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Charge Development in the Transition State for Decarboxylations in Water: Spontaneous and Acetone-Catalyzed Decarboxylation of Aminomalonate

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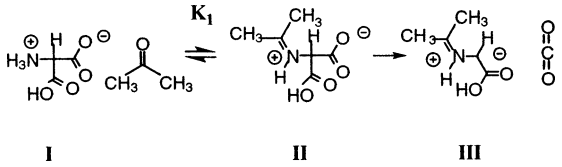
Richard and his associates have shown that acetone, like pyridoxal phosphate, catalyzes the racemization of amino acids in water.[1] Here, we report the ability of acetone to promote the decarboxylation of 2-aminomalonate (I, Scheme 1) and the existence

Scheme 1



of a relationship between the rates of decarboxylation of carboxylic acids and the carbon acidities of the products of their decarboxylation. That relationship implies the development of considerable negative charge in the transition state for decarboxylation in aqueous surroundings and correctly predicts that the benzoate ion should undergo decarboxylation in water at temperatures just below the critical point.

To determine the rate of its spontaneous decarboxylation, aminomalonate[2] (0.06 M) was incubated at 25 °C, in the presence of potassium phosphate, formate, malonate, or acetate buffers (0.8 M). At timed intervals, samples of the reaction mixture were removed for analysis and diluted with an equal volume of ^2H_2O to which pyrazine had been added as an integration standard. The progress of reaction was followed by ^1H NMR, monitoring the integrated intensities of the singlets corresponding to aminomalonate (4.1 ppm) and the product, glycine (3.5 ppm). No byproducts were observed. First-order rate constants were determined from semilogarithmic plots of the concentration of substrate remaining as a function of time (Figure 1a).

The zwitterionic form of aminomalonate was found to be most reactive, undergoing decarboxylation with a rate constant of 1.1×10^{-6} s^{-1} at 25 °C. Elimination of carbon dioxide would be expected to yield the ylide of glycine (^+H_3N-CH$^-$-COOH), the pK_{C-H} value of whose conjugate acid has been estimated as 21 by deuterium exchange.[3] At 25 °C, the aminomalonate zwitterion is decarboxylated 11 000 times more rapidly than is the monoanion of malonate,[4,5] indicating that the α-ammonium group reduces the free energy of activation by 5.4 kcals/mol. The nonreacting carboxylic acid substituent appears to lower the free energy of the transition state in water by 14.5 kcals/mol, as judged by comparison with the 10^{11}-fold slower decarboxylation of glycine.[6] Earlier work indicates that the cationic form of aminomalonate is decarboxylated much more slowly than the zwitterionic form at 45 °C;[7] and Figure 1b indicates that the anionic form, with both carboxyl groups ionized, is also resistant to decarboxylation.

Figure 1a shows that, in the presence of acetone (1.0 M), the rate of decarboxylation of aminomalonate (0.06 M) is enhanced ~100-fold and that, in the presence of acetone, the apparent rate of decarboxylation of aminomalonate varies with pH in a manner

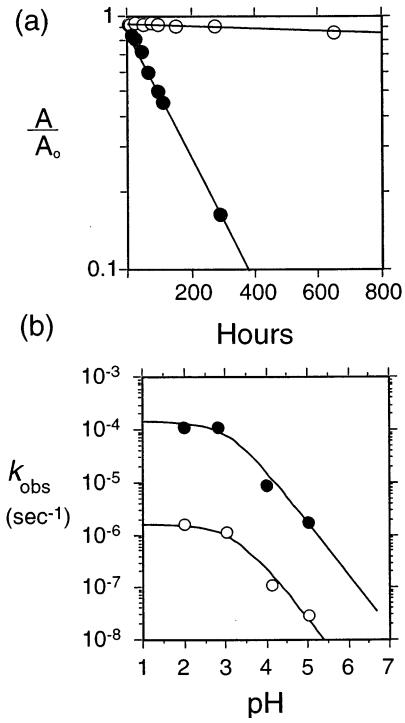


Figure 1. (a) Fraction of aminomalonate (0.06 M) remaining as a function of reaction time. The spontaneous reaction (open circles) was conducted in acetate (0.8 M) buffered solution at pH 5. Closed circles are data for the reaction under identical conditions with added acetone (1.0 M). (b) Observed rates of decarboxylation of aminomalonate (0.06 M) in the presence (closed circles) and absence (open circles) of 1.0 M acetone, plotted as a function of changing pH. The solid lines represent the behavior expected for reaction of the zwitterions of aminomalonate (pK_{COOH} 3.2) and iminomalonate (pK_{COOH} 3.1).

similar to its variation in the absence of acetone. That similarity of behavior is consistent with the view that, in both both cases, the reactant is an electrically neutral zwitterion and that acetone does not alter the pK_a value of the nonreacting carboxyl group significantly.

