Nuclear Magnetic Resonance Investigation of the Spontaneous Decarboxylation of Oxalo-2-propionic Acid

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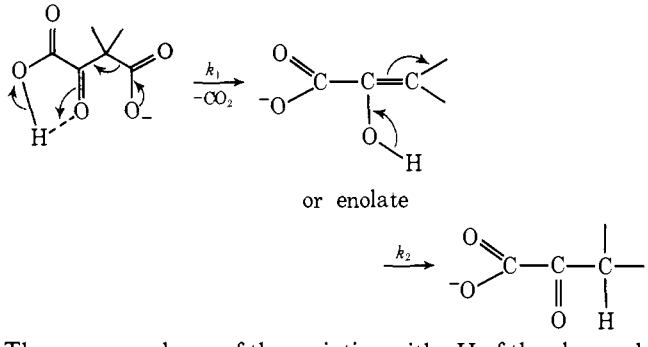
Abstract: The kinetics of the spontaneous decarboxylation of oxalo-2-propionic acid (OPA) has been investigated by NMR spectroscopy. The first kinetic evidence is presented for two first-order consecutive processes corresponding to the decarboxylation reaction and the subsequent ketonization of the enolate intermediate. The rate constants for the decarboxylation of diprotonated OPA and its monoanion were found to be 5.72×10^{-3} and 8.29×10^{-3} min^{-1}, respectively. The rate of the ketonization of the intermediate was found to be 1.47×10^{-3} M^{-1} min^{-1}. Equilibria as a function of pD were also measured on the keto-enol tautomerism of OPA as well as the hydration-dehydration steps. Results show that the hydrated form of OPA, which does not undergo decarboxylation, exists in appreciable concentration at low pD and could account for the retardation of the decarboxylation process when the fully protonated and partially ionized forms of OPA are the predominant species in solution. These studies demonstrate the usefulness of the NMR method as a tool for detecting the possible species and intermediates in this type of reaction system.

Introduction

The spontaneous,[1-7] metal-catalyzed,[3-12] and enzymatic[13] decarboxylation of β-keto acids that also contain α-keto acid groups have been the subject of several detailed kinetic studies and still continue to be of widespread scientific interest.

The mechanism of decarboxylation of oxaloacetic acid (OAA), dimethyloxaloacetic acid (DMOAA), and fluorooxaloacetic acid (FOAA) to pyruvic, α-ketoisovaleric acid, and fluoropyruvic acid has been discussed in terms of Scheme I.

Scheme I



The correspondence of the variation with pH of the observed rate constants for the first step to the degree of dissociation of OAA and DMOAA indicates that the decomposition proceeds most rapidly by way of the monoanion, in which hydrogen bonding between the carboxyl and the α-carbonyl oxygen may assist in the electron shift required for the rate-determining decarboxylation step. Evidence has been presented[4] for the retardation of the reaction when the fully ionized form is the predominant species in solution. While attention has been focused on the treatment of the observed rate constants in terms of degree of protonation of OAA and its analogues, little attention has been devoted to the other reactions which take place during the decarboxylation process.

In the case of OAA, conflicting reports have appeared from various laboratories concerning the numerical value of the equilibrium constant governing keto-enol tautomerization.[8,14-17] It now appears that the intensity of the ultraviolet band exhibited between 240 and 260 mμ, which had been used to estimate the enol species in enolization[18-20] and decarboxylation studies may not actually be an accurate measure of the concentration of this tautomer. The reason is that the molar absorbance at 255 mμ obtained in ether solution is used for estimating concentrations of enol species in aqueous solution, an assumption which may not be justified. Kun and co-workers[21] were successful in using NMR spectroscopy for determining the keto-enol tautomerization equilibrium, although they reported the presence of unknown species in addition to the enol and keto forms of OAA.

More recently Pogson and Wolfe[17] have shown that OAA exists in the hydrate form to an extent of 55% at pH 2. The rates of hydration-dehydration of OAA were found to be fast compared to its rate of decarboxylation. Since the hydrated form is not expected to exhibit any absorption in the uv and since spectrophotometric determinations of observed rate constants of decarboxylation of OAA and DMOAA were in excellent agreement with those obtained from manometric measurements, it follows that the rate determining step involves the keto form only. The retardation of the reaction at low pH could then very well be the result of the high concentration of the hydrated form. The hydrated form must also be taken into account in determining the percentage of the intermediate enolate species during the course of the reaction.

The application of NMR spectroscopy to the study of these systems should provide new information concerning not only the keto-enol tautomerization equilibrium and the role of the hydrate in the course of the reaction, but also the accurate determination of the rate constants k_1 and k_2 in Scheme I.

