

carbanion intermediate in which the rate of protonation to give aspartate is greater than the rate at which ammonia leaves the carbanion to give fumarate. Although the above experiments were carried out at pH 8 where the major reaction is NH_3 addition to fum^{0-} , similar results would be expected for NH_3 additions to fum^{00} and fum^{-} . The mechanism for the reversible deamination of aspartic acid may therefore be classified as an E1cB elimination and $\text{AdN}2$ addition.⁹

(9) $\text{AdN}2$ is an abbreviation for second-order nucleophilic addition going through a carbanion intermediate.

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The Kinetics of Hydration of Fumaric Acid between pH 0 and 6

Sir:

The reversible hydration of fumaric acid to malic acid, a reaction which is catalyzed by the enzyme fumarase, also occurs nonenzymatically at elevated temperatures. The kinetics and mechanism of the nonenzymatic reaction have been investigated at several temperatures in acid and basic solutions by Alberty and coworkers^{1,2}

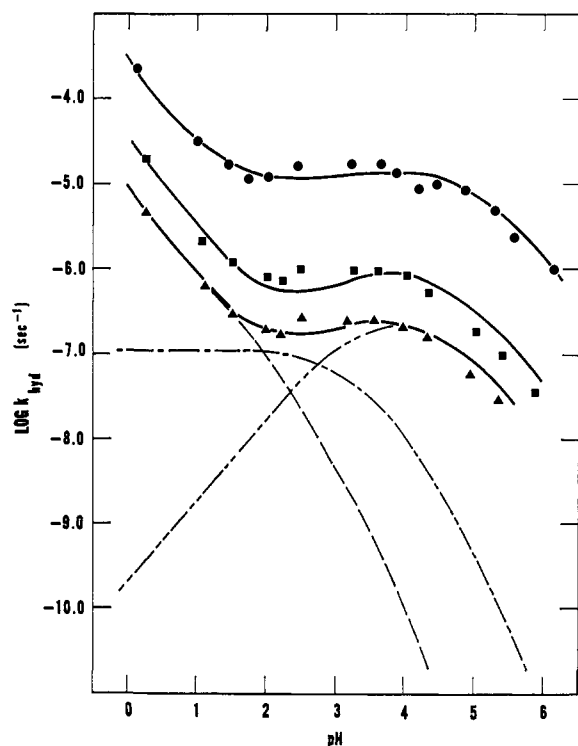


Figure 1. Rate of hydration of fumaric acid as a function of pH and temperature: —, rate for acid-catalyzed hydration; — — —, rate of H_2O addition to fum^{00} ; — — — —, rate of OH^- addition to fum^{00} ; \blacktriangle , rate at 117.8° ; \blacksquare , rate at 135.3° ; \bullet , rate at 175° .³

and between pH 0 and 6 at 175° by Bender and Connors.³ The mechanism proposed by Bender and Con-

- (1) L. T. Rozelle and R. A. Alberty, *J. Phys. Chem.*, **61**, 1637 (1957).
- (2) L. E. Erickson and R. A. Alberty, *ibid.*, **63**, 705 (1959).
- (3) M. L. Bender and K. A. Connors, *J. Amer. Chem. Soc.*, **83**, 4099 (1961); M. L. Bender and K. A. Connors, *ibid.*, **84**, 1980 (1962).

nors for the reaction between pH 2 and 6 involves the formation of the intermediate β -malolactonic acid, which undergoes rapid hydrolysis to malic acid. The β -malolactonic acid is formed from the isomerization of the monoanion of fumaric acid. This mechanism was based on the assumption that the kinetics of hydration between pH 2 and 6 could be interpreted in terms of the kinetic expression $k_{\beta\text{-malo}}(\text{fum}^{0-})$.⁴ On the basis of this assumption, Bender and Connors calculated values of 2.0 and 4.6 for $\text{p}K_1$ and $\text{p}K_2$ of fumaric acid at 175° .

