The Kinetics and Mechanism of the Reversible Nonenzymatic Deamination of Aspartic Acid[1a,b]

Jeffrey L. Bada[1c,d] and Stanley L. Miller

Contribution from the Department of Chemistry, University of California, San Diego, La Jolla, California 92037. *Received September 22, 1969*

Abstract: The kinetics of the reversible deamination of aspartic acid have been investigated between pH -1 and 13 over the temperature range 60–135°. The results indicate that the reaction is independent of pH for pH >10 and also between pH 4.5 and 7. The kinetics of the reverse reaction, the amination of fumaric acid, were calculated from the rates of deamination and the equilibrium constant for the reaction. Between pH 1 and 13, the amination reaction can be interpreted in terms of NH_3 additions to neutral fumaric acid, the fumarate monoanion, and the fumarate dianion. The calculated amination rates of neutral fumaric acid and the fumarate monoanion are consistent with the rates measured for the amination of fumaric diamide and fumaramate. Stereochemical studies of the addition of NH_3 to fumaric acid at pH 8 indicate that the addition is essentially nonstereoselective giving almost equal amounts of the *cis*- and *trans*-addition products. It was also found that the rate of exchange of the methylene protons of aspartic acid with D_2O is faster than the aspartic acid deamination rate. Both the stereochemical and exchange results support a carbanion mechanism. Between pH 1 and 13, the reversible deamination of aspartic acid is classified as an ElcB elimination and AdN2 addition. For pH values less than 1, the amination of fumaric acid takes place by a reaction analogous to the acid-catalyzed hydration of fumaric acid.

The synthesis of aspartic acid by heating the ammonium salts of malic, maleic, and fumaric acids was first demonstrated in 1850.[2] Since that time, the addition of ammonia and amines to fumaric acid has been extensively investigated as a means of synthesizing aspartic acid and aspartic acid derivatives. The reaction, which is reversible, can be written as

$$^{-}OOCCH_2CH(NH_3^+)COO^- \rightleftharpoons {}^{-}OOCCH{=}CHCOO^- + NH_4^+ \quad (1)$$

The reaction is catalyzed by the enzyme aspartase (L-aspartate ammonia-lyase EC4.3.1.1). Aspartase has been isolated from several microorganisms and a few higher plants, but has yet to be found in mammals or other vertebrates.[3] However, there are other enzymatic reactions which convert substituted aspartic acids to fumarate that occur in all organisms; these include the cleavage of argininosuccinic acid to arginine and fumaric acid and the cleavage of adenylosuccinic acid to fumaric and adenylic acids.

The reversible deamination of aspartic acid also takes place nonenzymatically.[1b,4,5] This deamination reaction differs from the decomposition of most amino acids. Most other amino acids undergo a slow irreversible decarboxylation;[6,7] the half-life for alanine decarboxylation at 25° is 10^{11} years. Of the twenty amino acids found in proteins, only aspartic acid and asparagine[8] are known to deaminate nonenzymatically. There are enzymes which will deaminate tyrosine,[9] histidine,[10] and phenylalanine.[11] However, these deaminations go almost to completion.

The equilibrium constant for eq 1 has been measured using the aspartase enzyme and also nonenzymatically at elevated temperatures.[5] The equilibrium constant and the kinetics of the nonenzymatic deamination of aspartic acid have been used to estimate the minimum ammonium ion concentration in the oceans of the primitive earth.[4]

A reaction analogous to the reversible deamination of aspartic acid is the reversible dehydration of malic acid. The kinetics and mechanism of this reaction have been investigated in acid[12] and basic[13] solutions and between pH 0 and 6.[14,15]

This paper reports a detailed investigation of the kinetics of deamination of aspartic acid as a function of pH and temperature. These kinetics and the equilibrium constant for eq 1 are used to calculate the kinetics of addition of ammonia to fumaric acid. The mechanism for the reaction is discussed and compared to the mechanism for the reversible hydration of fumaric acid.

Experimental Section

Materials. The DL-aspartic and L-aspartic acids were obtained from Calbiochem and the ND_4Cl, NaOD, and D_2O from BioRad. The fumaric diamide was prepared by the reaction of NH_3 with diethyl fumarate[16] (Aldrich Chem. Co.), and the fumaramic acid prepared[8] from monoethyl fumarate (Aldrich Chem. Co.). The

---

(1) (a) Taken in part from the Ph.D. Thesis of Jeffrey L. Bada, University of California, San Diego, Calif., 1968; (b) preliminary communication, J. L. Bada and S. L. Miller, *J. Amer. Chem. Soc.*, **91**, 3946 (1969); (c) National Aeronautics and Space Administration Fellow, 1967–1968; (d) Department of Geological Sciences, Hoffman Laboratory, Harvard University, Cambridge, Mass. 02138.

(2) V. Dessaignes, *Compt. Rend.*, **30**, 324 (1850); **31**, 432 (1850); J. Wolff, *Ann.*, **75**, 293 (1850).

(3) A. Meister, "Biochemistry of the Amino Acids," Academic Press, New York, N. Y., 1965, p 608.

