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A Non-enzymatic Olefinic Hydration under Neutral Conditions: the Kinetics and Mechanism of the Hydration of Fumaric Acid Monoanion[1]

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The monoanion of fumaric acid has been shown to undergo hydration uncatalyzed by any external agents, while crotonic acid anion does not undergo such a reaction. It is believed that the fumarate reaction is assisted by the carboxylic acid and carboxylate ion groups in a concerted intramolecular catalysis. The kinetics of the reversible hydration–dehydration reaction were investigated at 175° in the pH range 0 to 6, and the pH–rate constant curve describes a bell-shape which can be fitted with the rate equation 4 (for the hydration). Two mechanisms can account for the monoanion reaction: (i) a general acid–general base catalysis leading to malic acid in a single step, with *trans* addition of water; (ii) a general acid–nucleophile catalyzed isomerization to β-malolactonic acid, followed by hydrolysis with inversion to give over-all *cis* addition. At pH 5 the rate of disappearance of L-malate monoanion (followed polarimetrically) is 7.5 times faster than the rate of appearance of fumarate ion (followed spectrophotometrically); this result indicates that mechanism (ii) is the more probable one.

Introduction

The hydration of isolated carbon–carbon double bonds has been shown by many detailed studies to be subject to hydronium ion-catalysis.[3] The olefinic bond therefore participates as a nucleophile in this reaction. The olefinic bond in an α,β-unsaturated carbonyl compound, however, is rendered more electrophilic by conjugation with the electron-withdrawing group, and nucleophilic attack upon the olefin may occur. Thus the hydration of benzalacetone is catalyzed by hydroxide ion.[4] Perhaps the most interesting olefinic hydrations are those catalyzed by enzymes. The important features of these reactions are their very high rates, their stereospecificity, and their occurrence near neutrality. All of the unsaturated substrates of these enzymes appear to contain a carbon–carbon double bond in conjugation with an electron-withdrawing group. This group may be a carboxylic acid, a thiolester or a phosphate group.[5]

The hydration of fumaric acid, which is the subject of this paper, is catalyzed by hydronium ion,[6] hydroxide ion[7] and by the enzyme fumarase.[8] The enzymatic reaction occurs with *trans* addition of water, as has been shown by Gawron and Fondy and by Anet.[9] The effect of pH upon the rate of the fumarase-catalyzed hydration has been interpreted to mean that two ionizable groups, an acid and a base, are involved in the catalytic step. The enzyme functions in the neutral pH range, and this attribute is the most striking difference between the enzymatic and non-enzymatic reactions. Evidently neither the hydronium ion- nor the hydroxide ion-catalyzed hydration provides a helpful parallel to the enzymatic reaction.

There is no known example of a non-enzymatic olefinic hydration under neutral conditions.[10] A method for the study of enzyme models that has proved useful in the past is the incorporation of the pertinent catalytic functions into the organic molecule whose reaction is to be studied.[11] In this instance we are interested in the hydration of a double bond brought about by a combination of an acidic group and a basic group. The study described here was therefore undertaken to test the prediction that the monoanion of fumaric acid, which possesses both an acidic and a basic group suitably disposed about the double bond, could catalyze its own hydration near neutral pH.[12]

Experimental

Materials.—Fumaric acid (Fisher Scientific Co., purified grade) was recrystallized twice from water; m.p. 289.5–291.5° (sealed tube).[13] DL-Malic acid (Matheson, Coleman and Bell practical grade) was recrystallized from acetone and then recrystallized several times from an acetone-carbon tetrachloride mixture[13]; m.p. 127–128.5°. L-Malic acid[13] (Eastman Kodak Co., white label) was recrystallized twice from anhydrous ether; m.p. 104.5–106°, $[M]^{25}\text{D}$ −1.10° (water, c 0.468 M, pH 4.17) where molecular rotation $[M] = (\alpha(\text{deg.}))/(\text{d.(dm.)})(c(M))$. Crotonic acid (Eastman Kodak Co., practical grade) was recrystallized twice from water; m.p. 72–72.5°.

Hydrochloric acid, 1 M, was standardized against borax, and less concentrated solutions were prepared by dilution.

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[1] This research was supported by grants from the National Science Foundation and the National Institutes of Health. A brief report of the present work has been published: M. L. Bender and K. A. Connors, *J. Am. Chem. Soc.*, **83**, 4099 (1961). In the last line of text on p. 4099 the symbol k_h^{-1} should be written k'_h.

[2] (a) Alfred P. Sloan Foundation Research Fellow; (b) National Institutes of Health Postdoctoral Research Fellow.

[3] J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1956, Chap. 9; E. S. Gould, "Mechanism and Structure in Organic Chemistry," H. Holt and Co., New York, N. Y., 1959, Chap. 13; R. H. Boyd, R. W. Taft, Jr., A. P. Wolf and D. R. Christman, *J. Am. Chem. Soc.*, **82**, 4729 (1960).

[4] D. S. Noyce and W. L. Reed, *ibid.*, **81**, 624 (1959).

[5] B. G. Malmström, in "The Enzymes," Vol. 5, 2nd ed., P. D. Boyer, H. Lardy and K. Myrbäck, editors, Academic Press, Inc., New York, N. Y., 1961, Chap. 28.

[6] L. T. Rozelle and R. A. Alberty, *J. Phys. Chem.*, **61**, 1637 (1957).

[7] L. E. Erickson and R. A. Alberty, *ibid.*, **63**, 705 (1959).

[8] R. A. Alberty, Chap. 32 in ref. 5.

[9] O. Gawron and T. P. Fondy, *J. Am. Chem. Soc.*, **81**, 6333 (1959); F. A. L. Anet, *ibid.*, **82**, 994 (1960); O. Gawron, A. J. Glaid and T. P. Fondy, *ibid.*, **83**, 3634 (1961). The formation of aspartic acid by the aspartase-catalyzed addition of ammonia to fumaric acid involves the same stereochemistry as the hydration reaction: S. Englard and H. Breiger, *J. Biol. Chem.*, **233**, 1003 (1958); **235**, 1510 (1960).

