simeq 1.2$. Acidification of the product solution led to better separation of these peaks: nmr, D_2O, acidified: δ 1.28 (d, J = 7 Hz, $CH_3CHOHCD_2CO_2D$), 1.36 (d, J = 7 Hz, $CH_3CH(CD_3)ND_2^+CH_2CN$), 1.48 (d, J = 7 Hz, $NCCH_2ND^+$-$CH(CH_3)CD_2CO_2D$). Addition of 2-propanol to a portion of the acidified product solution gave a fourth doublet in this region; thus 2-propanol is not part of the reaction product. Addition of freshly distilled β-hydroxybutyric acid to the acidified product solution caused an increase in the intensity of the doublet at δ 1.28. Addition of *N*-isopropylcyanomethylamine hydrochloride to the acidified product solution caused the doublet at δ 1.36 and a singlet at δ 4.32 to increase in intensity. The third product, giving the doublet at δ 1.48, is assigned the structure β-cyanomethylaminobutyric acid; the doublet is at the same position as the doublet observed for β-aminobutyric acid (δ 1.50, J = 7 Hz).

Results

Spontaneous Decarboxylation. The rate of decarboxylation of acetoacetic acid was measured as a function of pH in buffered aqueous solution at 30° with ionic strength 0.1 M. The apparent first-order rate constants could be fitted to the equation

$$k_{obsd} = (k_a[H^+] + k_i K_a)/([H^+] + K_a)$$

where k_a is the rate constant for decarboxylation of neutral acetoacetic acid; k_i is the rate constant for decarboxylation of acetoacetate anion; and K_a is the acid ionization constant for acetoacetic acid. Fitting the data to this equation by the method of least squares gave $k_a = 29.1 \pm 0.6 \times 10^{-6}$ sec^{-1}, $k_i = 0.456 \pm 0.015 \times 10^{-6}$ sec^{-1}, and $K_a = 2.84 \pm 0.12 \times 10^{-4}$ (*i.e.*, $pK_a = 3.55 \pm 0.02$). These are in satisfactory agreement with earlier measurements.[5,26]

Primary Amines as Catalysts for Decarboxylation. To determine the optimum pK_a for an amine acting as a catalyst for the decarboxylation of acetoacetic acid, a series of primary amines of widely varying pK_a was studied. For these kinetics the evolution of CO_2 was followed manometrically. These results (Figure 1) clearly show that AAN is the best catalyst of the amines studied.[27] This amine was studied in more detail.

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(27) The pK_a's of the amines were: *p*-aminobenzoic acid,[28] 2.41; aniline,[28] 4.62; AAN,[29] 5.34; trifluoroethylamine,[30] 5.70; glycinamide,[31] 7.93; *n*-butylamine,[32] 10.60.
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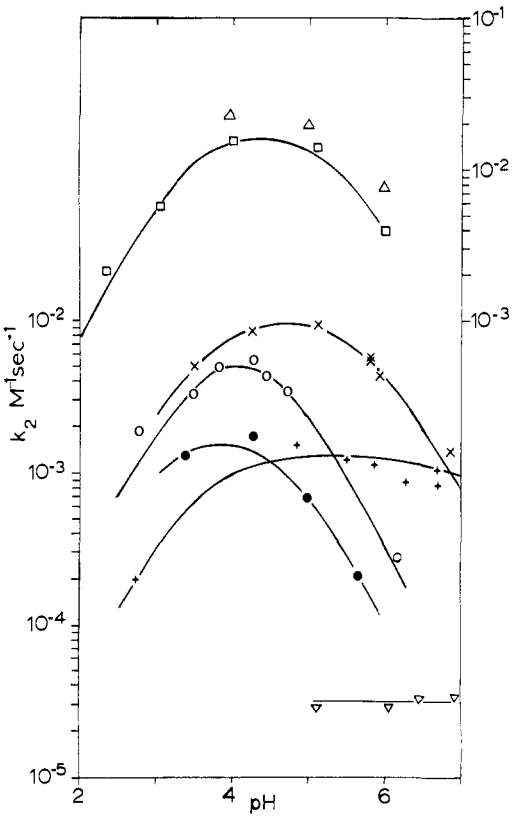

Figure 1. Second-order rate constants, expressed in terms of total amine and total acetoacetate concentrations, for amine-catalyzed decarboxylation of acetoacetate. For clarity, the points for AAN have been shifted vertically. Left-hand ordinate: (●) *p*-aminobenzoate; (○) aniline; (×) trifluoroethylamine; (+) glycinamide; (▽) butylamine. Right-hand ordinate: (□) AAN-catalyzed decarboxylation; (△) rate constants for imine formation from AAN and ethyl acetoacetate.

