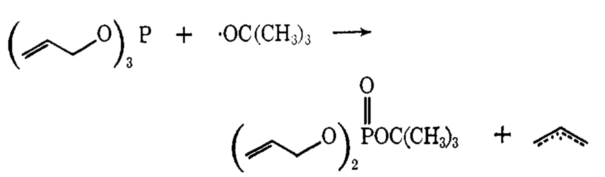
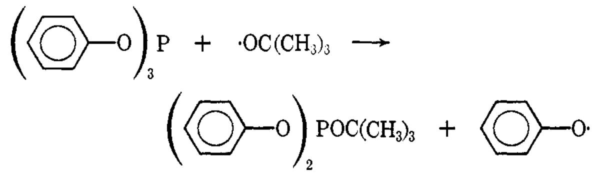
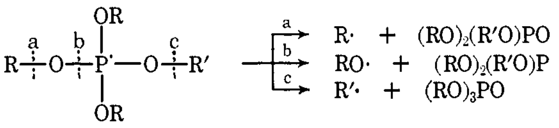

(8)

radical[10] was obtained when a dilute solution of triphenyl phosphite and di-*t*-butyl peroxide in cyclopropane was irradiated at $-85°$.[11]

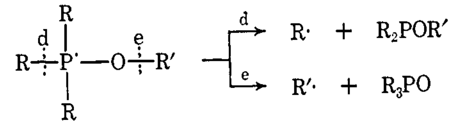

(9)

It is possible to rationalize the multiplicity of behavior of phosphite esters toward alkoxy radicals in the foregoing reactions (eq 7, 8, and 9), by considering a common four-coordinate phosphorus-centered radical intermediate such as II, which fragments to yield the most stable radical (eq 10).[12]


(10)

II

A phosphoranyl radical intermediate III similar to II can also account (eq 11) for the homolytic displacement of alkyl groups from trialkylphosphines by *t*-butoxy radicals (eq 3) and the sulfur-atom transfer from *t*-butylthiyl radicals to trialkylphosphines (eq 5). Except in the case of methylphosphines,[6] however, we have been unable to detect such an intermediate. The lifetimes of these phosphoranyl radicals are too short for detection and are presumably related to the ease with which alkyl radicals are expelled.[13] Finally, it should be added that the homolytic displacement reactions of eq 1 are not limited to phosphines but have been observed also with trialkylarsenic and trialkylantimony compounds. Thus, only the spectrum of ethyl radicals was detected when triethylarsenic and triethylantimony were subjected to *t*-butoxy radicals. The nitrogen analogs, on the other hand, do not generate alkyl radicals under the same conditions.[14,15]


(11)

III

Acknowledgment. We wish to thank Mr. K. Eaby for technical assistance.

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[10] The presence of a relatively long-lived radical species giving rise to a broad singlet obscured the spectrum of phenoxy radical (a_0 = 6.78, a_m = 1.90, and a_p = 10.04 G; $T = -55°$) on prolonged irradiation (*cf.* ref 4). Nmr analysis showed the absence of phenol in the sample prior to irradiation.

[11] Chemical studies lead to the same conclusion [W. G. Bentrude, *Tetrahedron Letters*, 3543 (1965); *J. Am. Chem. Soc.*, **87**, 4062 (1965)].

[12] Recent tracer studies have also pointed strongly to such a phosphoranyl radical intermediate [W. G. Bentrude and R. A. Wielesek, *ibid.*, **91**, 2406 (1969)].

[13] Whether the reaction proceeds by direct displacement [S$_H$2 mechanism; *cf.* W. A. Pryor, "Free Radicals," McGraw-Hill Book Co., Inc., New York, N. Y., 1966, p 9] or *via* a metastable four-coordinate species at this juncture is a moot point.

[14] For example, triethylamine does not afford ethyl radicals. A paramagnetic species is formed, however, and further studies of amines are in progress.

[15] Irradiation of a solid solution of hydrogen peroxide and triethylamine has been reported to form ethyl radicals [V. I. Mal'tsev and A. A. Petrov, *J. Org. Chem., USSR*, **3**, 205 (1967).].