[10] D. Pressman and H. J. Lucas, *J. Am. Chem. Soc.*, **64**, 1953 (1942), have shown that the hydration of acrolein to form hydroacrolein, although hydronium ion-catalyzed, will occur in neutral solution (unbuffered water). However, they state that the rate of hydration in water is negligible compared to the rate in acid solution. This situation differs from the present results, in which it is shown that the rate of hydration in neutral solution is comparable with that in acid solution.

[11] M. L. Bender, *Chem. Revs.*, **60**, 100 (1960).

[12] J. M. Weiss and C. R. Downs, *J. Am. Chem. Soc.*, **44**, 1118 (1922), have studied the conversion of fumaric acid to malic acid in aqueous solutions at 140° to 200°. The pH of these solutions was not controlled. These authors also give a thorough review of the earlier literature pertaining to this reaction.

[13] The physical properties of these acids have been studied by R. Descamps, *Bull. soc. chim. Belg.*, **48**, 201 (1939); **49**, 91 (1940); N. A. Lange and M. H. Sinks, *J. Am. Chem. Soc.*, **52**, 2602 (1930); N. A. Lange and H. Kline, *ibid.*, **44**, 2709 (1922); J. M. Weiss and C. R. Downs, *ibid.*, **45**, 2341 (1923). These last authors also summarize earlier work on fumaric and malic acids.

Sodium hydroxide solution was standardized against potassium biphthalate. Acetic acid solution was standardized against the sodium hydroxide. All water used was redistilled from alkaline permanganate. Deuterium oxide (General Dynamics Corp.) was > 99.5% D_2O. Sodium deuteroxide solution was made by dissolving sodium metal in deuterium oxide.[14]

Acetic acid–acetate buffers were prepared by mixing appropriate volumes of standard solutions of acetic acid and sodium hydroxide. The ionic strength of the solutions was brought to the desired value by the addition of reagent grade potassium chloride.

pH Control.—The hydronium ion concentration in these experiments was controlled with hydrochloric acid solutions or with acetic acid–acetate buffers. All of the kinetic and equilibrium measurements were made at 175°, and the hydronium ion concentration at this temperature was calculated from knowledge of the room temperature concentrations of the buffer components, the specific volume of water at 175°, which is 1.120,[15] and the pK_a' of acetic acid at 175°, which is 5.40.[16] In the pH_{175} range[17] 2 to 4.5 the solutions were practically unbuffered, and small changes in the pH_{175} probably occurred during the course of reaction. Measurements of pH_{25} (made with a Radiometer model 4c meter) showed that changes (from 0.02 to 0.2 unit) did occur in this range during the reaction.

Such changes in pH_{175} probably would have small effect upon the actual rate of reaction. The changes in pH_{25} might have an effect upon the apparent rate through an effect on the analytical method (*vide infra*).

Temperature Control.—The sample solutions, contained in ampules made from 18 mm. o.d. Pyrex tubing, were maintained at constant temperature in an insulated aluminum block. The block was operated with two heaters. A constant but variable source consisted of 80 ft. of no. 18 chromel wire wrapped around the outside of the aluminum cylinder and separated from it by asbestos cloth. The total power output of this heater was about 300 watts, and it was connected to the No. 2 input of a Sargent Thermonitor model S relay. At 175° the No. 2 heater was operated at 28% of maximum power. The regulating heater was tightly fitted into a hole drilled in the center of the aluminum block. This heater was a 120 volt, 240 watt Chromalox cartridge heater (model C418C, Edwin L. Wiegand Co., 7500 Thomas Boulevard, Pittsburgh 8, Pa.); it was connected to the No. 1 input of the Thermonitor relay.

The temperature sensor was a glass-enclosed thermistor (E. H. Sargent and Co., cat. no. S-81628). The temperature control in the block was ±0.2°. All studies reported here were carried out at 175°.

Kinetic and Equilibrium Measurements.—Most of the kinetic and equilibrium studies were made on solutions in which the initial reactant was fumaric acid. Because of its carbon–carbon double bond this molecule absorbs ultraviolet radiation much more strongly than does malic acid, and a spectrophotometric analysis is feasible. The absorbance of solutions at 270 mμ (measured with a Beckman DU spectrophotometer) was employed as a measure of the fumaric acid concentration; malic acid does not significantly absorb light of this wave length. The concentration of malic acid could be calculated by difference, since the initial concentration was known. Solutions of fumaric acid follow Beer's law at 270 mμ if the pH is held constant.

The initial concentration of fumaric or malic acid was varied from 0.90×10^{-3} to 4.33×10^{-3} mole/liter, though in most of the runs it was about 1.5×10^{-3} mole/liter. The ionic strength was held constant at 0.104 at room temperature, so that it was 0.093 at 175°. At the lowest pH (pH_{175} 0.14) the ionic strength was 0.73 (at 175°). In the calculation of ionic strength the contribution of the ionized substrates was neglected.

Usually from five to ten 4-ml. aliquots of sample solution were sealed in ampules for each kinetic run. Preliminary experiments (at pH 1) indicated that the presence of air caused erratic results, so all solutions were degassed on a vacuum line before sealing in ampules. This effect of air was not studied, but it was noted that at higher pH values the effect was much less pronounced.

Several runs were made with ampules containing only the buffer solutions in order to determine whether prolonged heating might alter the transparency of the solvent. When the ampules were cleaned as described below no change in absorbance at 270 mμ usually could be detected; however, an occasional sample did exhibit an increased absorption, amounting to 0.01–0.03 absorbance unit. Since this behavior was erratic, a correction could not be made for it, and it undoubtedly accounts for occasional high analytical results. The ampules were cleaned by heating in chromic acid–sulfuric acid cleaning solution, then by rinsing and boiling in four fresh changes of distilled water, and finally by prolonged steaming of the interior of each ampule.

The hydration kinetics for crotonic acid were carried out under the same conditions as for fumaric acid; the initial concentration of crotonic acid was 1.9×10^{-4} M. Spectrophotometric analysis at 225, 230 or 232 mμ was employed to follow the reaction.