The present study is concerned with the model substrate oxalo-2-propionic acid (OPA), which can enolize, hydrate-dehydrate, and decarboxylate. The choice of such a model is prompted by the fact that the methyl substituent should provide a direct NMR probe for the measurement of rates and equilibria of reactions in D_2O. Because it can enolize, OPA is a better model for OAA than is DMOAA.

This is the first of a series of reports describing the spontaneous metal-catalyzed, and pyridoxamine-catalyzed decarboxylation of OPA. These investigations are considered significant as models for biological systems, since OAA and its analogues are probable intermediates for the decarboxylation of the corresponding aminodicarboxylic acids, and may be formed by transamination of the latter.

Experimental Section

Materials. Oxalo-2-propionic acid was prepared from diethyl oxalopropionate (Aldrich Chemical Co.) according to Galegov's method.[22] α-Ketobutyric acid was obtained from Mann Laboratories and was used without further purification. D_2O was 99.8% pure from Mallinckrodt. Acetic-d_4 acid and sodium acetate-d_3 were 99.5 and 99 mol % pure, respectively, from Diaprep. Sodium deuterioxide was

prepared by dropping D_2O onto clean reagent-grade sodium metal. Reagent grade potassium nitrate was used to control ionic strength.

Measurements. NMR spectra were obtained with a Varian HA 100 nuclear magnetic resonance spectrometer. Chemical shifts are reported in parts per million with respect to hexamethyldisiloxane (HMDS) in an internal capillary. The pD was measured using a Beckman Research pH meter fitted with a combination glass electrode. The pH meter was standardized with strong acid and base so as to read directly in hydrogen ion concentration. The deuterium ion concentration was then computed by adding 0.40 to the observed reading on the meter.[23] Deuterioacetate buffers were prepared from 1.0 M acetic-d_4 acid and 1.0 M sodium acetate-d_3. The sample temperature was 32 ± 1 °C.

Treatment of NMR Data. The decarboxylation of OPA, the formation of the α-ketobutyrate intermediate, and the enol–keto conversion of the latter followed first-order kinetics as determined by direct integration of the β-CH_3 signal of OPA and the intermediate (see Results) in the course of three half-lives. The results for the decomposition of OPA were fitted to the equation

$$A = A_0 e^{-k_1 t} \tag{1}$$

where A_0 is the initial concentration of OPA determined by extrapolation to the time of mixing. The rate for the subsequent enol–keto tautomerization was calculated from the solution to two first-order (A → B → C) processes which express B as follows[24]

$$B = A_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \tag{2}$$

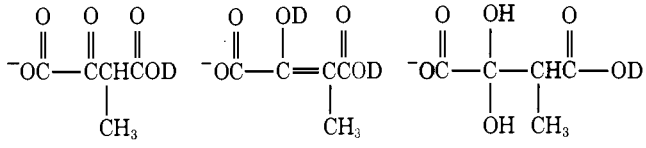
Values of k_2 were computed with a Compucorp 320/322 using the Newton Raphson method of iteration.[25] In eq 2, A_0 is known, k_1 is known from eq 1, and B is the direct integral of the β-CH_3 resonance of the α-ketobutyrate intermediate. The calculated t_{max} and B_{max} values are related to k_1 and k_2 by the following equations[24]

$$t_{max} = \left(\frac{1}{k_2 - k_1}\right) \ln \frac{k_2}{k_1} \qquad \ln \frac{A_0}{B_{max}} = k_2 t_{max}$$

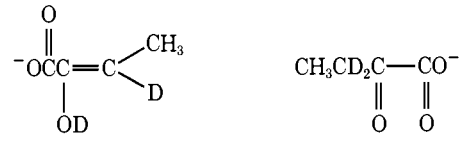
The percent of the enol form of OPA can be determined from the relative integrated intensities of OPA and its enol form.

Results and Discussion

In D_2O, the tautomers **1** and **2**, the hydrate **3**, the decomposition intermediate **4**, and the product from the ketonization of the latter (**5**) have different kinds of methyl groups and thus



1 **2** **3**

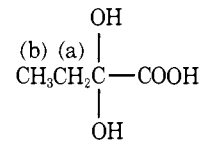


4 **5**

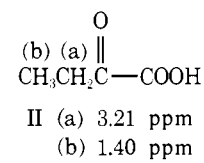
would be expected to give rise to different peaks in the NMR spectrum. In order to help identify the expected resonances, it was necessary to refer to the NMR spectra of α-ketobutyric acid (AKBA) in acid medium as well as in acetate buffer.