(4) J. L. Bada and S. L. Miller, *Science*, **159**, 423 (1968).

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

(6) P. H. Abelson, *Mem. Geol. Soc. Amer.*, **67**, 87 (1957); *Ann. N. Y. Acad. Sci.*, **69**, 276 (1957); *Progr. Chem. Org. Nat. Prod.*, **17**, 379 (1959); in "Researches in Geochemistry," P. H. Abelson, Ed., John Wiley & Sons, Inc., New York, N. Y., 1959, p 79.

(7) J. R. Vallentyne, *Geochim. Cosmochim. Acta*, **28**, 157 (1967); D. Povoledo and J. R. Vallentyne, *ibid.*, **28**, 731 (1967); D. Conway and W. F. Libby, *J. Amer. Chem. Soc.*, **80**, 1077 (1958).

(8) E. A. Talley, T. J. Fitzpatrick, and W. L. Porter, *ibid.*, **81**, 174 (1959).

(9) A. C. Neish, *Phytochemistry*, **1**, 1 (1961).

(10) Reference 3, p 826; V. R. Williams and M. Hiroms, *Fed. Proc.*, **26**, 387 (1967).

(11) Reference 3, p 921; E. A. Havir and K. R. Hanson, *Biochemistry*, **7**, 1904 (1968).

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

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

(14) 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).

(15) J. L. Bada and S. L. Miller, *ibid.*, **91**, 3948 (1969).

(16) D. T. Mowry and J. M. Butler in "Organic Synthesis," Coll. Vol. IV, N. Rabjohn, Ed., John Wiley & Sons, Inc., New York, N. Y., 1963, p 486.

erythro-3-deuterio-L-aspartic acid was generously provided by Dr. A. I. Krasna. All other chemicals were reagent grade.

Solutions for Kinetic Studies. Solutions of aspartic acid were buffered at the various pH values by either hydrochloric acid, succinate, phosphate, borate, carbonate, or NaOH. The final ionic strength of the solutions was adjusted to the desired values by adding sodium chloride. The solutions were deoxygenated and sealed under vacuum in ampoules constructed from either Pyrex or alkali resistant (Corning Glass 7280) glass, the latter being used for the very basic solutions. The ampoules were sterilized immediately after being sealed off.

The rates of addition of ammonia to fumaramic acid and fumaric diamide were determined in an ammonium chloride–ammonia buffer. The NH_3 concentrations were determined by titrating the solutions with HCl to the bromothymol blue end point. The initial concentrations of fumaramic acid and fumaric diamide were about 10^{-3} *M*. The solutions were also sealed in glass ampoules, but were not evacuated.

The ampoules were maintained (±0.2°) at 90, 100, 117, and 135° by refluxing solutions of ethanol–water, water, *n*-butyl alcohol, and water–propionic acid, respectively. The other temperatures were maintained by standard laboratory regulators to ±0.05°.

Rate Determinations and Analytical Procedures. The rates of deamination of aspartic acid were determined from measurements of both the rate of appearance of ammonia and fumaric acid. The ammonia was determined by Conway diffusion[17] and the fumaric acid by its ultraviolet absorption in 1–2 *M* hydrochloric acid solutions.[5] To correct for ultraviolet absorption by the buffer, a solution with the same concentration of buffer and salts was used as a blank. For the basic solutions the blank was heated for the same length of time to correct for substances dissolved off of the glass walls. The correction was never more than 10% of the total optical density of the solution. The rates determined from the ammonia measurements were always within ±5% of the rates calculated from the fumaric acid determinations except for pH values less than 4 where the rate of hydration of fumaric acid becomes significant.[12,14,15] Only the ammonia measurements were used to calculate the rates at pH values less than 4. The product of the 135° deamination reaction at pH 7 was shown to be fumaric acid by chromatography on silica.[18] Less than 0.2% maleic acid was found.

The rates of addition of ammonia to fumaramic acid and fumaric diamide were determined by observing the change in optical density of the solutions. The samples were diluted in HCl and measurements made at 240 and 230 mμ.

Buffer pH Values and p*K*'s of Fumaric Acid, Aspartic Acid, and Ammonia as a Function of Temperature. The pH values of the HCl, phosphate, borate, and carbonate buffers at the various temperatures were taken from Bates.[19] The pH's of the NaOH buffers as a function of temperature were calculated from the temperature variation of the pK_w of water.[20] The pH values of the succinate buffers as a function of temperature were determined from measurements described previously[5] and from the data of Bates and Gary.[21] The p*K* values of fumaric and aspartic acids and ammonia at the various temperatures were calculated from equations given previously.[5]

Stereochemical Studies of the Addition of NH_3 to Fumaric Acid. To determine the stereochemistry of the addition of ammonia to fumaric acid, 1.1 g of sodium fumarate was added to 5.0 ml of a D_2O solution which also contained ND_4^+ and ND_3 in an approximate 1:1 ratio. The pD of the solution was 9.3 at 25° and ND_3 concentration 2 *M*. The solution was heated at 116.3° for 6 hr, after which it was evaporated to dryness over concentrated H_2SO_4 in a vacuum desiccator. The resulting solid was redissolved in 1.0 ml of D_2O and adjusted to pH 6 with 6 *M* hydrochloric acid. The nmr spectra of this solution was obtained on a Varian HR-60 nmr spectrometer equipped with an NMR Specialities heteronuclear spin decoupler. The spectra were calibrated by the side-band technique. The nmr spectra of a D_2O solution containing 0.1 g of the *erythro*-3-deuterio-L-aspartic acid at pH 6 was also determined.