Nuclear Magnetic Resonance Study.—A sample of fumaric acid (0.00495 mole) was dissolved in 0.00500 mole of NaOD in D_2O solution and this solution was diluted to 25 ml. with D_2O. The pD_{25} of this solution was 4.02 as determined from a measurement using a S.C.E.–glass electrode system and the relationship[18]: pD = "meter pH" +0.40. Aliquots of this solution were degassed and sealed into Pyrex ampules, which were placed in the heating block at 175°. Ampules were removed after various periods of heating, and the malic acid was isolated by conventional means. In some cases the malic and fumaric acids were not separated. The acids were converted to their dipotassium salts with a KOD solution, this solution was evaporated to dryness, and the residue was dissolved in D_2O. The nuclear magnetic resonance spectra of these solutions were recorded at 60 Mc. with a Varian n.m.r. spectrometer.

Polarimetric Rate Study.—The rate of change of optical rotation of L-malic acid was measured at 175° in solutions about 0.5 M in L-malic acid. The malic acid was largely converted to the monoanion by the addition of sodium hydroxide, and the system provided its own buffer. The optical rotation was measured in a 2-dm. semi-microtube. Since the rotation is pH dependent,[19] the rate constant was determined from an initial rate measurement; over the time period studied the pH_{25} of the solution changed from 4.17 to 4.15. At constant pH the optical rotation is proportional to the concentration of L-malic acid over the small concentration range employed here.

In order to compare accurately the polarimetric rate constant with the dehydration rate constant, the latter quantity was measured spectrophotometrically on (diluted) aliquots of the same samples used for the rotation measurements.

Results

Kinetic Measurements.—Kinetic and equilibrium measurements of the hydration of fumaric acid to malic acid were carried out at 175° from pH_{175} 0.14 to 6.15. Measurements of the absorbance of solutions which had been removed at appropriate intervals from the heating block provided a measure of the fumaric acid concentration. The kinetic data fit a first-order rate equation. The over-all reaction may be written

$$\mathrm{F} \underset{k_d}{\overset{k_h}{\rightleftarrows}} \mathrm{M} \qquad (1)$$

where F and M represent total fumarate and malate. In the following treatment the concentration of water will be considered constant and will not be explicitly taken into account. The integrated rate equation for this first-order reversible

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[14] This standardized solution was kindly furnished by Dr. Erkki Euranto.

[15] M. G.-A. Hirn, *Ann. chim. phys.*, **10**, 32 (1867).

[16] A. A. Noyes, A. C. Melcher, H. C. Cooper, G. W. Eastman and Y. Kato, *J. Am. Chem. Soc.*, **30**, 335 (1908).

[17] The notation pH_{25} and pH_{175} will be employed for convenience in referring to the hydronium ion concentration function at 25° and 175°. It is understood that pH_{175} refers to the negative logarithm of the hydronium ion *concentration*.

[18] B. Zerner and M. L. Bender, *J. Am. Chem. Soc.*, **83**, 2267 (1961), and references therein.

[19] H. T. S. Britton and A. A. Moss, *J. Chem. Soc.*, 1487 (1936).

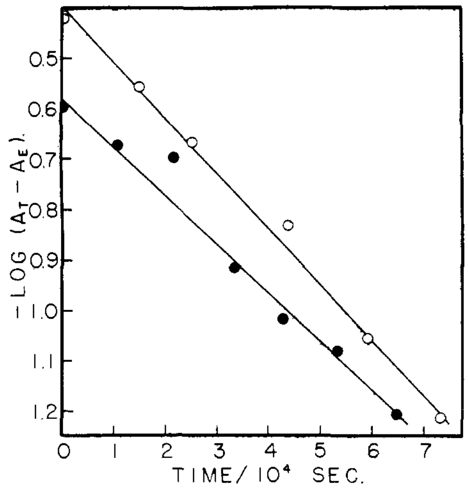

Fig. 1.—First-order plots of the hydration of fumaric acid at 175°: ●, $p\mathrm{H}_{175}$ 1.44; ○, $p\mathrm{H}_{175}$ 3.64.

reaction can be written in terms of the fumaric acid concentration[5,6]

$$\ln \frac{F_t - F_e}{F_0 - F_e} = -(k_h + k_d)t \qquad (2)$$

where F_0, F_t, F_e represent the fumaric acid concentrations at times zero, t and at equilibrium. Since Beer's law is followed by the system, these concentration terms may be directly replaced by the corresponding absorbances. Thus a plot of $\log\ (A_t - A_e)$ *versus* t should be linear if eq. 1 describes the reaction; from the slope of the line the sum $(k_h + k_d)$ is obtained. Some of these first-order plots are shown in Fig. 1. Many of the plots obtained in this work were better than those illustrated, and some were considerably worse. Within the precision of the measurements the points fall on a straight line. The initial concentration of reactant was varied over a 3.3-fold range without significant change in the observed first-order rate constant.

From the measurement of the equilibrium concentration of fumaric acid the equilibrium constant for reaction 1 can be calculated. With this

$$K = M_e/F_e = k_h/k_d \qquad (3)$$

additional relationship between k_h and k_d, these quantities can be separated. In Table I the kinetic results obtained by this treatment are listed. Table I also contains three rate constants at higher pH which were obtained from initial rate measurements, the reaction being too slow to follow until equilibrium. These calculations are based upon the absence of malic acid at zero time; the back reaction is therefore negligible, and the ratio of the initial rate (determined graphically) to the initial concentration of fumaric acid yields the constant k_h. The reproducibility of the rate constants in Table I is about ±10–20%, depending upon the pH and the absolute value of the constant. The data are therefore not expected to be very accurate.

Variation of the total acetate concentration (at $p\mathrm{H}_{175}$ 4.83) indicated that the components of the acetate buffer may cause an acceleration of the rate. However, at the acetate concentration employed in this work the effect is no greater than the experimental uncertainty of the measurements, and it can be neglected.