AKBA Acid and Deuterioacetate Buffer Spectra. AKBA acid spectra were investigated by Abbott and Martell.[26] Their results showed that AKBA is present as the keto and hydrate forms, and that the percent of hydrate decreases with increasing pD until at pD 4 the spectrum is nearly completely that of the keto form. The enolization of AKBA has been shown to be subject to general base catalysis by acetate ion and the second-order rate constant was reported to be (30–50) × 10^{-5} l. mol^{-1} min^{-1}.[27] When the spectrum of AKBA was run in 1.0 M deuterioacetate buffer at pD 4.60, it exhibited the same features as reported for the Zn(II), acetate system.[26] Originally, the spectrum exhibits the features of AKBA dimerization in H_2O and only later does the β-deuterated dimer form, as evidenced by the appearance of singlets shifted to higher field in place of the doublets and triplets. It is concluded then that in acetate buffer, dimerization of AKBA is favored.

OPA Acid Spectra. The spectrum of OPA in H_2O at pH 1.60, after 10 min from the time of mixing, consists of doublets at 1.57, 1.68, and 2.04 ppm, a singlet at 1.84 ppm, and quartets at 3.44 and 2.66 ppm. As the reaction proceeds, a decrease in the intensity of the doublets at 1.57 and 1.68 ppm and the singlet at 1.84 ppm is noticeable, while the doublet at 2.04 ppm gains intensity. This is coupled with the appearance of triplets at 1.40 and 1.24 ppm and quartets at 2.22 and 3.21 ppm. The doublet at 2.04 ppm keeps gaining intensity until late in the course of the reaction, when it starts disappearing slowly, while the triplets and quartets at 2.22 and 3.21 ppm continue to gain intensity and the singlet and other doublets lose intensity. This behavior is consistent with the formation of an enol intermediate which subsequently ketonizes to the keto and hydrate forms of AKBA. Thus the triplets at 1.24 and 1.40 ppm and the quartets at 2.22 and 3.21 ppm are assigned to the hydrate and keto forms, I and II, respectively, of AKBA. The doublet

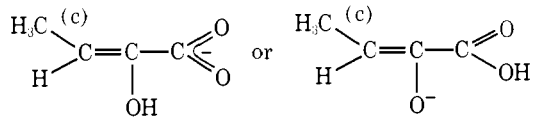


I (a) 2.22 ppm
(b) 1.24 ppm



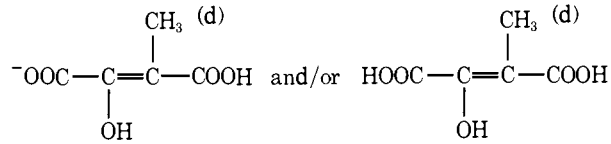
II (a) 3.21 ppm
(b) 1.40 ppm

at 2.04 ppm is almost certainly due to the methyl resonance of the intermediate III resulting from the decomposition of



III (c) 2.04 ppm

OPA. The expected quartet from the proton resonance of III is buried under the H_2O peak. The pair of doublets at 1.57 and 1.68 ppm must result from two forms of OPA existing in equilibrium, since their intensity ratio remains constant throughout the course of the reaction. Moreover, the intensity ratio of the singlet at 1.84 ppm to the sum of the integrated intensities of the doublets remains constant as well. Thus the singlet at 1.84 ppm is assigned to the enol form of OPA, IV,



IV (d) 1.84 ppm

and the doublets at 1.57 and 1.68 ppm are assigned to the hydrate V and the keto form VI of OPA. This assignment is based on the finding that OAA is partially hydrated in solution[17] and the fact that varying the pH caused a variation in the integral intensity of VI to V; VI being favored as the pH is raised, while the equilibrium constant governing this transformation remains the same. The quartets at 3.44 and 2.66 ppm then belong to the single proton resonances of VI and V, respectively.

In D_2O, all quartets vanish and the three doublets at 1.57, 1.68, and 2.04 ppm are replaced by broad singlets at 1.55, 1.67, and 2.03 ppm, while the singlet originally at 1.84 ppm in H_2O shows up at 1.82 ppm. The AKBA hydrated and keto forms