In recent investigations of the equilibrium constant for the reversible deamination of aspartic acid to fumaric acid and ammonia,⁶ the $\text{p}K$'s of fumaric acid were measured between 0 and 95° . Extrapolation of these measurements to 175° gives $\text{p}K_1 = 3.53$ and $\text{p}K_2 = 4.98$. The extrapolated $\text{p}K_2$ value differs slightly from the calculated $\text{p}K_2$ value of Bender and Connors. There is, however, a large difference in the extrapolated $\text{p}K_1$ value and that calculated from the kinetic data. Using the extrapolated $\text{p}K$ values in the rate equation given by Bender and Connors gives a poor fit to their experimental data. Therefore, the rate equation used by Bender and Connors is either incorrect or incomplete. This in turn casts doubt on the β -malolactonic acid mechanism.

This communication reports measurements of the kinetics of the nonenzymatic hydration reaction between pH 0 and 6 at 118 and 135° . The lower temperatures were used to reduce the extrapolation of the measured $\text{p}K$'s of fumaric acid.

Solutions of fumaric acid were buffered by either hydrochloric acid or succinate.⁷ Sodium chloride was added to the solutions to adjust the ionic strength to 0.1. The reaction solutions were deoxygenated and sealed under vacuum in Pyrex glass ampoules. The rate of disappearance of fumaric acid was determined spectrophotometrically at 118 and 135° . From these measurements, the first-order rate constants for hydration, k_{hyd} , were determined as a function of pH. The results are shown in Figure 1; also shown are the k_{hyd} values at 175° determined by Bender and Connors.

The results in Figure 1 can be interpreted by the rate expression

$$\frac{d(\text{malic acid})}{dt} = k_{\text{hyd}}(\text{fum})_{\text{T}} = k_{00}^{\text{H}^+}(\text{fum}^{00})(\text{H}^+) + k_{00}(\text{fum}^{00})(\text{H}_2\text{O}) + k_{00}^{\text{OH}^-}(\text{fum}^{00})(\text{OH}^-) \quad (1)$$

The k 's are the rate constants for the various reactions. Equation 1 can be rewritten as

$$k_{\text{hyd}} = \frac{k_{00}^{\text{H}^+}(\text{H}^+)}{\left(1 + \frac{K_{1\text{fum}}}{(\text{H}^+)} + \frac{K_{1\text{fum}}K_{2\text{fum}}}{(\text{H}^+)^2}\right)} + \frac{k_{00}^{\text{H}_2\text{O}}}{\left(1 + \frac{K_{1\text{fum}}}{(\text{H}^+)} + \frac{K_{1\text{fum}}K_{2\text{fum}}}{(\text{H}^+)^2}\right)} + \frac{k_{00}^{\text{OH}^-}K_{\text{w}}}{(\text{H}^+)\left(1 + \frac{K_{1\text{fum}}}{(\text{H}^+)} + \frac{K_{1\text{fum}}K_{2\text{fum}}}{(\text{H}^+)^2}\right)} \quad (2)$$

(4) The abbreviations used in this communication are the same as those given previously.⁵

(5) J. L. Bada and S. L. Miller, *J. Amer. Chem. Soc.*, **91**, 3946 (1969).

(6) J. L. Bada and S. L. Miller, *Biochemistry*, **7**, 3403 (1968).

(7) The determination of the pH values of the buffers at 118 and 135° has been described in previous publications.^{5,6}

where $k_{00}^{\text{H}_2\text{O}} = k_{00}(\text{H}_2\text{O})$ and K_w is the ionization constant of water. Using the measured pK 's of fumaric acid,² the k_{hyd} data shown in Figure 1 were fitted by the method of least squares to obtain equations in the form of eq 2. The solid curves shown in Figure 1 were calculated from these equations. The values determined for the rate constants at 117.8° are $k_{00}^{\text{H}^+} = 7.85 \times 10^{-6} \text{ sec}^{-1} M^{-1}$, $k_{00}^{\text{H}_2\text{O}} = 1.09 \times 10^{-7} \text{ sec}^{-1}$, and $k_{00}^{\text{OH}^-} = 2.17 \times 10^2 \text{ sec}^{-1} M^{-1}$. Also shown in Figure 1 are the rate constants at 117.8° for the individual reactions as a function of pH.