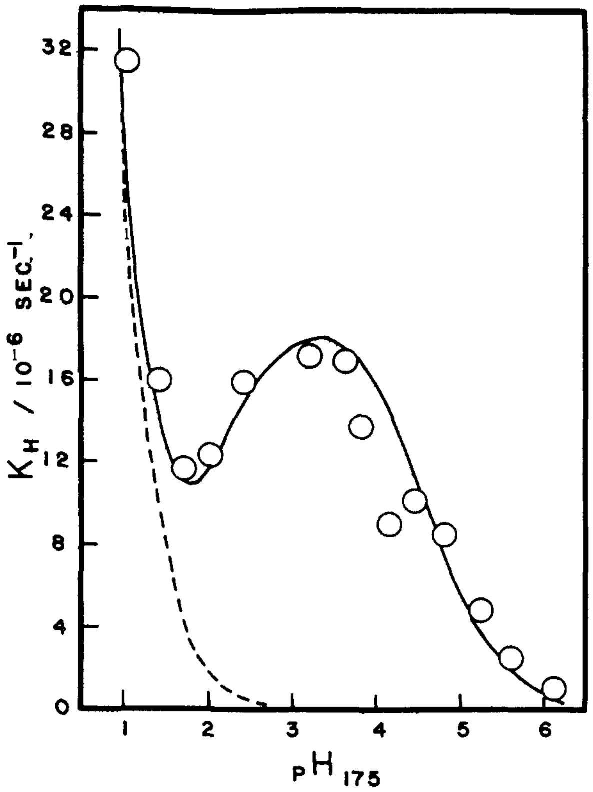

Fig. 2.—The hydration of fumaric acid at 175°: ———, calculated curve assuming reaction of monoanion; – – – –, calculated assuming no reaction of monoanion.

A plot of k_h against $p\mathrm{H}_{175}$ is shown in Fig. 2; k_d is a similar function of pH. Evidently the data cannot be explained solely on the basis of a hydronium ion-catalyzed reaction of the un-ionized substrates plus a hydroxide ion-catalyzed reaction of the doubly-ionized molecules. The data can be accounted for by assuming that in addition to these two known catalytic reactions a third process, an "uncatalyzed" reaction of the monoanionic forms, occurs. The rate of hydration can then be written[20]

$$V_h = k_h^0[\mathrm{H_2F}][\mathrm{H^+}] + k_h^-[\mathrm{HF^-}] + k_h^=[\mathrm{F^=}][\mathrm{OH^-}] \qquad (4)$$

and a similar equation (involving constants k_d^0, k_d^- and $k_d^=$) describes the dehydration rate, V_d. The experimental result is given by eq. 5.

$$V_h = k_h[\mathrm{F}] \qquad (5)$$

where F represents the total fumarate concentration. The acid–base equilibria in the system are described by eq. 6–8

$$K_{1F} = [\mathrm{H^+}][\mathrm{HF^-}]/[\mathrm{H_2F}] \qquad (6)$$
$$K_{2F} = [\mathrm{H^+}][\mathrm{F^=}]/[\mathrm{HF^-}] \qquad (7)$$

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(20) The term $k_h^-[\mathrm{HF^-}]$ in eq. 4 could be replaced by either of the kinetically equivalent terms $k'[\mathrm{H^+}][\mathrm{F^=}]$ or $k''[\mathrm{OH^-}][\mathrm{H_2F}]$, where $k' = k_h^-/K_{2F}$ and $k'' = k_h^-K_{1F}/K_w$. Although a choice between these terms cannot be made on the basis of kinetic measurements, one can calculate (with the data given below) that k' and k'' would have the approximate values 1 l./mole-sec. and 10^4 l./mole-sec., respectively, and these values are so unexpectedly large that they seem very improbable in this system. Moreover, a mechanistic interpretation of these alternate terms leads to essentially the same picture implied by the term $k_h^-[\mathrm{HF^-}]$.

$$K_w = [H^+][OH^-] \qquad (8)$$

Equations 4–8 can be combined to give 9, which relates k_h to the hydronium ion concentration. A

$$k_h = \frac{k_h^0[H^+]^3 + k_h^- K_{1F}[H^+] + k_h^= K_{1F}K_{2F}K_w/[H^+]}{[H^+]^2 + K_{1F}[H^+] + K_{1F}K_{2F}} \qquad (9)$$

similar equation gives k_d as a function of $[H^+]$. Equation 9 correctly describes the shape of the *p*H–rate curve in Fig. 2. The smooth line in Fig. 2 has been calculated according to eq. 9 using the following values[21] for the constants $k_h^0 = 3.3 \times 10^{-4}$ l./mole-sec., $k_h^- = 2.0 \times 10^{-5}$ sec.$^{-1}$, $K_{1F} = 1.0 \times 10^{-2}$ ($pK_{1F} = 2.0$), $K_{2F} = 2.5 \times 10^{-5}$ ($pK_{2F} = 4.6$). The dashed line in the figure is drawn using the same values for k_h^0 K_{1F} and K_{2F}, and taking $k_h^- = 0$. The dehydration rate constant curve can be constructed with these values[21b] for the constants: $k_d^0 = 1.1 \times 10^{-4}$ l./mole-sec., $k_d^- = 1.1 \times 10^{-5}$ sec.$^{-1}$, $K_{1M} = 6.3 \times 10^{-3}$ ($pK_{1M} = 2.2$), $K_{2M} = 1.6 \times 10^{-5}$ ($pK_{2M} = 4.8$).

TABLE I

RATE CONSTANTS FOR HYDRATION AND DEHYDRATION AT 175°

pH_{175}[a]	$k_d \times 10^6$, sec.$^{-1}$	$k_h \times 10^6$, sec.$^{-1}$	pH_{175}[a]	$k_h \times 10^6$, sec.$^{-1}$	$k_d \times 10^6$, sec.$^{-1}$
Fumaric acid–malic acid system			Crotonic acid–hydroxybutyric acid system		
0.14	216	72	1.04	25.4	22.4
1.04	31.4	14.3	4.84	0.1	..
1.44	15.9	7.0	5.66	0.33	..
1.74	11.6	5.0			
2.04	12.3	5.6			
2.44	15.9	7.4			
3.22	17.2	9.4			
3.64	16.9	9.4			
3.84	13.7	8.6			
4.18	9.0	5.6			
4.47	10.1	6.4			
4.83	8.5	5.6			
5.27	4.8	..			
5.64	2.4	..			
6.15	1	..			