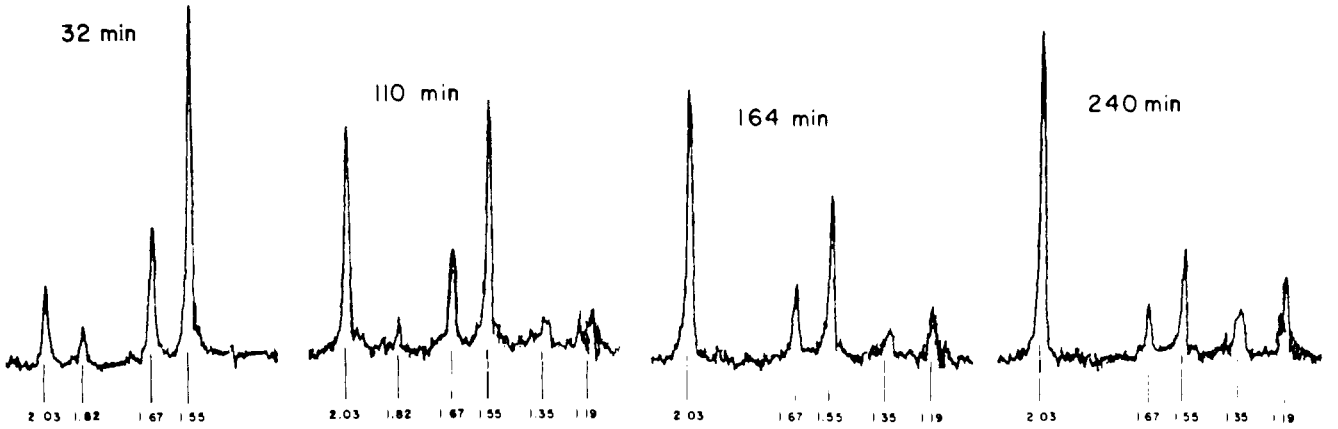
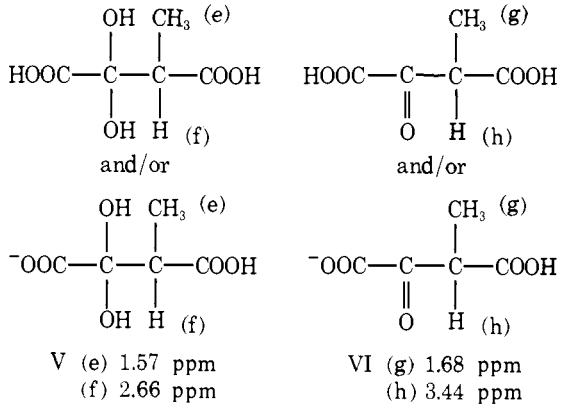


Figure 1. Nuclear magnetic resonance spectra, determined at different times, of the solution obtained upon decarboxylation of OPA in D_2O at pD 1.74 and 32 ± 1 °C.



appear at 1.19 and 1.35 ppm, respectively. The change in the NMR spectra during the course of the reaction is shown in Figure 1. The single β proton of OPA must be acidic enough to exchange rapidly with solvent, converting all the corresponding methyl resonances to broad singlets. Note that the singlet at 2.03 ppm is assigned to the intermediate III and the singlets at 1.55, 1.67, and 1.82 ppm are assigned to the deuterated forms of V, VI, and IV, respectively. Since the ratio of the integrations of VI and V remains constant during the course of the reaction, the equilibrium constant governing the hydration-dehydration transformation can be determined. The value of K was found to be 2.59 in the range of total initial OPA concentrations of 0.10–0.25 M, 0.50 ionic strength, and pD range of 1.30–1.92. The percent enol present can be evaluated from the relative integrated intensities of the $-CH_3$ resonances of the enol form IV and the corresponding $-CH_3$ resonances of OPA present as forms V and VI. The percentages of enol were found to be 2.1, 4.5, 4.8, 5.6, and 6.4 at pD values 1.30, 1.57, 1.66, 1.74, and 1.92, respectively.

The first-order rate constants of decarboxylation of OPA were determined by direct analysis of integrated intensities. Since the relative intensity of IV to the sum of the intensities of V and VI and the relative intensity of V to that of VI remain constant during the course of the reaction, the rate constant k_1 for the decarboxylation of the keto form of OPA can be evaluated from eq 1. A_0 was obtained by extrapolating the integrated intensity of VI to the time of mixing, and A is its integral intensity at time t. This method of treatment assumes that the rate-determining step involves the keto form of OPA. This is justified, since the rates of hydration-dehydration and enolization were found to be fast for the analogous acid OAA compared to its rate of decarboxylation. As mentioned earlier, the agreement between spectrophotometric and manometric determinations of k_1 suggests that the hydrate form is not involved in the rate-determining step and, therefore, the decrease in the integrated intensity of V with time is in accordance with the equilibrium condition. The values obtained for k_1 at five different pD values are listed in Table I together with the corresponding k_2 values for the rate of ketonization of the intermediate III, and the time (t_{max}) required to reach the maximal concentration (B_{max}) of this intermediate. The values of k_2 were calculated from eq 2 using the integrated intensity of III. A characteristic curve showing the decarboxylation of OPA and the subsequent ketonization of the intermediate is shown in Figure 2.