The rate constants determined in these investigations for the acid-catalyzed hydration reaction ($k_{00}^{\text{H}^+}$) are very similar to those determined by Rozelle and Alberty.¹ The ratio of the rate constants for the addition of hydroxide ion to neutral fumaric acid ($k_{00}^{\text{OH}^-}$) and the fumarate dianion² ($k_{--}^{\text{OH}^-}$) can be compared to $k_{00}^{\text{NH}_3}$ and $k_{--}^{\text{NH}_3}$ calculated for the amination reaction.⁵ For both OH^- and NH_3 , the rates of addition to fum^{00} are much greater than the rates of addition to fum^{--} . However, at 135° $k_{00}^{\text{OH}^-}/k_{--}^{\text{OH}^-} = 2.8 \times 10^7$ while $k_{00}^{\text{NH}_3}/k_{--}^{\text{NH}_3} = 2.7 \times 10^4$. This 10^3 difference can be explained as follows. The ratio $k_{00}^{\text{OH}^-}/k_{00}^{\text{NH}_3}$ gives the relative nucleophilicity of OH^- compared to NH_3 . This ratio is 2.1×10^3 at 135.3°. However, $k_{--}^{\text{OH}^-}/k_{--}^{\text{NH}_3}$ at this temperature is only 1.9. The ratio $k_{--}^{\text{OH}^-}/k_{--}^{\text{NH}_3}$ is smaller than $k_{00}^{\text{OH}^-}/k_{00}^{\text{NH}_3}$ because of an electrostatic term in the free energy of activation for OH^- addition to fum^{--} . This electrostatic free energy, $\Delta F_{\text{el}}^\ddagger$, is the free energy required to bring together OH^- and fum^{--} from infinite separation. For OH^- addition to fum^{00} and NH_3 addition to both fum^{00} and fum^{--} , $\Delta F_{\text{el}}^\ddagger$ can be approximated as zero. This electrostatic free energy term can be written as

$$\begin{aligned} \Delta F_{\text{el}}^\ddagger &= (\Delta F_{--}^\ddagger - \Delta F_{00}^\ddagger)_{\text{OH}^- \text{ addn}} - \\ &\quad (\Delta F_{--}^\ddagger - \Delta F_{00}^\ddagger)_{\text{NH}_3 \text{ addn}} \\ &= RT \ln \{2.81 \times 10^7 / 2.73 \times 10^4\} = 5.6 \text{ kcal/mol} \end{aligned}$$

$\Delta F_{\text{el}}^\ddagger$ can be estimated theoretically from^{8,9}

$$\Delta F_{\text{el}}^\ddagger = \frac{Z_{\text{OH}^-} Z_{\text{fum}^{--}} e^2 N}{Dr_\ddagger}$$

Substituting $\Delta F_{\text{el}}^\ddagger = 5.6 \text{ kcal/mol}$ in this expression gives $Dr_\ddagger = 1.2 \times 10^{-6} \text{ cm}$. Using the value¹⁰ $D = 45$ at 135° gives $r_\ddagger = 2.7 \text{ \AA}$ which is of the expected magnitude. Therefore most of the 10^3 difference between $k_{00}^{\text{OH}^-}/k_{--}^{\text{OH}^-}$ and $k_{00}^{\text{NH}_3}/k_{--}^{\text{NH}_3}$ can be accounted for by the electrostatic free energy.

Although the term $k_{0-}(\text{fum}^{0-})(\text{H}_2\text{O}) = k_{0-}^{\text{H}_2\text{O}}(\text{fum}^{0-})$ is kinetically equivalent to $k_{00}^{\text{OH}^-}(\text{fum}^{00})(\text{OH}^-)$, it does not appear in the rate expression given in eq 1. This can be accounted for on the following basis. The ratio of $k_{00}^{\text{NH}_3}/k_{0-}^{\text{NH}_3}$ at 135.3° is 5.75.⁵ Assuming the same ratio for $k_{00}^{\text{H}_2\text{O}}/k_{0-}^{\text{H}_2\text{O}}$ gives an estimated value for $k_{0-}^{\text{H}_2\text{O}}$ at 135.3° of $4.5 \times 10^{-8} \text{ sec}^{-1}$. At pH 4, this gives $3.2 \times 10^{-8} \text{ sec}^{-1}$ for the maximum rate of addition of H_2O to fum^{0-} at 135.3°. This is about 4% of the measured k_{hyd} at this pH. On the basis of these

(8) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley & Sons, Inc., New York, N. Y., 1961, p 144.