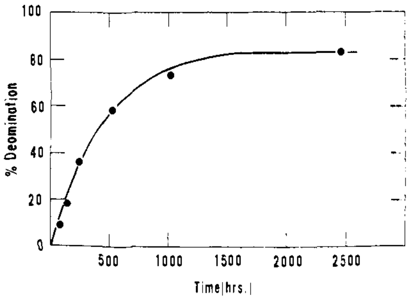


Figure 1. Deamination of aspartic acid as a function of time. The solid curve was calculated from eq 3 and the values determined for x_∞ and K_{DL}^{app}; $pH_{116.3°} = 5.86$, $A_0 = 4.87 \times 10^{-3}$ *M*.

Exchange of Aspartate Methylene Protons in D_2O. The rate of exchange of the methylene protons of aspartic acid with D_2O at 116.3° was investigated in solutions buffered by either phosphate, borate, or NaOD; the exchange rate of the methylene protons of malate at $OD^- = 0.4$ *M* was also measured. After dissolving the aspartic acid in the D_2O buffer, the solution was evaporated to dryness in a vacuum desiccator to remove the hydrogen from the buffer. The residue was redissolved in D_2O and the solutions were sealed under vacuum in Pyrex ampoules.

The nmr spectrum of the solutions were obtained using a Varian T-60 nmr spectrometer. The rate of deamination of aspartic acid in the buffered D_2O solutions was determined as described previously.

The pH values of the phosphate and borate D_2O buffer solutions were measured on a Radiometer 25 SE pH meter. These pH values were adjusted to 116.3° and the pD of the solutions at 116.3° calculated by adding[22] 0.40 to these adjusted pH's.

Results and Discussion

Kinetics of Deamination of Aspartic Acid. On the basis that the deamination of aspartic acid is a first-order reaction, the rate law for eq 1 is

$$-\frac{d(\text{DL-aspartic acid})}{dt} = \frac{d(\text{fumaric acid})}{dt} = \frac{d(\text{ammonia})}{dt} = k_{\text{deam}}(\text{DL-aspartic acid}) - k_{\text{addn}}(\text{fumaric acid})(NH_3) \quad (2)$$

where k_{deam} is the first-order rate constant for the deamination of aspartic acid and k_{addn} the second-order rate constant for the addition of ammonia to fumaric acid. The parentheses refer to the molal concentrations of the respective quantities. Integration of eq 2 yields

$$\ln\left\{\frac{1 - \dfrac{x}{x_\infty}}{1 + \dfrac{x}{x_\infty + K_{DL}^{app}}}\right\} = -k_{\text{deam}}\left(1 + \frac{4A_0}{K_{DL}^{app}}\right)^{1/2} t \quad (3)$$

where x and x_∞ are the fumaric acid or ammonia concentrations at time t and infinite time, respectively, A_0 the initial aspartic acid concentration, and K_{DL}^{app} the apparent equilibrium constant[5] for eq 1. The rate of appearance of fumaric acid and ammonia at several pH values was followed to equilibrium at 116° at an ionic strength of 0.1. The results at pH 5.9 are shown in Figure 1. There was less than 0.2% decarboxylation to α- or β-alanine in the samples heated for 5 half-lives. The solid line in Figure 1 was calculated from eq 3 and the values determined for x_∞ and K_{DL}^{app}.

---

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

(18) W. A. Bulen, J. E. Varner, and R. C. Burrell, *Anal. Chem.*, **24**, 187 (1952).

(19) R. G. Bates, G. D. Pinching, and E. R. Smith, *J. Res. Nat. Bur. Std.*, **45**, 418 (1950); R. G. Bates, "Determination of pH," John Wiley & Sons, Inc., New York, N. Y., 1964, p 67.

(20) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworth & Co., London, 1959, p 544.

(21) R. G. Bates and R. Gary, *J. Res. Nat. Bur. Std.*, **65*A***, 495 (1961).