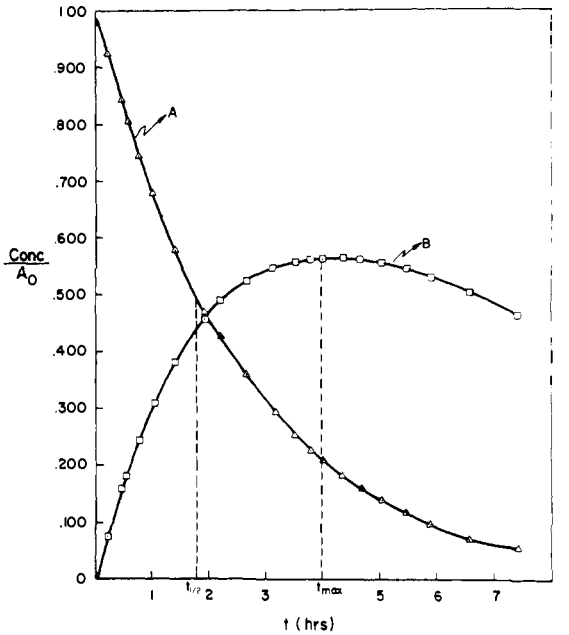


Figure 2. Graphical representation of the variation of concentration for two consecutive first-order reactions, obtained on decarboxylation of OPA in D_2O at pD 1.74 and 32 ± 1 °C: A = OPA; B = intermediate.

OPA in Deuterioacetate Buffer. The spectrum of OPA in deuterioacetate buffer at pD 4.65 after 10 min from the time of mixing consists of singlets at 1.34, 1.59, and 1.99 ppm. As the reaction proceeds, a decrease in the integrated intensity of the singlet at 1.59 ppm is noticeable, while both other singlets gain intensity. However, in contrast to the unbuffered decomposition of OPA, the integral of the singlet at 1.99 ppm reached its maximum value in a much shorter time and the appearance of products exemplified by the singlet at 1.34 ppm was much faster. From these observations and earlier studies on AKBA (vide supra) the singlet at 1.99 ppm is assigned to the intermediate III. The singlet at 1.59 ppm belongs then to

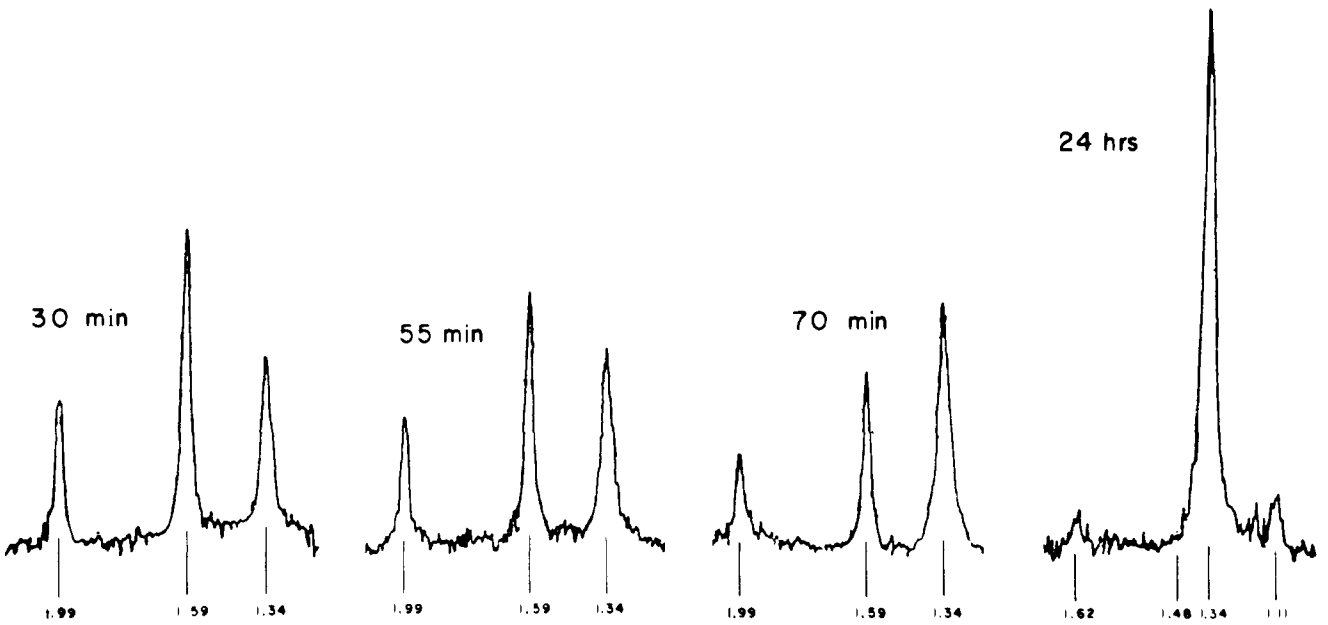


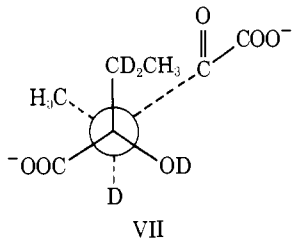
Figure 3. Nuclear magnetic resonance spectra, determined at several times, of the solution obtained on decarboxylation of OPA in 1.0 M deuterioacetate buffer at pD 4.60 and 32 ± 1 °C.