[a] Hydrochloric acid solutions from pH_{175} 0.14–2.44; acetate buffers for rest of solutions. Total acetate concentration was 0.0905 *M* in all acetate buffers. Ionic strength = 0.73 at pH_{175} 0.14, and 0.093 at all others.

Equilibrium Measurements.—The equilibrium constant is defined by eq. 3, and can be calculated from a knowledge of the absorbance at equilibrium, the absorptivity of fumaric acid, and the total concentration of substrate (fumaric plus malic acids). The equilibrium constants are less reproducible than the rate constants; the effect of traces of air may be partly responsible, as may the occasional spectral interference traceable to the solvent (see above). The average equilibrium constants may be obtained from Table I.

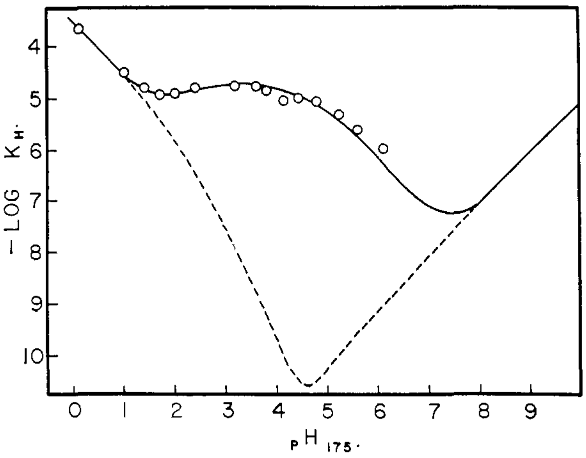

Fig. 3.—The hydration of fumaric acid at 175°: ———, calculated curve assuming reaction of monoanion; – – – –, calculated assuming no reaction of monoanion.

Discussion

The high reactivity of the fumaric acid hydration over a wide *p*H range is impressively demonstrated by Fig. 3, in which the solid line has been calculated by means of eq. 9 using $k_h^0 = 3.3 \times 10^{-4}$ l./mole-sec., $k_h^- = 2.0 \times 10^{-5}$ sec.$^{-1}$, $k_h^= = 1.5 \times 10^{-4}$ l./mole-sec.[6], $pK_{1F} = 2.0$, $pK_{2F} = 4.6$ and $K_w = 3.0 \times 10^{-12}$ ($pK_w = 11.53$ at 175°).[22] The dashed line shows the curve to be expected if $k_h^- = 0$ and the other constants have the values given above. It will be noted that neutrality is at pH_{175} 5.77, so that the hydration–dehydration reaction occurs under truly neutral conditions. The extreme reactivity which apparently must be attributed to the inclusion of the monoanion (k_h^-) term may be gauged by taking the vertical distance, in logarithmic units, between the solid and dashed lines in Fig. 3; each unit represents an order of magnitude in the ratio of observed rate to the rate calculated for the absence of the monoanionic reaction. Thus at *p*H 4.5 the calculated rate enhancement is about 3×10^5-fold.

Since the kinetic data for hydration can be quantitatively accounted for by rate equation (4)[23], it seems that the monoanionic forms of fumaric acid and of malic acid undergo reaction uncatalyzed by an external reagent, and that these forms are the only ones that do so. This suggests that the presence of both the –COOH and –COO⁻ group in the molecule is necessary for this reaction. This hypothesis was tested by studying the kinetics of hydration of crotonic acid anion, which is a model for the fumarate monoanion, the carboxylic acid group having been replaced by a methyl group. The limited data obtained (Table I) can be accounted for by the simple rate equation[24]

$$V_h = k_H[HC][H^+] + k_{OH}[C^-][OH^-]$$

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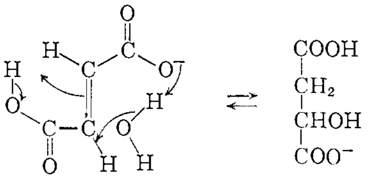
[21] (a) In the pH_{175} range shown in Fig. 2 the contribution from the hydroxide ion-catalyzed term is negligible. (b) The pK_a values of these molecules at room temperature are (at 25°): pK_{1F} 3.0, pK_{2F} 4.4 (W. L. German, A. I. Vogel and G. H. Jeffery, *Phil. Mag.*, **22**, 790 (1936)); and (at 20°): pK_{1M} 3.4, pK_{2M} 4.9 (F. Auerbach and E. Smolczyk, *Z. physik. Chem.*, **110**, 65 (1924)). Since the pK_a values at 175° are not known, these quantities were treated as adjustable parameters in achieving the best agreement between the experimental and calculated curves.

[22] K. Schwabe, *Chem. Tech.* (*Berlin*), **11**, 554 (1959).

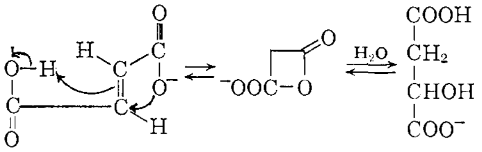
[23] The qualitative correspondence between eq. 4 and the data is clear; the quantitative agreement has not been strictly demonstrated, of course, since the constants of the equation were treated as adjustable parameters rather than determined in independent measurements.

[24] D. Pressman and H. J. Lucas, *J. Am. Chem. Soc.*, **61**, 2271 (1939), have shown that the hydronium ion-catalyzed hydration is first order with respect to the hydronium ion concentration.

with k_H = 2.8 × 10^{-4} l./mole-sec. and k_{OH} = 0.3 l./mole-sec. If it is supposed that the entire reaction at pH 4.84 is due to an uncatalyzed anion reaction, which is *most unlikely*, it can be calculated that the rate constant for the fumarate monoanion hydration is *at least* 200 times greater than the (hypothetical) one for crotonate. It therefore seems that both the $-COOH$ and $-COO^-$ groups are essential for an appreciable reaction rate.