(22) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

Table I. First-Order Rate Constants (sec^{-1}) for Deamination of Aspartic Acid

60.1°			80.1°			89.8°			100.1°			116.3°			135.3°		
pH 25°	pH 60.1°	$k_{deam} \times 10^{10}$	pH 25°	pH 80.1°	$k_{deam} \times 10^{9}$	pH 25°	pH 89.8°	$k_{deam} \times 10^{8}$	pH 25°	pH 100.1°	$k_{deam} \times 10^{8}$	pH 25°	pH 116.3°	$k_{deam} \times 10^{7}$	pH 25°	pH 135.3°	$k_{deam} \times 10^{6}$
3.53	3.53	1.07	1.02	1.02	0.388	3.52	3.52	1.25	1.02	1.02	0.487	−0.08	−0.08	0.0624	−0.97	−0.97	0.0189
4.61	4.65	1.39	2.01	2.01	1.10	4.45	4.53	1.61	2.01	2.01	2.05	1.04	1.04	0.306	−0.71	−0.71	0.0243
5.49	5.53	1.28	2.71	2.71	2.22	5.40	5.56	1.80	2.71	2.71	3.38	2.01	2.01	1.45	−0.39	−0.39	0.0305
7.74	7.70	1.94	3.53	3.53	2.05	6.87	6.87	1.81	3.53	3.55	4.79	2.78	2.78	2.22	−0.08	−0.08	0.0481
9.16	8.90	4.90	4.64	4.69	3.20	7.78	7.78	1.95	4.64	4.74	6.59	3.54	3.58	3.88	0.30	0.30	0.0897
9.78	9.52	5.04	5.49	5.60	3.18	9.22	8.86	2.64	5.49	5.67	7.05	4.62	4.77	4.67	0.70	0.70	0.206
12.85	11.74	8.36	6.55	6.52	3.63	9.85	9.49	2.85	6.48	6.51	7.09	5.52	5.78	4.80	1.02	1.02	0.303
13.58	12.47	9.93	7.83	7.81	4.04				7.74	7.77	7.67	6.56	6.65	5.14	2.01	2.01	1.67
			9.27	8.94	7.82				9.27	8.89	8.67	7.75	7.84	4.93	2.71	2.71	2.39
			11.46	10.51	10.07				9.87	9.50	9.51	9.16	8.77	4.28	3.53	3.61	3.49
									11.49	10.31	8.93	9.78	9.39	4.09	4.64	4.86	4.24
									13.09	11.22	8.77	11.26	9.95	3.82	5.49	5.85	3.90
												12.85	10.74	3.81	6.55	6.72	4.08
												13.58	11.47	3.64	7.83	8.00	3.79
															9.27	8.88	2.75
															9.88	9.49	2.81
															11.49	10.07	2.55
															13.82	11.51	2.77

When the per cent deamination is small, eq 2 simplifies to

$$-\frac{d(\text{aspartic acid})}{dt} = \frac{d(\text{fumaric acid})}{dt} = \frac{d(\text{ammonia})}{dt} = k_{deam}(\text{aspartic acid}) \quad (4)$$

Values of k_{deam} ($I = 0.1$) calculated from this equation at several pH values and temperatures are shown in Table I. A plot of log k_{deam} *vs.* pH has been given previously.[1b] The data are the average values of rate constants determined from samples heated for 2 or more different lengths of time, except at the low temperatures where the rates were so slow that in most cases only one measurement was made. The experimental uncertainty of the measurements is about ±5%. The results indicate that the reaction rate is independent of pH in base (pH greater than 10) and between pH 4.5 and 7. An Arrhenius plot of the rate constants at pH >10 and at pH 7 are shown in Figure 2. These data were fitted by the method of least squares to give

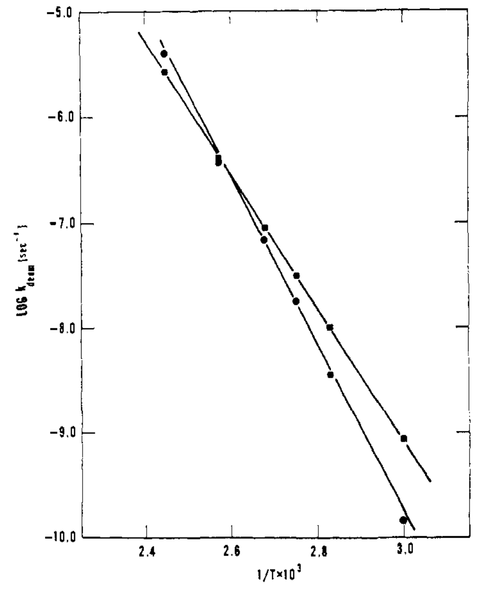


Figure 2. Values of k_{deam} *vs.* $1/T$ (°K): ●, k_{deam} values at pH >10; ●, k_{deam} at pH 7.

$$\log k_{deam}(\text{sec}^{-1})\ (\text{pH } 7.0) = 14.35 - 8047.5/T \quad (5)$$

$$\log k_{deam}(\text{sec}^{-1})\ (\text{pH} > 10) = 9.69 - 6248.5/T \quad (6)$$

The values of E_a, the Arrhenius activation energy, calculated from these equations are 36.8 and 28.6 kcal mol^{-1}, respectively.

The rate of deamination of aspartic acid was investigated as a function of ionic strength at pH 1 and 6.5. The results indicated that log k_{deam} varies linearly with ionic strength at both pH values. The ratio of $k_{deam}(\mu = 0.1)/k_{deam}(\mu = 1.0)$ at pH 1 and 6.5 are 1.25 and 1.24, respectively. The reaction therefore has only a slight dependence on ionic strength at both pH values.