Table I. Rate Constants for Decarboxylation and Ketonization

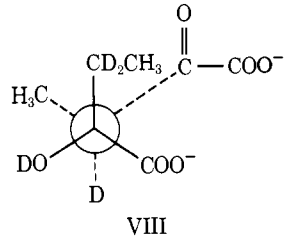
pD	$k_1 \times 10^3$, min^{-1}	$k_2 \times 10^3$, min^{-1}	% hydrate	% B_{max} calcd	% B_{max} obsd	t_{max} calcd, min	t_{max} obsd, min
1.30	5.97	6.86	81	30	31	156	158
1.57	6.31	4.23	70	44	45	192	191
1.66	6.42	3.15	68	50	48	217	214
1.74	6.52	2.31	65	57	57	246	240
1.92	6.78	1.56	58	62	62	267	269

the methyl resonance of OPA. No resonances corresponding to the enol or hydrate forms of OPA were observed at this pD. However, at pD 4.44, a broad singlet at 1.44 ppm was observed, the intensity of which decreased during the course of the reaction. This was assigned to the hydrated form of OPA present as 11% at this pD. That the enol form is not observed separately under our experimental conditions indicates that the equilibrium between the enol and keto forms of OPA is rapid. For comparison, the half-life for the rate of ketonization of the enol form of OAA lies between 10 and 30 s in 0.10 M acetate buffer at 1.5 °C.[19]

As the keto form builds up in concentration, three broad singlets appear at 1.11, 1.48, and 1.62 ppm (Figure 3). These peaks are assigned to the dimeric forms of AKBA. The broad singlet at 1.11 ppm is assigned as previously reported[26] to the methyl protons adjacent to the -CD_2- group in the two possible dimeric forms, VII and VIII, while the broad singlet at 1.62



VII



VIII

ppm and the partially buried singlet at 1.48 ppm are assigned to the methyl protons adjacent to the -CD- group.

Table II lists the observed first-order rate constants together with the observed and calculated times required to reach the maximal concentration of the intermediate.

The results obtained from the spontaneous decarboxylation of OPA have been analyzed in terms of two consecutive first-order processes. The choice of this model is prompted by the fact that the rate of enolization of AKBA has been shown to be very slow (6.8×10^{-5} min^{-1}),[27] while the steps involving hydration-dehydration and enol-keto tautomerization of OPA are fast and are considered to be in rapid preequilibrium with the rate-determining step involving the active keto form.

The observed first-order rate constant of decarboxylation for the total reaction ($k_{1,obsd}$) is related to the separate rate constants for the acid (K_{H_2A}), the univalent ion (k_{HA^-}), and the bivalent ion, $k_{A^{2-}}$:

$$k_{obsd} = k_{H_2A} + (k_{HA^-} - k_{H_2A})\alpha_1 + (k_{A^{2-}} - k_{H_2A})\alpha_2$$

The degrees of ionization to univalent (α_1) and bivalent (α_2) ions are in turn related to the ionization constants of OPA:

$$\left(\frac{\alpha_1}{1 - \alpha_1 - \alpha_2}\right) = \frac{K_1}{[H^+]}$$

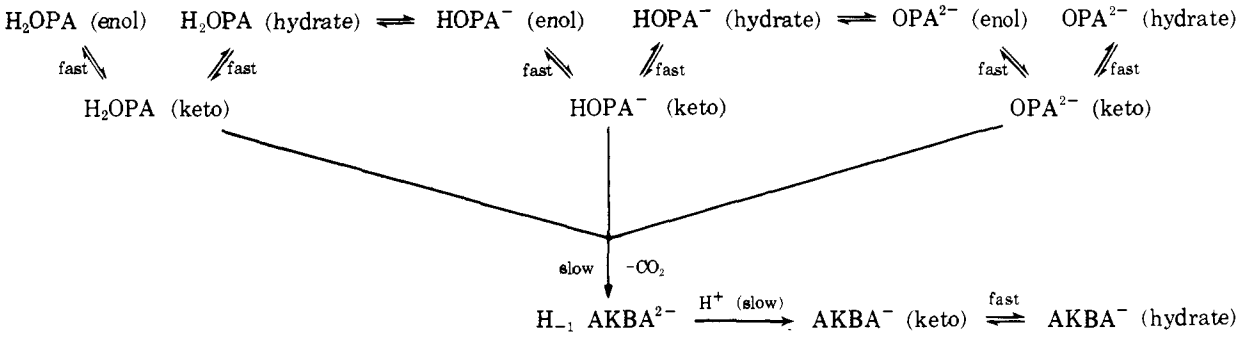
$$\left(\frac{\alpha_2}{1 - \alpha_1 - \alpha_2}\right) = \frac{K_1K_2}{[H^+]^2}$$