The apparent second-order rate constants for AAN-catalyzed decarboxylation were independent of amine concentration or buffer concentration within experimental error (see Table I).

If the rate-determining step for AAN-catalyzed decarboxylation of LAA is imine formation, then the rate

Table I. Aminoacetonitrile-Catalyzed Decarboxylation of Acetoacetic Acid[a]

pH	Ionic strength	k_2^{app}
	(a) Manometric Kinetics[b]	
2.36	0.1	0.0021 ± 0.0002
3.07	0.1	0.0056 ± 0.0005
4.00	0.1	0.0152 ± 0.0010
5.10	0.1	0.0137 ± 0.0007
6.00	0.1	0.0040 ± 0.0002
	(b) Spectrophotometric Kinetics	
6.00	0.5	0.0055
5.00	0.5	0.0149

[a] In water at 30.0 ± 0.1°, ionic strength maintained with KCl. [b] At each pH the buffer concentration was varied over a 4-fold range to test for buffer catalysis, and the amine concentration was varied over a 7-fold range. The second-order rate constants were independent of amine or buffer concentration.

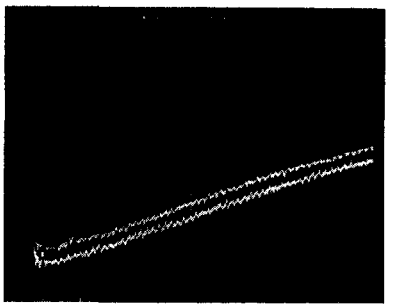

Figure 2. Oscilloscope picture from a stopped-flow kinetics experiment with 0.0802 *M* AAN and 0.01 *M* acetoacetate, pH 6.00 in H_2O, μ = 0.5 (KCl); time scale 0.1 sec/division.

constants for decarboxylation would be expected to resemble closely those for the reaction of AAN with ethyl acetoacetate to form an imine. The product of the reaction of AAN with ethyl acetoacetate is the enamine, ethyl β-cyanomethylaminocrotonate. It has been shown[33] that at high buffer concentrations the rate-determining step for the hydrolysis of this enamine is the acid-catalyzed addition of water to the imine; tautomerization of enamine to imine is a rapid preequilibrium under these conditions. Therefore, in the reverse direction, imine formation is rate determining for enamine formation.

From the rate constants for imine hydrolysis determined in the enamine hydrolysis study and the apparent equilibrium constant for enamine formation, the rate constants for imine formation from acetoacetate and AAN shown in Figure 1 were calculated. These are somewhat greater than the rate constants for decarboxylation, but are closely similar and respond similarly to changes in pH. The close similarity of the rate constants for imine formation from the ester, and amine-catalyzed decarboxylation, strongly suggests that for the latter process imine formation is also at least partly rate limiting.

Spectrophotometric Study of AAN-Catalyzed Decarboxylation. Rates of decarboxylation could also be measured by following the disappearance of absorbance at 270 nm (due to the enol of acetoacetate). Rate constants measured in this way were consistent with those measured manometrically. However, there was also an initial rapid increase in absorbance. When the initial phase of the reaction was studied by stopped-flow techniques, it became clear that in addition to the rise in optical density, which followed a first-order rate law, there was a very rapid process, manifesting itself as a lag before the optical density began to rise. This effect is shown in Figure 2.

Control experiments showed that this lag was not a result of the dead time of the instrument but was a real characteristic of the reaction system and must therefore represent another kinetically significant process. By extrapolating the observed line after the lag, the extent of the deviation could be seen to show a first-order decay, and a half-life for this process could be estimated; see Table II. These results indicate that the integrated rate law describing absorbance as a function of time is of the form

$$A = a + be^{-\lambda_1 t} + ce^{-\lambda_2 t} + de^{-\lambda_3 t}$$

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Table II. Aminoacetonitrile-Catalyzed Decarboxylation: the Fast Reaction[a]

pH	AAN, *M*	λ_3,[b] sec^{-1}	λ_2,[c] sec^{-1}	λ_1,[c,d] sec^{-1}	ΔOD[e]
4.99	0.321	0.00478	2.00	71	0.41
5.00	0.161	0.00239	1.44	28	0.21
5.00	0.0802	0.00119	1.17	31	0.10
6.00	0.161	0.000890	0.636	37	0.15
6.00	0.0802	0.000445	0.513	29	0.075
5.39[f]	0.321	0.00354	0.520	38	0.41