The rate of deamination was also studied at several different concentrations of succinate and phosphate buffer, to determine whether the reaction was general acid or general base catalyzed. No general acid or general base catalysis was found at either pH 3 or 6. The rate of deamination in 0.84 *M* succinate buffer (pH 3.1) was the same within experimental error as the rate in a 0.043 *M* buffer of the same pH and ionic strength. Similar results were obtained with phosphate buffers at pH 6.

Kinetics of Addition of Ammonia to Fumaramic Acid and Fumaric Diamide. The rates of addition of ammonia to fumaramic acid and fumaric diamide were determined with the ammonia in an approximate 100-fold excess. The reaction should therefore obey a pseudo-first-order rate expression. A plot of log {(fumaric diamide)/(fumaric diamide)$_0$} *vs.* time for the addition of NH_3 to fumaric diamide at 80° is shown in Figure 3. The plot indicates that the reaction does follow pseudo-first-order kinetics. The rate of addition was also shown to be first order in NH_3.

The respective rate constants determined for the addition of ammonia to fumaramic acid and fumaric diamide at the various temperatures are shown in Table II. At the pH of these measurements, the ionic

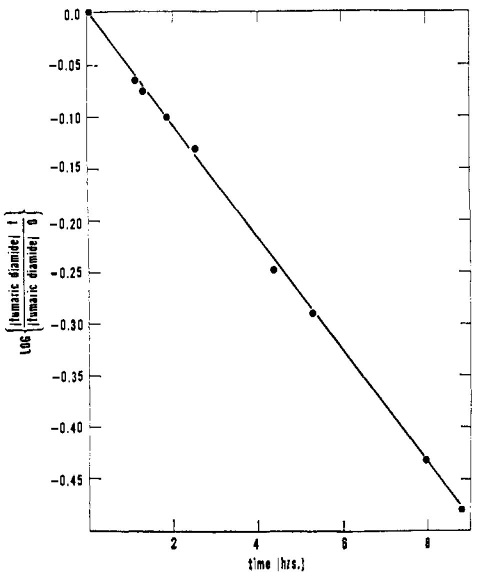


Figure 3. Rate of addition of ammonia to fumaric diamide at 80°. Ammonia concentration was 0.512 *M* and initial fumaric diamide concentration 10^{-3} *M*.

form of fumaramic acid[23] is fumar⁻. Fumaric diamide is neutral since it has no protons which ionize at pH's less than 14. The rate constants in Table II therefore refer to the addition of ammonia to these forms. At the pH's and temperatures of these measurements, the rates of hydrolysis[24] of fumaramic acid and fumaric diamide are about 50 times slower than the rates of amination.

Table II. Second-Order Rate Constants for the Addition of Ammonia to Fumaramic Acid and Fumaric Diamide

Temp, °C	Ammonia addn to fumaramic acid pH 9.7 at 25° k_{fumar^-} (sec^{-1} M^{-1})	Ammonia addn to fumaric diamide pH 9.7 at 25° k_{diamide} (sec^{-1} M^{-1})
26.8	2.53×10^{-7}	5.33×10^{-7}
40.1	1.07×10^{-6}	2.10×10^{-6}
60.0	7.50×10^{-6}	1.50×10^{-5}
79.9	3.82×10^{-5}	7.15×10^{-5}
100.4	1.56×10^{-4}	

Kinetics of Exchange of the Methylene Protons of Aspartic Acid. The exchange reaction for the aspartate methylene protons in D_2O can be written as

$$\text{aspartate} \xrightarrow{2k_{\text{exch}}} \text{3-deuterioaspartate}$$

$$\text{3-deuterioaspartate} \xrightarrow{k_{\text{exch}}} \text{3,3-dideuterioaspartate}$$

The total area under the methylene proton peaks in the nmr spectra of the aspartate solutions is a measure of the concentration of aspartate and 3-deuterioaspartate.

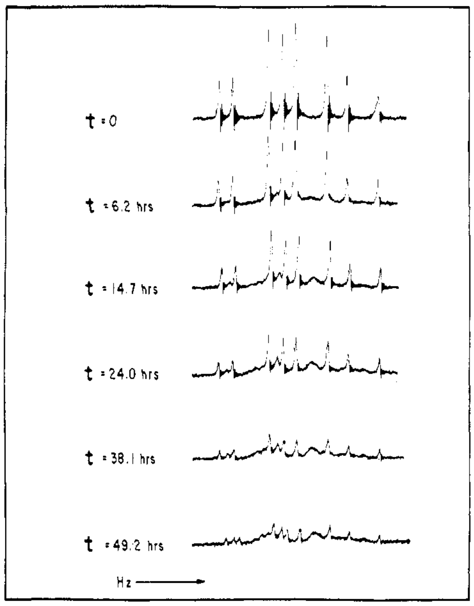


Figure 4. Exchange of methylene protons of DL-aspartate with D_2O at $pD_{116.3°}$ 10.8.