If we assume that α_2 is negligible in the pD region 1.30–1.92, then α_1 can be calculated using the deuterium ion concentration and a value for the equilibrium constant K_1 that will give the best fit to the plot of k_{obsd} as a function of α_1. Such a plot will then yield k_{H_2A} as the intercept and ($k_{HA^-} - k_{H_2A}$) as the slope. Figure 4 represents such a plot and yields a value of 5.72×10^{-3} min^{-1} for k_{H_2A} and 8.29×10^{-3} min^{-1} for k_{HA^-} while the best value for K_1 was found to be 8.32×10^{-3}. A comparison of the first-order rate constants k_{H_2A} and k_{HA^-} suggests that the rate of decomposition of H_2A and HA^- proceeds with

Table II. Rate Constants for Decarboxylation and Tautomerization

pD	$k_1 \times 10^2$, min^{-1}	$k_2 \times 10^2$, min^{-1}	% B_{max} calcd	% B_{max} obsd	t_{max} calcd, min	t_{max} obsd, min
4.44	2.56	5.84	25	23	25	27
4.60	2.33	5.46	22	21	27	28
4.75	2.12	5.15	22	20	29	31

Scheme II



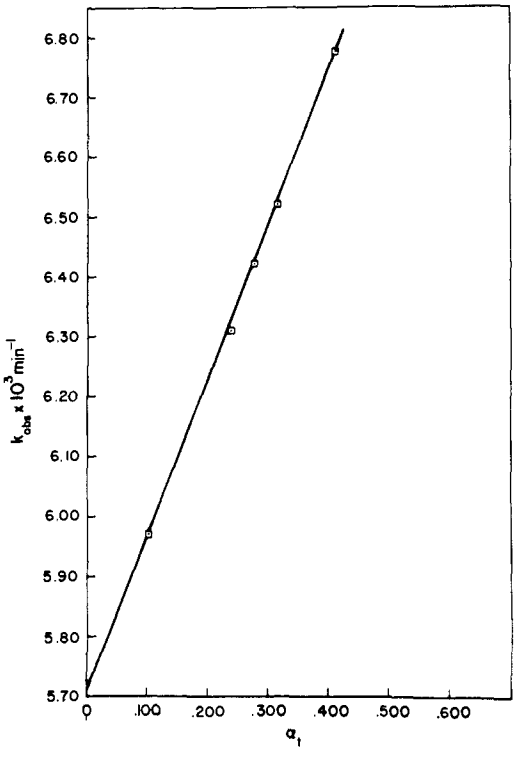


Figure 4. Variation of the observed first-order rate constant $k_{1,obsd}$ with the degree of ionization, α_1, of OPA at 32 ± 1 °C.

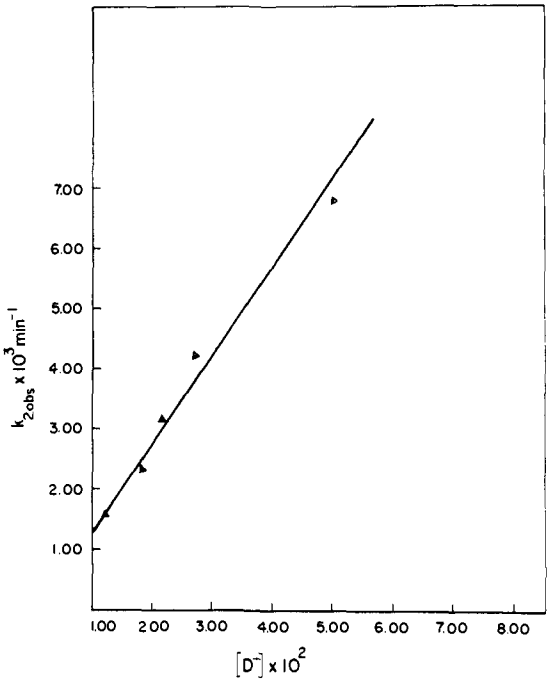


Figure 5. Variation of the observed second-order rate constant k_2 with the deuterium ion concentration of solutions of OPA in D_2O at 32 ± 1 °C.

comparable magnitude. By contrast, Pedersen[5] and Gelles[2] have shown that the univalent ion of OAA decomposes 44 times faster than the undissociated form, although they reported a discrepancy between the observed and calculated rates when the OAA concentration was different from 0.015 M. The reaction scheme for decarboxylation, the enol-keto tautomerization, and the hydration-dehydration reactions can then be written as shown in Scheme II. The calculated times required to reach the maximal concentration of the enol or enolate intermediate agree very well with the observed values. It is seen from Tables I and II that the maximum amount of the intermediate is smaller when the decomposition occurs in acetate buffer. This has been attributed to the catalysis of the ketonization step by OH_3^+ and OH^-, acetic acid and acetate ion.[18] However, when the maximal concentration of the OPA intermediate is compared to that of DMOAA[4] in acid solution or acetate buffer, a difference in the magnitude of the numbers for B_{max} is noticed.