[a] In water at 30.0 ± 0.1°; μ = 0.5 *M* (KCl). [b] Obtained from experiments in which the "slow" reaction was studied. [c] Measured by stopped-flow techniques. [d] Because of the very small amplitude of the λ_1 process the precision of these numbers is low; errors are estimated at 30–40%. [e] The amount of increase in optical density for the λ_2 process; determined in "slow" kinetics experiments. ΔOD is expressed in terms of a 1-cm path as was used for "slow" experiments and not the 2-cm path used in stopped-flow experiments. [f] In D_2O; pD = pH meter reading + 0.41.[g] [g] A. K. Corington, M. Paabo, R. A. Robinson, and R. G. Bates, *Anal. Chem.*, **40**, 700 (1968).

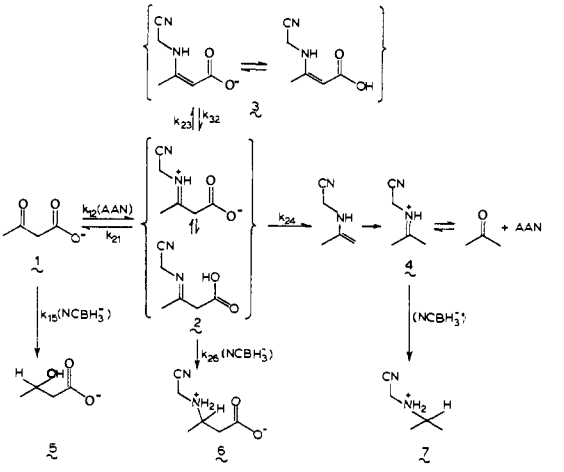
Cyanoborohydride Trapping Experiments. Cyanoborohydride is stable[34] in aqueous solution at pH 5 or 6 and is known to be much more reactive towards iminium ions than toward ketones.[35] Control experiments[36] showed that LAA was reduced slowly by cyanoborohydride ion at pH 5 and that reductive amination of acetone in the presence of 0.321 *M* AAN was 50 times faster than simple reduction in the absence of AAN. Therefore it seemed reasonable to hope that cyanoborohydride would trap the imine formed from AAN and LAA. In fact, when AAN and LAA were allowed to react in the presence of cyanoborohydride, the products, identified on the basis of nmr spectral evidence, are **5**, **6**, and **7**. The kinetics of the reaction were followed spectrophotometrically with variable amounts of $NCBH_3^-$ present; see Table III. These results clearly show that there is a nonlinear dependence of observed rate constants on $NCBH_3^-$ concentration. The set of reactions shown in Scheme I leads to eq 1 for the observed pseudo-first-order rate constant for the disappearance of LAA as a function of cyanoborohydride concentration

Table III. Trapping of the Imine with Cyanoborohydride[a]

pH	$NaBH_3CN$, *M*	k_{obsd}, sec^{-1}	Parameters from least-squares fitting to eq 1[b]
4.98	0	0.00469	a = 0.03 ± 0.39
4.99	0.0384	0.00597	b = 0.2 ± 1.4
4.99	0.0621	0.00747	c = 16 ± 80
4.99	0.102	0.00964	d = 43 ± 530
5.00	0.156	0.0104	
5.01	0.211	0.0118	
5.00	0.236	0.0151	
5.00	0.317	0.0143	
4.99	0.388	0.0171	
5.00	0.478	0.0178	
4.99	0.530	0.0200	
6.00	0	0.00174	a = 0.0052 ± 0.0017
6.01	0.0411	0.00232	b = 0.0031 ± 0.0010
5.98	0.0684	0.00267	c = 17 ± 10
6.02	0.102	0.00293	d = 1.8 ± 0.6
6.04	0.180	0.00345	
6.02	0.336	0.00462	
6.02	0.508	0.00500	
6.01	0.414	0.00490	
6.51	0	0.000617	a = 0.00006 ± 0.00032
6.53	0.0336	0.000866	b = 0.0018 ± 0.0003
6.52	0.0652	0.00107	c = 15 ± 6
6.52	0.112	0.00124	d = 2.9 ± 0.4
6.52	0.213	0.00138	
6.52	0.340	0.00152	
6.52	0.533	0.00162	

[a] In aqueous solution, at 30.0 ± 0.1°, with ionic strength 1.0 *M* (KCl), AAN = 0.321 *M*. [b] Parameters are defined in the text.