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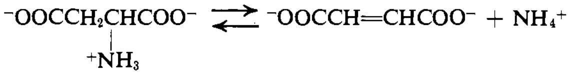
The Kinetics and Mechanism of the Nonenzymatic Reversible Deamination of Aspartic Acid

Sir:

The reversible deamination of aspartic acid, which yields fumaric acid and ammonia, is catalyzed by aspartase, an enzyme which has been isolated from several microorganisms and a few higher plants. This deamination reaction also occurs nonenzymatically at elevated temperatures.[1] The reaction can be written as



$$^{-}OOCCH_2\underset{+NH_3}{\underset{|}{C}}HCOO^{-} \rightleftharpoons {}^{-}OOCCH{=}CHCOO^{-} + NH_4^{+} \quad (1)$$

The equilibrium constant for the reaction has been measured[2] between 5 and 135°. This reversible deamination of aspartic acid differs from the decomposition of the other amino acids found in proteins. Most other amino acids undergo a slow irreversible decarboxylation.[3] The kinetics for this nonenzymatic reversible deamination of aspartic acid and the equilibrium constant have been used to estimate the minimum ammonium ion concentration in the oceans of the primitive earth.[1] We report here further investigations of the kinetics and mechanics of this reaction.

The rate of deamination of aspartic acid was studied between pH -1 and 13 over the temperature range 60 to 135°. Solutions of DL-aspartic acid, buffered by either hydrochloric acid, succinate, phosphate, borate, or NaOH, were used for the determinations.[4] The final ionic strength was adjusted to 0.1 by adding sodium chloride. The solutions were deoxygenated and sealed under vacuum in glass ampoules. Values of k_{deam}, the first-order rate constant for deamination, were determined from measurements of the rate of appearance

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[1] J. L. Bada, and S. L. Miller, *Science*, **159**, 423 (1968).

[2] J. L. Bada and S. L. Miller, *Biochemistry*, **7**, 3403 (1968).

[3] P. H. Abelson in "Researches in Geochemistry," P. H. Abelson, Ed., John Wiley & Sons, Inc., New York, N. Y., 1959, p 79; J. R. Vallentyne, *Geochem. Cosmochim. Acta*, **28**, 157 (1964); D. Conway and W. F. Libby, *J. Amer. Chem. Soc.*, **80**, 1077 (1958).

[4] The pH values of the HCl, phosphate, borate, and carbonate buffers at the various temperatures were determined by extrapolation of data taken from R. G. Bates, G. D. Pinching, and E. R. Smith, *J. Res. Natl. Bur. Std.*, **45**, 418 (1950), and R. G. Bates, "Determination of pH," John Wiley & Sons, Inc., New York, N. Y., 1964, p 76; the pH values of the succinate buffers as a function of temperature were determined from measurements described previously[2] and from the data of R. G. Bates and R. Gary, *J. Res. Natl. Bur. Std.*, **65A**, 495 (1961); the pH of the NaOH buffers at the elevated temperatures were calculated from the temperature variation of pK_w given in R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworth & Co. (Publishers), London, 1959, p 544.

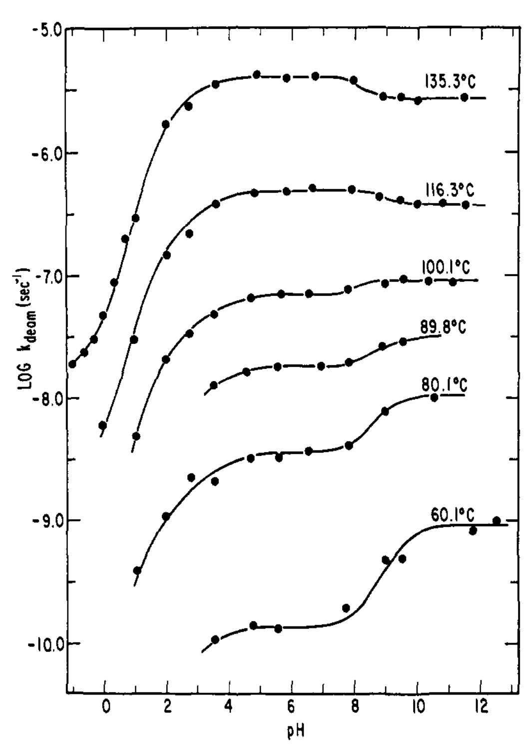

Figure 1. Rate of deamination of aspartic acid as a function of pH and temperature.

of ammonia[5] and confirmed by the ultraviolet absorption of fumaric acid.[2] The results are shown in Figure 1. The experimental uncertainty of the measurements is about ±5%. Figure 1 indicates that the reaction is independent of pH in base (pH greater than 10) and between pH 4.5 and 7. The values of E_a, the Arrhenius activation energy, calculated for the reaction at pH values greater than 10 and at pH 7 are 28.6 and 36.8 kcal/mol, respectively.