(10)

Two possibilities must be considered in accounting for the effectiveness of these groups in accelerating the rate: the groups either do not actively participate in the catalysis, or they do function as catalysts. The first of these alternatives requires that the electronic and steric properties of the $-COOH$ and $-COO^-$ groups are responsible for an enhancement of the rate many orders larger than is commonly ascribable to such causes. It is extremely doubtful that the monoanion reaction is markedly assisted by substituent effects.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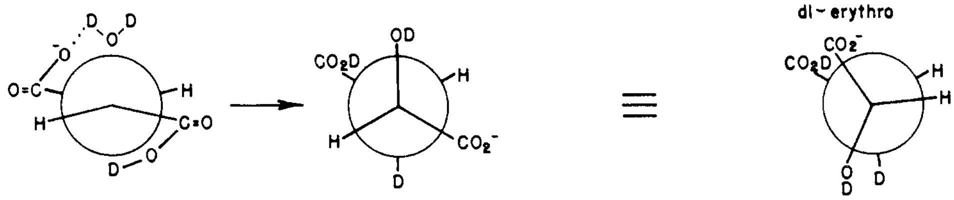
The second possibility, that the groups perform as catalysts, seems the more likely one. Two mechanisms can be drawn in which both groups, acting in a concerted manner, are necessary.



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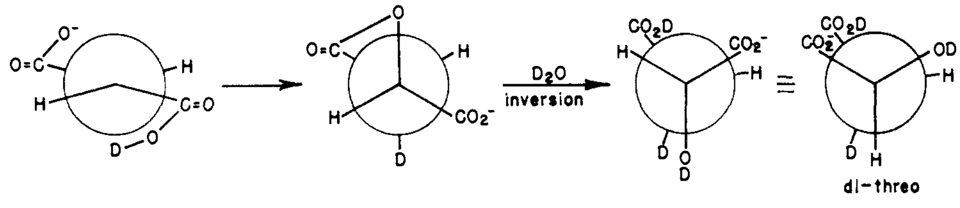

(13)

(i) One of these possible pathways can be described as an intramolecular general acid–general base catalysis. The elements of water are included in the transition state. In this mechanism the $-COOH$ group acts as an acid, providing a proton, while the $-COO^-$ accepts a proton from a water molecule. In the dehydration reaction the movement of electrons would be the reverse of that pictured in reaction 10.

(ii) The other mechanism is a general acid–nucleophile catalyzed isomerization to β-malolactonic acid, which then undergoes rapid hydrolysis to malic acid. The carboxylate group attacks carbon in the rate-determining step, and the solvent is not directly involved in the slow step of the hydration.

It is also possible that the catalytic groups function not in concert but consecutively. Such catalysis would be similar to those pictured above, and would be difficult to distinguish from them.

The stereochemical results of mechanisms i and ii are different. Equations 12 and 13 show the course of the reaction, as carried out in deuterium oxide solution, according to both postulated mechanisms. *Via* pathway i the addition is seen to be *trans*, resulting in the formation of *dl-erythro*-monodeuterio-malic acid. Pathway ii, on the other hand, leads to an over-all *cis* addition with the formation of *dl-threo*-monodeuterio-malic acid, since β-malolactonic acid is known to hydrolyze in mildly acidic solution with inversion.[25] Both of these stereoisomers are known, and their nuclear magnetic resonance spectra have been determined; the *threo* compound has been studied by Anet[26a] and Gawron[9] and the *erythro* by Alberty and Bender.[26b] The reaction was accordingly carried out in D_2O and the n.m.r. spectrum of the dipotassium salt of the malic acid formed was determined. The spectrum did not resemble either of the published spectra, appearing to lack an absorption band for the methylene proton. In a separate experiment an authentic sample of DL-malic acid was subjected to the same conditions of temperature and solvent, and the n.m.r. spectrum of the remaining malate showed a single absorption band, corresponding to the proton in the 2-position. This experiment indicated that the exchange of methylene protons is much more rapid than the dehydration reaction. It was therefore not possible to determine by means of nuclear magnetic resonance experiments whether the addition is *cis* or *trans*, or even to demonstrate that it is stereospecific.

The mechanisms 10 and 11 may be schematically represented as 14 and 15, respectively. If the initial reactant is an optically active malic acid, a comparison of the rate of disappearance of malic acid, measured polarimetrically, with the rate

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[25] H. N. K. Rørdam, *J. Chem. Soc.*, 2931 (1932); W. A. Cowdrey E. D. Hughes, C. K. Ingold, S. Masterman and A. D. Scott, *ibid.*, 1252 (1937).

[26] (a) F. A. L. Anet, *J. Am. Chem. Soc.*, **82**, 994 (1960); (b) R. A. Alberty and P. Bender, *ibid.*, **81**, 542 (1959).

$$M \underset{k_h}{\overset{k_d}{\rightleftarrows}} F \qquad (14)$$

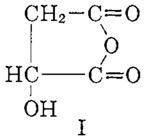
$$M \underset{k_2}{\overset{k_1}{\rightleftarrows}} L \underset{k_4}{\overset{k_3}{\rightleftarrows}} F \qquad (15)$$

of appearance of fumaric acid, measured spectrophotometrically, may permit differentiation between these mechanisms. If mechanism 14 is operative these two rates must be equal.

In eq. 15, if $k_1 \cong k_d$ and $k_4 \cong k_h$, the same equality of rates of reaction of L-malic acid will be expected, since return of the optically active lactone to reactant will produce L-malic acid; loss of optical activity will therefore occur at the same rate as production of fumaric acid. If, however, $k_4 \cong k_h$ and $k_3 \cong k_d$, with both k_1 and k_2 large compared with k_h and k_d, it is possible that mechanism 15 will result in a difference between the rates of production of fumaric acid and loss of optically active malic acid. The magnitude and sign of rotation by the lactone will determine whether this differentiation can be realized. In the present case the postulated intermediate shows rotation opposite in sign to L-malic acid ($[\alpha]_D = +41°$ for β-malolactonic acid,[27] while $[\alpha]_D = -8°$ (pH 4) for L-malic acid), so that the differentiation may be possible if this assignment of rate constants is valid.