As in the case of glycine racemization,[1] acetone presumably functions as an electrophilic catalyst, forming a covalent imminium adduct (II) that destabilizes substituents on the α carbon atom. Elimination of carbon dioxide from II would yield the ylide of iminoglycine (III). The equilibrium constant for aminomalonate addition to acetone (K_1) is unfavorable. It seems reasonable to assume that K_1 may be similar to that observed for glycine, 3×10^{-3} M^{-1},[1] since the ionization of the second carboxyl group, which distinguishes aminomalonate from glycine, appears to be insensitive to acetone addition (Figure 1b). Thus, we estimate that the rate constant for decarboxylation of II exceeds that of I by a factor of 3×10^4.[8]

Comparison of these rate constants for spontaneous and acetone-catalyzed decarboxylation of aminomalonate indicates an imminium

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Table 1. First-Order Rate Constants Estimated from Reported Values for Spontaneous Decarboxylation of Carboxylate Ions and the pK_a Values of Carbon Acids Generated by Their Decarboxylation, 25 °C

carboxylate ion	$k_{25°C}$, (s^{-1})	$\sim t_{1/2}$	pK_{C-H}
iminomalonate	0.03[16]	22 s	14[1]
acetoacetate	4.5×10^{-7} [17]	18 days	19.16[18]
aminomalonate	1.1×10^{-6} [16]	8 days	21[3]
trichloroacetate	9×10^{-9} [19,20]	2 years	25[21]
malonate monoanion	9.5×10^{-11} [4,5]	230 years	25.6[22]
cyanoacetate	3×10^{-12} [20]	7000 years	28.9[11]
1-methylorotate	3×10^{-16} [13]	78,000,000 years	34[14]

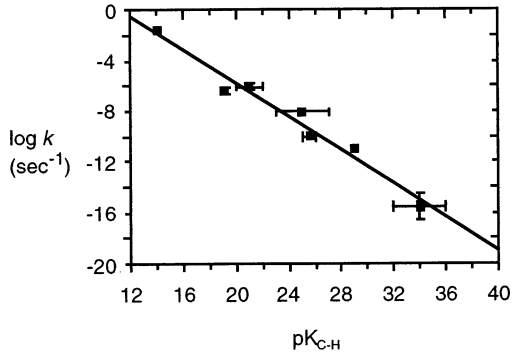

Figure 2. First-order rate constants estimated for decarboxylation of carboxylate ions plotted as function of product C−H acidity (Table 1).

substituent effect of −6.1 kcal/mol on the free energy of activation for decarboxylation, somewhat less pronounced than the imminium effect of −9.5 kcal/mol on the carbon acidity of glycine.[1] That effect is consistent with the development of a substantial amount of negative charge at the α-carbon atom in the transition state for decarboxylation. A Brønsted plot of rate constants for decarboxylation of aminomalonate, iminomalonate, and other carboxylic acids (Table 1), as a function of the carbon acidities of the products generated by their decarboxylation, exhibits a slope (β_{lg}) of −0.7 (Figure 2).

If the pK_{C-H} value of benzene is 43,[9] extrapolation of the plot in Figure 2 indicates that the benzoate ion should undergo decarboxylation in water with a rate constant of $\sim 10^{-22}$ s^{-1} at 25 °C. If we assume that $\Delta S^{\ddagger}$ for benzoate decarboxylation is the same as the average value observed for the other acids in Table 1 (0.010 kcal K^{-1} mol^{-1}), then the decarboxylation of the aqueous benzoate ion should be detectable at temperatures below the critical point of water. We verified that prediction experimentally by observing the accumulation of benzene in aqueous solutions of potassium benzoate (0.05 M) at 344 °C, indicating an apparent rate constant of 4×10^{-6} s^{-1}.[10] By the same criterion, the decarboxylation of the acetate ion (methane pK_{C-H} 49)[11,12] is expected to be slower than that of benzoate by a factor of 10 000. We found that solutions of sodium acetate (0.1 M), showed no trace of decarboxylation after incubation at 344 °C for 15 days.

Enzymes that catalyze amino acid decarboxylation use cofactors such as PLP to stabilize the carbanion generated by elimination of CO_2. In contrast, orotidine 5′-phosphate (OMP) decarboxylase acts purely as a protein catalyst. This enzyme converts OMP to UMP with a rate constant (k_{cat}) of 20 s^{-1}, whereas the spontaneous decarboxylation of OMP occurs with a rate constant of 3×10^{-16} s^{-1} in the absence of enzyme.[13] The anion produced by decarboxylation of OMP is extremely unstable, with an estimated pK_{C-H} value of 34 for the 6-CH group of product UMP.[14] In effect, this enzyme finds a way of stabilizing the 6-carbanion of UMP through binding interactions that are not yet fully understood. If the k_{cat} value for yeast ODCase is placed on the Brønsted plot in Figure 2, one is led to infer that the "effective" pK_{C-H} value of UMP, at the active site of yeast ODCase, is 9.5. The results of point mutation experiments suggest that the anion of UMP may be stabilized by interaction with a basic residue (Lys-93 in the yeast enzyme).[15] It would be of interest to know the extent to which the pK_a value of that residue may be perturbed from its value in solution, in the central complexes that arise during catalysis.

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