The kinetics and mechanism of the reverse reaction, the addition of NH_3 to fumaric acid, are easier to visualize than those of the deamination reaction. The second-order rate constants for NH_3 addition to fumaric acid were calculated from the rates of deamination and the equilibrium constant.[2] These calculations indicate that the addition reaction is independent of pH for values greater than 10, has a maximum near pH 6, and decreases rapidly with pH for values less than 5. From this observed pH dependence, the following rate expression can be written to describe the observed rates of addition[6]

$$\frac{d(\text{aspartic acid})}{dt} = k_{\text{addn}}(\text{fum})_T(NH_3)_T = k_{00}^{NH_3}(\text{fum}^{00})(NH_3) + k_{0-}^{NH_3}(\text{fum}^{0-})(NH_3) + k_{--}^{NH_3}(\text{fum}^{--})(NH_3) \quad (2)$$

where $k_{00}^{NH_3}$, $k_{0-}^{NH_3}$, and $k_{--}^{NH_3}$ are the second-order rate constants for the addition of ammonia to neutral fumaric acid, the fumarate monoanion, and the fumarate dianion, respectively. Using the pK's of fumaric acid[2] and the pK_a of ammonia,[2,7] the values of k_{addn}, calculated from k_{deam} and the equilibrium constant, were fitted by the method of least squares to obtain equations in the form of eq 2. The values determined for $k_{00}^{NH_3}$, $k_{0-}^{NH_3}$, and $k_{--}^{NH_3}$ at 116.3° are 5.68×10^{-2} sec^{-1} M^{-1}, 1.45×10^{-2} sec^{-1} M^{-1}, and 1.88×10^{-6} sec^{-1} M^{-1}, respectively. Figure 2 shows the values of k_{addn} at 116.3° along with the curves calculated for the individual reactions of ammonia with fum^{00}, fum^{0-}, and fum^{--}. The ratio $k_{--}^{NH_3}$:$k_{0-}^{NH_3}$:$k_{00}^{NH_3}$ at 116.3° is 1:7700:30,000; the ratio at 135° is 1:4700:27,000.

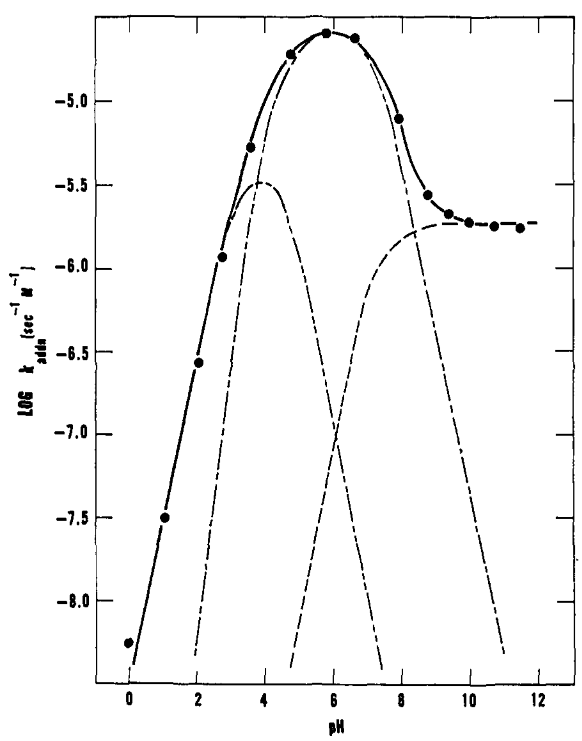

Figure 2. Rate of addition of ammonia to fumaric acid as a function of pH at 116.3°: — — — —, rate of addition of NH_3 to fum^{00}; — — —, rate of addition of NH_3 to fum^{0-}; — —, rate of addition of NH_3 to fum^{--}; ●, values of k_{addn} calculated from k_{deam} and the equilibrium constant.

These calculations indicate that between pH 1 and 13, the amination of fumaric acid is composed of ammonia additions to the various ionic forms of fumaric acid. In studies of OH^- addition to fum^{--}, Erickson and Alberty[8] suggested a mechanism for the reaction which involved a carbanion intermediate. The experiment which supported this mechanism was that when disodium malate was heated in D_2O and 0.5 M NaOD, the malate CH_2 protons exchanged rapidly with D_2O. We have carried out a similar experiment using aspartic acid. A buffered solution of DL-aspartate was heated at 116.3° in D_2O at pH$_{116°}$ 8.1. The aspartate CH_2 protons also exchanged rapidly with solvent, suggesting that a carbanion intermediate is formed in the reaction. The stereochemistry of the addition of ND_3 to fumaric acid in D_2O at 116° and pH 8 was also investigated, and found to give about equal amounts of *cis* and *trans* addition, the products being *threo*- and *erythro*-3-deuterio-DL-aspartic acid, respectively. The *threo* and *erythro* isomers were distinguished by their nmr spectra. Both of these results support a mechanism involving a planar

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(5) E. J. Conway, "Microdiffusion Analysis and Volumetric Error," Chemical Publishing Co., New York, N. Y., 1963, p 98.