The rate expression for the exchange reaction is

$$\frac{A}{A_{t=0}} = e^{-(k_{\text{exch}} + k_{\text{deam}})t} \tag{7}$$

where A and $A_{t=0}$ are the total areas under the methylene proton peaks at time t and $t = 0$, respectively. The values of k_{exch} were determined at the various pH values from the nmr spectra of the solutions and eq 7. The nmr spectra of solutions heated for various lengths of time at $pD_{116°}$ 10.8 are shown in Figure 4. The values of k_{exch} determined at the various pD values are given in Table III; also included in Table III are the deamination rates of aspartic acid measured at each pD and the dehydration rate and methylene proton exchange rate of the malate dianion (mal²⁻) at pD 12.5.

Table III. First-Order Rate Constants (sec^{-1}) for the Exchange of the Methylene Protons of Aspartic Acid and Deamination of Aspartic Acid in D_2O at 116.3°

$pH_{25°}$ or OD^- concentration	$\sim pD_{116.3°}$	k_{exch} $\times 10^6$	k_{deam} $\times 10^6$
5.78	6.27	5.70	0.263
7.05	7.54	3.92	0.447
8.36	8.38	3.74	0.503
9.51	9.53	3.50	0.709
12.37	10.77	4.25	0.973
0.14 *M* OD^-	11.87	4.53	1.20
0.60 *M* OD^-	12.64	17.0	1.06
0.40 *M* OD^-	12.47	93.3[a]	5.91[a]

[a] Exchange and dehydration rate constants determined for malic acid.

Kinetics of Addition of Ammonia to the Various Ionic Forms of Fumaric Acid. 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 the addition to ammonia to fumaric acid k_{addn}, can be calculated from the values k_{deam} and $K_{\text{DL}}^{\text{app}}$ These calculations indicate that the rate of the addition

---

[23] The following abbreviations will be used: asp^{0+0}, $HOOCH_2CH(NH_3^+)COOH$; asp^{0+-}, $HOOCH_2CH(NH_3^+)COO^-$; asp^{-+-}, $^-OOCCH_2CH(NH_3^+)COO^-$; asp^{-0-}, $^-OOCCH_2CH(NH_2)COO^-$; asparagine^{0-}, $NH_2OCCH_2CH(NH_2)COO^-$; fum^{00}, $HOOCCH{=}CHCOOH$; fum^{0-}, $HOOCCH{=}CHCOO^-$; fum^{2-}, $^-OOCCH{=}CHCOO^-$; $fumar^-$, $NH_2OCCH{=}CHCOO^-$; mal^{2-}, $^-OOCCH_2CH(OH)COO^-$. $(Fum)_T$ and $(NH_3)_T$ are the total concentrations of the various ionic forms of fumaric acid and ammonia, respectively.

[24] Vigneron-Voortman, P. Croay, and A. Bruylants, *Bull. Soc. Chim. Belg.*, 73, 753 (1964).

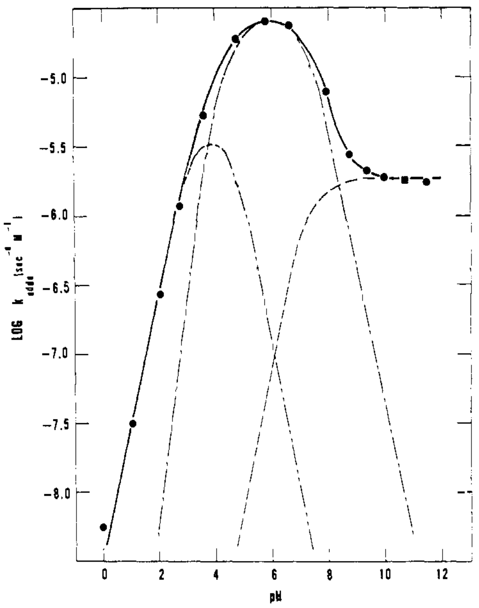


Figure 5. Rate of addition of ammonia to fumaric acid as a function of pH at 116.3°: – – – –, rate of addition of NH_3 to fum^{00}; —·—, rate of addition of NH_3 to fum^{0-}; — —, rate of addition of NH_3 to fum^{2-}; ●, values of k_{addn} calculated from k_{deam} and the equilibrium constant.

reaction is independent of pH for pH values greater than 10, has a maximum near pH 6, and decreases rapidly with pH for pH values less than 5. The following equation can be written to describe the observed rate of the addition of NH_3 to fumaric acid between pH 1 and 13.[25]

$$\frac{d(\text{aspartic acid})}{dt} = k_{addn}(\text{Fum})_T(NH_3)_T = k_{00}{}^{NH_3}(\text{fum}^{00})(NH_3) + k_{0-}{}^{NH_3}(\text{fum}^{0-})(NH_3) + k_{2-}{}^{NH_3}(\text{fum}^{2-})(NH_3) \quad (8)$$