Scheme I



$$k_{obsd} = a[NCBH_3^-] + b(1 + c[NCBH_3^-])/(d + c[NCBH_3^-]) \quad (1)$$

where $a = k_{15}$; $b = k_{12}$(AAN); $c = k_{26}/k_{24}$; and $d = 1 + k_{21}/k_{24}$. The data could be fitted to this equation, although the results at pH 5 did not lead to well-defined parameters; see Table III.

The results of this curve fitting can be tested for internal consistency since the kinetics scheme requires that $d = 1 + k_{21}/k_{24}$ and $c = k_{26}/k_{24}$ be pH independent.

The values obtained for $1 + k_{21}/k_{24}$ are: pH 5.00, 44 ± 530; pH 6.00, 1.8 ± 0.6; pH 6.52, 2.9 ± 0.4. These values are the same within the estimated standard deviations, and the value from the data at pH 6.52 is the most precise, as is expected since these showed the most definite curvature. The value of k_{26}/k_{24} should also be pH independent, as was found; the values were: pH 5, 16 ± 80; pH 6, 17 ± 10; pH 6.52, 15 ± 6. The weighted average value of $1 + k_{21}/k_{24}$ is 2.6 ± 0.3.

Discussion

The initial rise in optical density which is observed when AAN and LAA are mixed is reasonably ascribed to formation of the enamine; there is no chemically plausible route for direct decarboxylation of the enamine so it must tautomerize to the imine, which has previously been considered to be the compound which actually decarboxylates.[37a] Thus the minimum ki-

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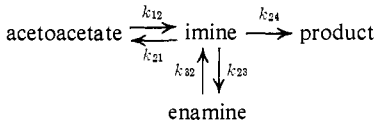
(37) (a) Imine formation is expected to proceed by way of an intermediate carbinolamine, but studies of imine formation in simple systems have shown that the carbinolamine should only be present at very low concentrations.[37b] The kinetic data did not require the inclusion of carbinolamine formation and decomposition as discrete steps: k_{12} and k_{21} should of course be interpreted not as simple rate constants, but as the combinations of microscopic rate constants appropriate for a two-step process proceeding *via* a transient intermediate. (b) J. Hine and F. A. Via, *J. Amer. Chem. Soc.*, **94**, 190 (1972).

Table IV. Values of the Rate and Equilibrium Constants for the Reaction of Aminoacetonitrile with Acetoacetic Acid[a]

	[AAN], M					
	0.321	0.161	0.080	0.321[b]	0.161	0.080
	pH 5.00			pD 5.39	pH 6.00	
k_{12}', M^{-1} sec^{-1}	0.038 ± 0.005	0.039 ± 0.005	0.039 ± 0.002	0.029 ± 0.004	0.014 ± 0.002	0.014 ± 0.002
k_{21}, sec^{-1}	27 ± 11	12 ± 5	15 ± 5	20 ± 2	17 ± 7	14 ± 6
$K_1 = k_{12}'/k_{21}$, M^{-1}	0.00142 ± 0.0008	0.0033 ± 0.0018	0.0026 ± 0.0007	0.0015 ± 0.0003	0.0008 ± 0.0004	0.0010 ± 0.0006
k_{23}, sec^{-1}	27 ± 12	8 ± 3	7 ± 3	5.1 ± 0.8	9 ± 4	6 ± 2
k_{32}, sec^{-1}	3.3 ± 0.5	2.0 ± 0.2	1.5 ± 0.2	0.60 ± 0.06	0.84 ± 0.09	0.64 ± 0.07
$K_2 = k_{23}/k_{32}$	8 ± 5	4 ± 2	5 ± 3	9 ± 2	11 ± 6	9 ± 4
k_{24}, sec^{-1}	17 ± 7	8 ± 3	9 ± 4	12 ± 2	11 ± 5	9 ± 4

[a] In H_2O at 30.0 ± 0.1°, μ = 0.5 (KCl). [b] In D_2O.

netic scheme is



This scheme will lead to an integrated rate law with three exponential terms, which is the behavior which was observed. The fact that some enamine was formed requires that k_{23} be at least competitive with k_{24}. If decarboxylation of the imine is slow enough that enamine formation is competitive with it, then hydrolysis of the imine may also compete with decarboxylation. In order to test whether k_{21} and k_{24} were comparable, experiments were carried out in which the imine was trapped before it could decarboxylate. This was done by allowing AAN and LAA to react in the presence of $NCBH_3^-$, and measuring the rate of consumption of LAA.