(6) The following abbreviations will be used; fum^{00}, HOOCCH=CHCOOH; fum^{0-}, HOOCCH=CHCOO$^-$; fum^{--}, $^-$OOCCH=CHCOO$^-$; (fum)$_T$ and $(NH_3)_T$ are the total concentrations of the various ionic forms of fumaric acid and ammonia, respectively.

(7) R. G. Bates and G. D. Pinching, *J. Res. Natl. Bur. Std.*, **42**, 419 (1949).

(8) L. E. Erickson and R. A. Alberty, *J. Phys. Chem.*, **63**, 705 (1959).

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 ElcB elimination and AdN2 addition.[9]

[9] AdN2 is an abbreviation for second-order nucleophilic addition going through a carbanion intermediate.

[10] This work was supported by Grant GB 2687 from the National Science Foundation.

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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] and between pH 0 and 6 at 175° by Bender and Connors.[3] The mechanism proposed by Bender and Connors 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 pK_1 and pK_2 of fumaric acid at 175°.

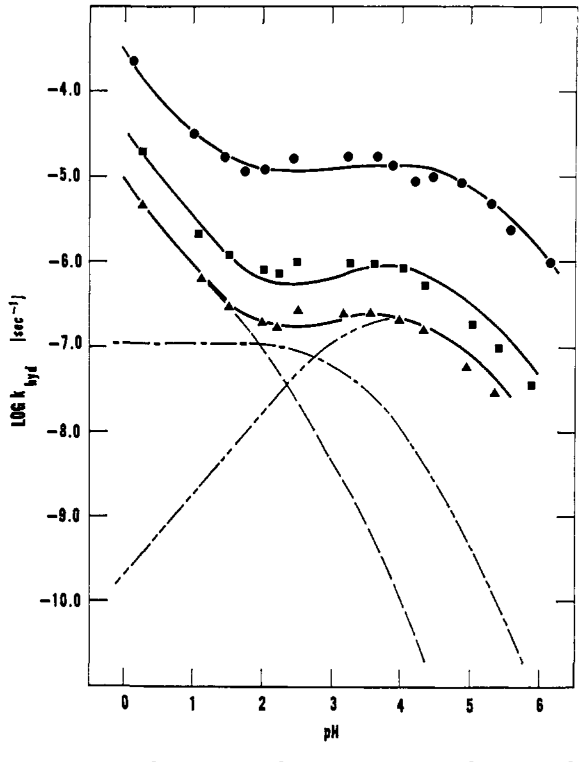

Figure 1. Rate of hydration of fumaric acid as a function of pH and temperature: — —, rate for acid-catalyzed hydration; — - —, rate of H_2O addition to fum^{00}; — - - —, rate of OH^- addition to fum^{00}; ▲, rate at 117.8°; ■, rate at 135.3°; ●, rate at 175°.[3]

In recent investigations of the equilibrium constant for the reversible deamination of aspartic acid to fumaric acid and ammonia,[6] the pK's of fumaric acid were measured between 0 and 95°. Extrapolation of these measurements to 175° gives $pK_1 = 3.53$ and $pK_2 = 4.98$. The extrapolated pK_2 value differs slightly from the calculated pK_2 value of Bender and Connors. There is, however, a large difference in the extrapolated pK_1 value and that calculated from the kinetic data. Using the extrapolated pK 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 pK'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

$$\frac{d(\text{malic acid})}{dt} = k_{hyd}(\text{fum})_T = k_{00}{}^{H^+}(\text{fum}^{00})(H^+) + k_{00}(\text{fum}^{00})(H_2O) + k_{00}{}^{OH^-}(\text{fum}^{00})(OH^-) \quad (1)$$

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

$$k_{hyd} = \frac{k_{0c}{}^{H^+}(H^+)}{\left(1 + \frac{K_{1fum}}{(H^+)} + \frac{K_{1fum}K_{2fum}}{(H^+)^2}\right)} + \frac{k_{00}{}^{H_2O}}{\left(1 + \frac{K_{1fum}}{(H^+)} + \frac{K_{1fum}K_{2fum}}{(H^+)^2}\right)} + \frac{k_{00}{}^{OH^-}K_W}{(H^+)\left(1 + \frac{K_{1fum}}{(H^+)} + \frac{K_{1fum}K_{2fum}}{(H^+)^2}\right)} \quad (2)$$

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[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).

[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]