(9) The Z 's are the respective charges on OH^- and fum^{--} , e the charge on an electron, N Avogadro's number, D the dielectric constant of the surrounding media, and r_\ddagger the equilibrium distance between OH^- and fum^{--} in the activated complex.

(10) C. G. Malmberg and A. A. Maryott, *J. Res. Natl. Bur. Std.*, **56**, 1 (1956).

considerations, $k_{0-}^{\text{OH}^-}(\text{fum}^{00})(\text{OH}^-)$ appears to be the dominant term with only a small contribution from the kinetically equivalent term $k_{0-}^{\text{H}_2\text{O}}(\text{fum}^{0-})$.

These results cannot exclude the β -malolactonic acid mechanism. However, the addition of NH_3 to fum^{0-} has been shown to be nonstereoselective⁵ which is inconsistent with the stereochemistry expected for the β -malolactonic acid mechanism.¹¹ It seems unlikely that there should be two different mechanisms for the amination and hydration of fumaric acid. The mechanism⁵ for the amination of fumaric acid has been classified as AdN_2 , and a similar mechanism can be written for the hydration reaction. The relative rates of the amination and hydration of the various ionic forms of fumaric acid differ because of the different nucleophilicities of NH_3 , OH^- , and H_2O and because of electrostatic effects.¹²

(11) The β -malolactonic acid mechanism proposed by Bender and Connors predicts stereoselective *cis* addition.⁹

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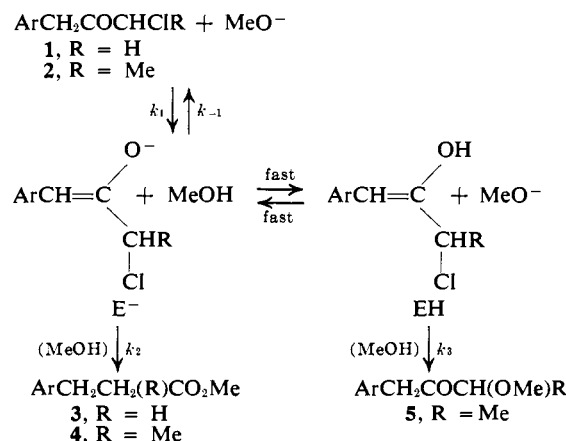
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Mechanistic Change in the Favorskii Rearrangement on Methyl Substitution

Sir:

Recently it was demonstrated that in the ionization of X^- from the enolate ion $\text{ArCH}=\text{C}(\text{O}^-)\text{CH}_2\text{X}$ the $\text{C}-\text{X}$ bond developed a high degree of ionic character in the transition state ($\rho \cong -5$).¹ It follows that substitution of a methyl group for hydrogen, as in $\text{ArCH}=\text{C}(\text{O}^-)\text{CHXMe}$, should greatly facilitate this ionization.² This prediction has now been confirmed and, as a consequence of this rate change, a dramatic change in the mechanism and products formed under Favorskii rearrangement conditions has been observed (Table I).

The differences summarized in Table I can be accommodated by a common mechanistic scheme for **1** and **2** in which the presence of the methyl group plays a decisive role on the course of the reaction.



(1) F. G. Bordwell, W. R. Springer, and R. G. Scamehorn, *J. Am. Chem. Soc.*, **91**, 2087 (1969).

(2) For *lim* solvolyses the rate acceleration brought about by methyl substitution is of the order of 10^4 ; see, e.g., A. Streitwieser, Jr., "Solvolytic Displacement Reactions," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 74.