The results showed that the imine could be trapped and analysis of the kinetics of the disappearance of LAA in the presence of AAN and varying amounts of cyanoborohydride led to a value of 2.6 ± 0.3 for the quantity $(1 + k_{21}/k_{24})$, showing that k_{21} and k_{24} are indeed very similar.

There is now sufficient information in hand to solve the kinetic equations describing the spectrophotometric decarboxylation studies. This system requires five rate constants k_{12}, k_{21}, k_{23}, k_{24}, and k_{32}. Thus its solution requires the measurement of five independent quantities, which are λ_1, λ_2, λ_3, ΔOD (the magnitude of the initial rise in optical density), and $1 + k_{21}/k_{24}$. Approximate equations relating unknown and known quantities can be derived (see Appendix). Solving these equations leads to the rate and equilibrium constants in Table IV.

These results may be interpreted in terms of the chemical species involved. The apparent equilibrium constant K relates the concentration of the kinetically significant form of the imine to the total amine and acetoacetate concentrations. If the values of K are corrected for protonation of aminoacetonitrile, then it is clear that K_1 is 10 times larger at pH 5. This requires that the kinetically significant form of the imine be the neutral species (which is to some extent zwitterionic). From the known pK_a of acetoacetic acid, the equilibrium constant for the reaction of the neutral species can be calculated.

$$K = [CH_3C(CH_2COOH){=}NCH_2CN]/[CH_3COCH_2COOH][NCCH_2NH_2] = 0.25$$

The apparent equilibrium constant, K_2, relates the neutral form of the imine and the total enamine (since both neutral and anionic forms of the enamine will absorb). The extinction coefficient has been assumed to be the same for both forms as for the ester of the enamine, ethyl β-cyanomethylaminocrotonate.[38] This assumption has a serious effect on k_{23} and K_2 *only*, the other values being relatively insensitive to the value used for the extinction coefficient. K_2 is relatively imprecise, so that the values at pH 5 and 6 are not significantly different. This requires that the pK_a of the enamine be greater than 5. Considering the powerfully electron releasing properties of NH conjugated to the carboxyl group this is reasonable.[38] Carbamic acid has recently been shown[39] to have a pK_a of 5.25.

From K_2 and the equilibrium constant calculated above for imine formation from neutral AAN and acetoacetic acid, one obtains an equilibrium constant of 1 for enamine formation from AAN and acetoacetic acid, in gratifying agreement with the value for enamine formation from ethyl acetoacetate[33] (K = 0.94). It is noteworthy that the only rate constants which are sensitive to AAN concentration are k_{32} and k_{23}, which correspond to C-protonation and deprotonation. The analogous process for ethyl cyanomethylaminocrotonate has been shown[33] to be very sensitive to general acid catalysis.

The key results of this analysis are: (1) decarboxylation is partially rate determining for the AAN-catalyzed decarboxylation; (2) the specific rate constant for the decarboxylation of the imine is *ca.* 10 sec^{-1}.

It is noteworthy that the neutral imine **2** is 3 × 10^5 times as reactive as neutral acetoacetic acid at 30°. This enormous rate factor is responsible for the catalytic action of amines, despite the unfavorable equilibrium constants for imine formation.

Recently O'Leary and Baughn[19] have measured a ^{13}C isotope effect of *ca.* 1.03 for the AAN-catalyzed decarboxylation of acetoacetate, requiring that the decarboxylation step be at least partially rate determining. Assuming reasonable values for the isotope effect on k_{24}, they estimated k_{24}/k_{21} to be *ca.* 0.1–0.7,

(38) A pK_a for *trans*-$CH_3C(NH_2){=}CHCOOH$ can be estimated as follows. Charton[40] has shown that for a series of compounds, *trans*-$CH_3CX{=}CHCOOH$, the pK_a's correlate with σ_p, with $\rho = -3.29$. The enol of acetoacetic acid is more acidic than this correlation line predicts; this is attributable to the acid-strengthening effect of intramolecular hydrogen bonding.[41] Assuming that the effect of hydrogen bonding is the same for the amino compound, a pK_a of 5.4 is estimated, using σ_p for NH_2.

(39) S. L. Johnson and D. L. Morrison, *J. Amer. Chem. Soc.*, **94**, 1323 (1972).

(40) M. Charton, *J. Org. Chem.*, 30, 974 (1965).