The structures of OPA and DMOAA are similar enough that one would not expect a considerable difference in the concentration of the intermediate. There is a possibility that hydrate formation in acid solution could influence the calculation of the concentration of the intermediate. This possibility should be investigated.

The ketonization of the intermediate III exhibits specific acid catalysis, and the rate law is of the form

$$-d(B)/dt = k_D(D^+)$$

where k_D is the rate constant arising from the deuterium ion catalyzed path. From the plot of the first-order rate constant k_2 as a function of (D^+), shown in Figure 5, the value of 1.47 $\times 10^{-3}$ M^{-1} min^{-1} is obtained for k_D.

It is noticeable from Table I that the hydrated form exists to the extent of as much as 81% at pD 1.30. This could very well retard the rate of decarboxylation of OPA when the partially dissociated or undissociated forms are the predominant species in solution. In this connection, it is interesting that a plot of the variation of k_1 with pD seems to resemble a plot of the percent of hydrate vs. pD more than it does the degree of dissociation of the diprotonated species (α_1) vs. pD. This interesting observation suggests that further quantitative studies of these effects should be carried out. This interpretation of the involvement of hydrate formation in rate retardation is in accord with the concept that the five-membered hydrogen-bonded chelate ring in Scheme I is the active intermediate in the decarboxylation process.

In summary, the findings in this work indicate: (1) the decarboxylation of β-keto acids that also contain α-keto acid functions must involve consideration of the hydrated form; (2) the NMR method provides an effective means of determination of the first-order rate constants of ketonization and evaluation of the equilibrium constant governing the keto-enol equilibrium. The use of NMR to sort out these microscopic rates and equilibria makes possible a more precise determination of rates of decarboxylation in these systems.

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Stereospecific Aliphatic Hydroxylation by Iron–Hydrogen Peroxide. Evidence for a Stepwise Process[1]

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Abstract: Treatment of a mixture of cyclohexanol and ferrous perchlorate with hydrogen peroxide in acetonitrile led to a mixture of cyclohexanediols and cyclohexanone. In acetonitrile containing perchloric acid, *cis*-1,3-cyclohexanediol accounted for 71.9% of the diol produced. Under these conditions, hydrogen removal at C-3 occurred stereoselectively cis to the hydroxyl group. A mechanism for this aliphatic hydroxylation which involves an initial directed hydrogen abstraction (k_H/k_D = 1.18), free radical oxidation by Fe^{3+}, and stereoselective carbonium ion capture was proposed. Evidence for the stepwise nature of this process was derived from analysis of the Fe^{2+}/H_2O_2 oxidation of 7-hydroxynorbornane (**6**). Hydrogen abstraction from **6** was found to occur stereoselectively syn to the hydroxyl group and subsequent 2,6-hydride transfers result in a 1:1 ratio of syn,exo and anti,exo diols (**8** and **9**).

The oxidative transformations mediated by the biological oxidases have attracted much attention both for their mechanistic complexity and potential synthetic utility.[2] Of particular importance are the heme-iron mixed function oxidases such as cytochrome P-450, which are known to effect olefin epoxidation, aromatic hydroxylation, and hydroxylation of saturated carbon centers in a wide variety of organic compounds. Aliphatic hydroxylation is of interest since there exist at present no general, direct, synthetic methods for specific replacement of an unactivated carbon-bound hydrogen with an hydroxyl group. Indeed, biological fermentation is often the method of choice for such transformations.

These enzyme systems are known to activate molecular oxygen by sequential two-electron reduction to the formal oxidation state of hydrogen peroxide.[3] The subsequent processes, O-O and C-H bond scission, are extremely rapid and mechanistic details have remained obscure. If the extensive chemistry of iron-hydrogen peroxide systems is used for precedent and guidance, the closest historical "model" for aliphatic hydroxylation is Fenton's reagent,[4] a mixture of ferrous ion and hydrogen peroxide. According to the generally held mechanism first proposed by Haber and Weiss,[5] the Fenton's reagent oxidation of organic compounds proceeds by initial one-electron reduction of hydrogen peroxide to yield free hydroxyl radicals and subsequent hydrogen abstraction (Scheme I).

There has been understandable reluctance to embrace hydroxyl radical as the active oxygen species in biological oxi-