Using the pK's of fumaric acid and the pK_a of ammonia, eq 8 can be rewritten

$$k_{addn} = \frac{k_{00}{}^{NH_3}}{\left[1 + \frac{H^+}{K_a}\right]\left[1 + \frac{K_{1fum}}{H^+} + \frac{K_{1fum}K_{2fum}}{(H^+)^2}\right]} + \frac{k_{0-}{}^{NH_3}}{\left[1 + \frac{H^+}{K_a}\right]\left[1 + \frac{H^+}{K_{1fum}} + \frac{K_{2fum}}{H^+}\right]} + \frac{k_{2-}{}^{NH_3}}{\left[1 + \frac{H^+}{K_a}\right]\left[1 + \frac{H^+}{K_{2fum}} + \frac{(H^+)^2}{K_{1fum}K_{2fum}}\right]} \quad (9)$$

where $k_{00}{}^{NH_3}$, $k_{0-}{}^{NH_3}$, and $k_{2-}{}^{NH_3}$ are the second-order rate constants for the addition of ammonia to neutral fumaric acid (fum^{00}), the fumarate monoanion (fum^{0-}), and the fumarate dianion (fum^{2-}), respectively. The values of k_{addn}, calculated from k_{deam} and $K_{DL}{}^{app}$, were fitted by the method of least squares to obtain equations in the form of eq 9. The values of $k_{00}{}^{NH_3}$, $k_{0-}{}^{NH_3}$, and $k_{2-}{}^{NH_3}$ determined from these calculations are shown in Table IV. The uncertainty in the values is about ±10%. The relative ratio of $k_{2-}{}^{NH_3}$:$k_{0-}{}^{NH_3}$:$k_{00}{}^{NH_3}$ at 116.3° is 1:7700:30,000. The values of $k_{00}{}^{NH_3}$ determined at 60 and 89.8° are only approximate because of the limited data in the low pH range at these temperatures. Figure 5 shows the values of k_{addn} at 116.3° along with the curves calculated for the addition of ammonia to fum^{00}, fum^{0-}, and fum^{2-}. Also shown in Figure 5 is the curve calculated from eq 9 and the values of $k_{00}{}^{NH_3}$, $k_{0-}{}^{NH_3}$, and $k_{2-}{}^{NH_3}$.

Table IV. Values of $k_{00}{}^{NH_3}$, $k_{0-}{}^{NH_3}$, and $k_{2-}{}^{NH_3}$ Calculated from Equation 9

Temp, °C	$k_{00}{}^{NH_3}$ (sec^{-1} M^{-1})	$k_{0-}{}^{NH_3}$ (sec^{-1} M^{-1})	$k_{2-}{}^{NH_3}$ (sec^{-1} M^{-1})
60.1	$\sim 1.2 \times 10^{-3}$	3.18×10^{-4}	3.24×10^{-8}
80.1	3.88×10^{-3}	1.38×10^{-3}	1.72×10^{-7}
89.8	$\sim 1.6 \times 10^{-2}$	3.40×10^{-3}	3.53×10^{-7}
100.1	2.02×10^{-2}	6.35×10^{-3}	7.46×10^{-7}
116.3	5.68×10^{-2}	1.45×10^{-2}	1.88×10^{-6}
135.3	2.02×10^{-1}	3.52×10^{-2}	7.40×10^{-6}

The values of $k_{00}{}^{NH_3}$ and $k_{0-}{}^{NH_3}$ may be compared to the second-order rate constants for the addition of ammonia to fumaric diamide and fumaramic acid. The values of E_a (kcal mol^{-1}) calculated from Arrhenius plots of $k_{00}{}^{NH_3}$, $k_{0-}{}^{NH_3}$, $k_{2-}{}^{NH_3}$, $k_{diamide}$, and k_{fumar^-} are 18.9, 17.1, 19.2, 19.6, and 19.5, respectively. Table V gives the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ calculated from the various rate constants at 100°. The heat of activation, $\Delta H^{\ddagger}$, was calculated using the relation $\Delta H^{\ddagger} = E_a - RT$, and the $\Delta S^{\ddagger}$ value calculated from

Table V. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for $k_{00}{}^{NH_3}$, $k_{0-}{}^{NH_3}$, $k_{2-}{}^{NH_3}$, $k_{diamide}$, and k_{fumar^-} at 100°

Rate constant	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu
$k_{00}{}^{NH_3}$	18	−19
$k_{0-}{}^{NH_3}$	16	−26
$k_{2-}{}^{NH_3}$	18	−38
$k_{diamide}$	19	−25
k_{fumar^-}	19	−27

$$\Delta S^{\ddagger} = 4.576\left(\log k - 10.73 - \log T + \frac{E_a}{4.596T}\right)$$