The spectrophotometrically measured rate constant k_d in a solution of L-malic acid monoanion was 4.3×10^{-6} sec.$^{-1}$ (from the data in Table I it can be concluded that $pH_{175} = 5.0$ in this solution). The first-order rate constant k_r for the rate of loss of malic acid (measured polarimetrially) in the same solution was 3.2×10^{-5} sec.$^{-1}$, so that $k_r/k_d = 7.5$. This large difference in rate constants cannot be due to hydroxide ion-catalyzed racemization[7] of L-malate, for this process is negligibly slow at pH 5. A racemization reaction for the monoanion which is not associated with the dehydration reaction is possible, but a mechanism involving removal of the hydrogen atom is ruled out since the n.m.r. experiment described above indicated that the hydrogen on the asymmetric carbon atom does not exchange with the solvent. It is concluded that the difference between k_r and k_d is real, and that this evidence probably eliminates the one-step mechanism shown in eq. 10 as the operative one in the present reaction. On the basis of these results we feel that eq. 11 probably represents the course of the reaction.

Some analogies and reservations pertinent to this conclusion must be pointed out. No account has been taken of the possible intermediate formation of malic acid anhydride (I) which might be



I

produced from β-malolactonic acid *via* a transannular attack, by the free carboxyl group, on the ring carbonyl. Substituted β-malolactonic acids are known to yield the corresponding anhydrides upon distillation.[28] If I is indeed produced and then undergoes hydrolysis to malic acid, as shown in scheme 16, then the optical rotation change

$$\begin{tikzcd} \text{L-malic acid} \arrow[r] & \text{D-malolactonic acid} \arrow[r] \arrow[d] & \text{fumaric acid} \\ \text{D-malic acid} & \text{D-malic acid anhydride} \arrow[l] & \end{tikzcd} \qquad (16)$$

will not be related to only the rate constants in eq. 15, and the case in which $k_1 \cong k_d$ and $k_4 \cong k_h$ is not necessarily ruled out by the polarimetric study. This scheme is consistent with the result $k_r > k_d$ and does not require the buildup of relatively large concentrations of the lactone in solution.

The hydrolysis of β-malolactonic acid to malic acid, which in any mechanism employing the scheme 15 will be postulated to be a fast reaction, has been studied by Holmberg,[27] who gives as the first-order rate constant at 25° in neutral solution $k = 3.4 - 4.0 \times 10^{-4}$ min.$^{-1}$. One may therefore consider k_2 (in eq. 15) as very large compared with either k_h or k_d.

A further aspect of the chemistry of β-malolactonic acids must be briefly noted. Only one such compound, the β,β-di-*n*-propyl-β-malolactonic acid, has been reported to be formed from the corresponding hydroxy acid,[29a] and it has been suggested by Zaugg[29b] that this exceptional behavior may be apparent only, and that Bains and Thorpe actually did not have the β-lactone. Thus no good evidence exists to show that such a transformation as that postulated in the present work is possible. The conditions employed here are, however, quite different from those used in earlier studies[29a]; moreover, the production of β-malolactonic acid in concentrations too low to permit isolation can account for all of the kinetic results observed here.

The other transformation postulated in mechanism 11, the isomerization of a lactone to an unsaturated acid, is a known reaction. This phenomenon (termed "lacto-enoic" tautomerism by Linstead[30]) has been observed to be reversible for some acid–lactone systems, though in no case was a β-lactone involved. Under the conditions of these studies crotonic acid gave no lactone.[31] Since most of these reactions were carried out in strongly acid solution they provide little evidence which is helpful in the present study. It is interesting that the reversible conversion of an unsaturated acid (*cis-cis-* or *cis-trans*-muconic acid) to the lactone can be effected by an enzyme.[32]

Conclusions.—The reaction reported in this paper appears to be the first observed non-enzymatic olefinic hydration under neutral conditions. Its rate is extremely small, so the system does not parallel the fumarase-catalyzed reaction very closely. However, the pH dependence of the kinetics is in qualitative accord with that of the enzymatic reaction, and the implication of an acidic

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(27) B. Holmberg, *Svensk. Kem. Tidskr.*, **30**, 190, 215 (1918).

(28) A. Kandiah, *J. Chem. Soc.*, 1215 (1932).

(29) (a) L. Bains and J. F. Thorpe, *ibid.*, 2742 (1923); (b) H. E. Zaugg, "Organic Reactions," Vol. VIII, J. Wiley and Sons, Inc., New York, N. Y., 1954, p. 317.

(30) R. P. Linstead and H. N. Rydon, *J. Chem. Soc.*, 580 (1933).

(31) E. J. Boorman and R. P. Linstead, *ibid.*, 577 (1933).

(32) Y. J. Topper, in "The Enzymes," Vol. 5, 2nd ed., P. D. Boyer, H. Lardy and K. Myrbäck, editors, Academic Press, Inc., New York, N. Y., 1961, Chap. 25.

and a basic group in the non-enzymatic catalysis indicates that a similar, but more efficient, assistance may be responsible for the enzymatic catalysis.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF MICHIGAN, ANN ARBOR, MICH.]

The Mechanism of the Decompositions of *t*-Butyl 3-Phenylperpropionate and *t*-Butyl 4-Phenylperbutyrate

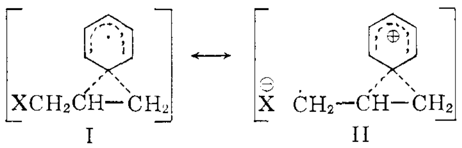
BY MICHAEL M. MARTIN

RECEIVED DECEMBER 5, 1961

A kinetic study of the free radical decompositions of *t*-butyl 3-phenylperpropionate and 4-phenylperbutyrate has established that these peresters decompose by a two-step, non-concerted mechanism. This is evidence against the existence of bridged, "non-classical" free radicals.