(41) F. H. Westheimer and O. T. Benfey, *J. Amer. Chem. Soc.*, **78**, 5309 (1956).

which is in agreement with the value measured in this work, *i.e.*, 0.5 ± 0.2. Performing the calculation in the other direction, we may calculate from the two sets of data that $k_{24}^{12}/k_{24}^{13} = 1.05$.

The rate of the acetoacetate decarboxylase catalyzed decarboxylation (*i.e.*, k_{cat}) is 100 times faster than the rate of decarboxylation of the *N*-cyanomethylimine of acetoacetate.[42,43] Electronic effects on the rate of the reaction should be small, since the pK_a of the catalytic amino group of the enzyme is[44] 5.9, which is close to that of AAN. Thus the enzyme must accelerate the actual decarboxylation step, as well as facilitating imine formation. It has been suggested[19] that a local solvent polarity effect may be involved. It is also possible that the enzyme forces the zwitterion of the imine into a conformation in which the C–COO^- bond is perpendicular to the plane of the iminium ion. This is expected to be the reactive conformation because the C–C bond which must break is parallel to the π orbitals of the iminium ion; it is expected to be less stable than the planar form because of the greater separation of opposite charges and the loss of the intramolecular hydrogen bond.

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Appendix: Derivation of Equations Leading to an Analytical Solution for the Kinetics of AAN-Catalyzed Decarboxylation

Since the system involved five rate constants, its solution requires measuring five independent quantities related to these rate constants. A suitable set is λ_1, λ_2, λ_3, the three "rate constants," describing the absorbance–time curve, which are defined in the text; ΔOD, the increase in OD from the initial value to the maximum (evaluated from the cyanoborohydride trapping experiments).

$$1 + r = 1 + \frac{k_{21}}{k_{24}} \tag{2}$$

One can derive approximate equations relating λ_1 and λ_2 to the microscopic rate constants, starting from the rigorously valid conditions[45]

$$\lambda_1 + \lambda_2 + \lambda_3 = k_{32} + k_{12} + k_{21} + k_{23} + k_{24}$$

$$\lambda_1\lambda_2\lambda_3 = k_{24}k_{12}k_{32}$$

Since

$$\lambda_3 = \frac{k_{12}}{1 + k_{21}/k_{24}} \ll \lambda_1, \lambda_2 \tag{3}$$

then

$$\lambda_1 + \lambda_2 \simeq k_{32} + k_{21} + k_{23} + k_{24}$$

and

$$\lambda_1\lambda_2 = k_{32}(k_{21} + k_{24})$$

Since $\lambda_2 \ll \lambda_1$, and since it turns out that k_{32} is small relative to λ_1

$$\lambda_1 = k_{21} + k_{23} + k_{24} \tag{4}$$

$$\lambda_2 = k_{32}(k_{21} + k_{24})/(k_{21} + k_{23} + k_{24}) \tag{5}$$

At steady state

$$\Delta OD = \epsilon_{enamine}k_{23}k_{12}[LAA]_0/k_{32}(k_{21} + k_{24}) \tag{6}$$

Now with (2)–(6) we have five linear equations in five unknowns, which may be solved to give

$$k_{12} = \lambda_3(1 + r)$$

$$k_{32} = 1/(1/\lambda_2 - a)$$

$$k_{24} = \lambda_1(1 - \lambda_2 a)/(1 + r)$$

$$k_{23} = \lambda_1\lambda_2 a$$

$$k_{21} = \lambda_1(1 - \lambda_2 a)/(1 + 1/r)$$

with

$$a = \Delta OD/\{\lambda_3(1 + r)\epsilon_{enamine}[LAA]_0\}$$

The extinction coefficient of the enamine **3** is assumed to be the same as that for ethyl β-cyanomethyaminocrotonate,[33] *i.e.*, 17,100 ± 700.

(42) k_{cat} is 1.68×10^3 sec^{-1}: S. M. Coutts, Ph.D. Thesis, Harvard University, 1967.

(43) The ^{13}C isotope effect for the enzyme catalyzed decarboxylation is[19] 1.02, requiring partially rate-determining loss of CO_2.

(44) D. E. Schmidt, Jr., and F. H. Westheimer, *Biochemistry*, **10**, 1249 (1971).

(45) Derived by following standard procedures[46] for the solution of systems of first-order reactions.

(46) A. A. Frost and R. G. Pearson, "Kinetics and Mechanisms," 2nd ed, Wiley, New York, N. Y., 1961, pp 173–177.