Introduction

In the course of studies currently in progress relating to polar effects in free radical-forming reactions, it became essential that the question of the existence or lack of existence of bridged, "non-classical" radicals, analogous to the phenonium ions which intervene during some of the solvolytic reactions of β-phenyl-substituted benzenesulfonates and substituted benzenesulfonates,[1,2] be settled. The transition state leading to such a bridged radical formed by the addition of a free radical to allylbenzene is depicted in structure I, or, employing the polar model for the transition state of a free radical-forming reaction, suggested by Walling and Mayo,[3] and utilized by many others,[4] by structure II.



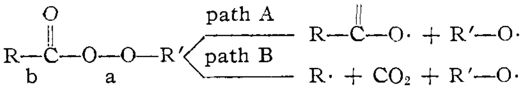
I II

To date, no evidence has been presented which requires phenyl participation in radical-forming reactions at a carbon atom beta to a benzene ring, but neither has any been presented which rigorously excludes it. In their studies on the decarbonylation of β-phenylated propionaldehydes, Curtin and Hurwitz[5] observed that for a 1,2-phenyl shift to occur, the migration origin must either be tertiary, or if secondary, both groups must be aryl. The authors recognized that no conclusions regarding the concerted or non-concerted nature of the decarbonylation could be drawn, however.

The most compelling evidence against the existence of bridged radicals was presented by Seubold,[6] who found that the degree of rearrangement of the 2-phenyl-2,2-dimethylethyl radical, generated by the decarbonylation of 3-phenyl-3,3-dimethylpropionaldehyde, depends upon the concentration of the hydrogen donor, thus implicating the simultaneous existence of at least two interconvertible radical species. However, it is still possible that the first-formed radical is bridged, and that this radical may then abstract a hydrogen atom from the hydrogen donor or rearrange to a non-bridged radical. Thus, although this work precludes a bridged radical as the product-determining species, it does not rigorously exclude bridging at the rate-determining stage of radical formation.

Overberger and Gainer[7] studied the rates of decomposition of three *p*-substituted 2-azo-bis-3-methyl-3-phenylbutanes at 255°, and found the rate to be insensitive to the electronic nature of the *p*-substituent. This result is consistent with the lack of participation by a β-phenyl group, but does not rigorously exclude it, since, as Leffler[8] has pointed out, the rates of a reaction for a series of related compounds can be relatively insensitive to changes in structure or solvent when the particular reaction is observed near the isokinetic temperature because of compensating changes in ΔH^* and ΔS^*.

Nowhere has the behavior of ΔH^* and ΔS^* been utilized so effectively in gaining an understanding of the nature of a transition state than in perester decompositions (reaction 1). In the elegant work of Bartlett's group,[9] the factors which cause a perester to decompose in a stepwise (path A) or concerted fashion (path B) are established. When



$$\underset{\mathrm{III}}{\mathrm{R}\overset{\mathrm{b}}{—}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}—\mathrm{O}\overset{\mathrm{a}}{—}\mathrm{O}—\mathrm{R}'} \begin{cases} \xrightarrow{\text{path A}} \mathrm{R}—\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}—\mathrm{O}\cdot + \mathrm{R}'—\mathrm{O}\cdot \\ \xrightarrow{\text{path B}} \mathrm{R}\cdot + \mathrm{CO_2} + \mathrm{R}'—\mathrm{O}\cdot \end{cases} \qquad (1)$$

III

the alkyl radical R· is of a stable type (benzyl, *t*-butyl or trichloromethyl), there is simultaneous cleavage of bonds a and b, producing carbon dioxide and the alkyl radical in the rate-determining step.

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[1] D. Cram, *J. Am. Chem. Soc.*, **71**, 3863 (1949); **74**, 2129, 2137 2159 (1952).

[2] (a) S. Winstein, B. K. Morse, E. Grunwald, K. C. Schreiber and J. Corse, *ibid.*, **74**, 1113 (1952); (b) S. Winstein and B. K. Morse, *ibid.*, **74**, 1133 (1952); (c) S. Winstein, M. Brown, K. C. Schreiber and A. H. Schlesinger, *ibid.*, **74**, 1140 (1952); (d) S. Winstein and K. Schreiber, *ibid.*, **74**, 2165, 2171 (1952).

[3] (a) F. R. Mayo and C. Walling, *Chem. Revs.*, **46**, 191 (1950); (b) C. Walling, "Free Radicals in Solution," John Wiley and Sons, Inc., New York, N. Y., 1957; (c) C. Walling and B. Miller, *J. Am. Chem. Soc.*, **79**, 4181 (1957); (d) C. Walling and B. Jacknow, *ibid.*, **82**, 1756 (1960).

[4] (a) G. A. Russell, *J. Org. Chem.*, **23**, 1407 (1958); (b) E. S. Huyser, *J. Am. Chem. Soc.*, **82**, 394 (1960); (c) P. D. Bartlett and C. Rüchardt, *ibid.*, **82**, 1756 (1960).

[5] D. Y. Curtin and M. J. Hurwitz, *ibid.*, **74**, 5381 (1952).

[6] F. H. Seubold, *ibid.*, **75**, 2532 (1953).

[7] C. G. Overberger and H. Gainer, *ibid.*, **80**, 4561 (1958).

[8] (a) J. E. Leffler, *J. Org. Chem.*, **20**, 1202 (1955); (b) M. G. Alder and J. E. Leffler, *J. Am. Chem. Soc.*, **76**, 1475 (1954).

[9] (a) P. D. Bartlett and R. R. Hiatt, *ibid.*, **80**, 1398 (1958); (b) P. D. Bartlett and D. M. Simon, *ibid.*, **82**, 1753 (1960); (c) P. D. Bartlett, E. P. Benzing and R. E. Pincock, *ibid.*, **82**, 1762 (1960); (d) P. D. Bartlett and R. E. Pincock, *ibid.*, **82**, 1769 (1960).