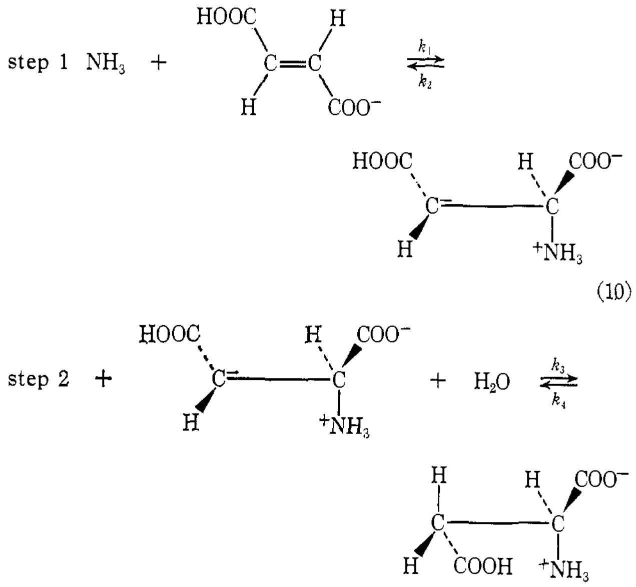
where k is the respective rate constant. It would be expected from the $k_{00}{}^{NH_3}$ and $k_{0-}{}^{NH_3}$ values for fumaric acid that $k_{diamide} > k_{fumar^-}$ and this is what is observed. The $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values are also similar for $k_{00}{}^{NH_3}$ and $k_{diamide}$, and for $k_{0-}{}^{NH_3}$ and k_{fumar^-}. However, the ratio k_{fumar^-}/k_{0-} is 0.028 at 80°, extrapolating to 0.027 at 30°. The difference is due to the substitution of carboxamide group for the carboxyl group. In a study of the rates of addition of glycine to acrylamide

---

(25) The equation $k_{addn}(\text{Fum})_T(NH_3)_T = k_{00}{}^{NH_3}(\text{fum}^{00})(NH_3) + k_{00}{}^{NH_2^-}(\text{fum}^{00})(NH_2^-) + k_{0-}{}^{NH_2^-}(\text{fum}^{0-})(NH_2^-)$ is kinetically indistinguishable from eq 8, both giving rate$_{deam}$ = k'(asp^{-0-}) for the deamination rate of aspartic acid at high pH. However, the nucleophilic addition of amide ion is not consistent with the data for the deamination of asparagine. At high pH values, if NH_3 is the leaving group, rate$_{deam(asparagine)}$ = k''(asparagine^{0-}) while for NH_2^- as a leaving group rate$_{deam(asparagine)}$ = k'''(asparagine^{0-})(OH$^-$). The deamination rate of asparagine was investigated at several pH values at 60°. The rate constants (sec^{-1}) at pH 7.94, 8.67, and 10.24 are 3.89×10^{-8}, 8.11×10^{-8}, and 9.20×10^{-8}, respectively. The data show that the asparagine deamination reaction is independent of pH for pH values greater than ~9. The results indicate that NH_3 is the leaving group in basic solution and not NH_2^-. This is the expected result since NH_2^- should be a very poor leaving group. This suggests that NH_3 is also the nucleophile in the amination of fumaric acid.

and methyl acrylate, Friedman, *et al.*,[26] found $k_{\text{amide}}/k_{\text{ester}} = 0.033$ at 30° and pH 8.1. Assuming that the rate of glycine addition to the ester is nearly equal to the rate of addition to the acid, these results are consistent with the $k_{\text{fumar}^-}/k_{\text{O}^-}$ ratio.

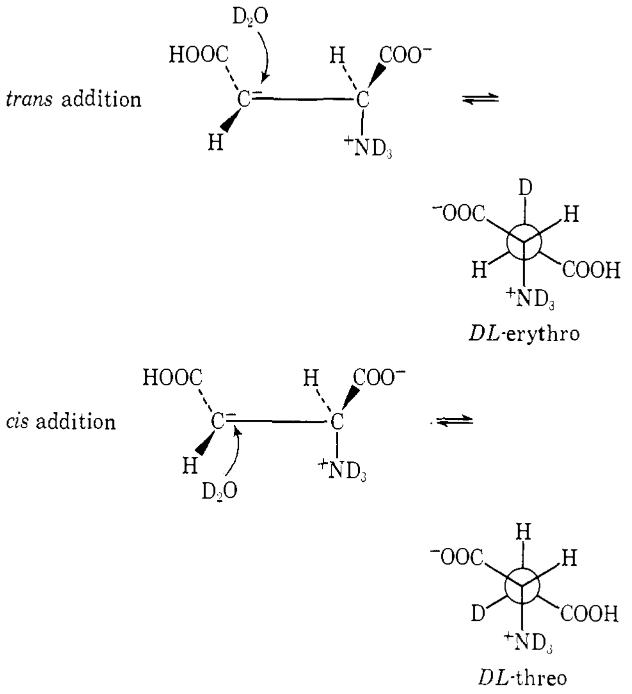
Mechanism for the Reversible Amination of Fumaric Acid. Between pH 1 and 13 the amination of fumaric acid is composed of NH_3 additions to fum^{00}, fum^{0-}, and fum^{2-}. For the addition of OH^- to fum^{2-}, Erickson and Alberty[13] suggested that the reaction proceeded through a carbanion intermediate. Similar carbanion mechanisms can be written for the amination of fumaric acid. For NH_3 addition to fum^{0-} the mechanism is



(10)

Similar carbanion pathways can be written for NH_3 additions to fum^{00} and fum^{2-}.

These carbanion mechanisms are supported by studies of the stereochemistry of the aspartic acid produced from the addition of ND_3 to fumarate in D_2O. Proton addition, step 2 in eq 10, can give two stereochemical products, depending on whether the addition is *cis* or *trans* to the ammonia and assuming there is no free rotation of the carbanion. The reactions may be written as



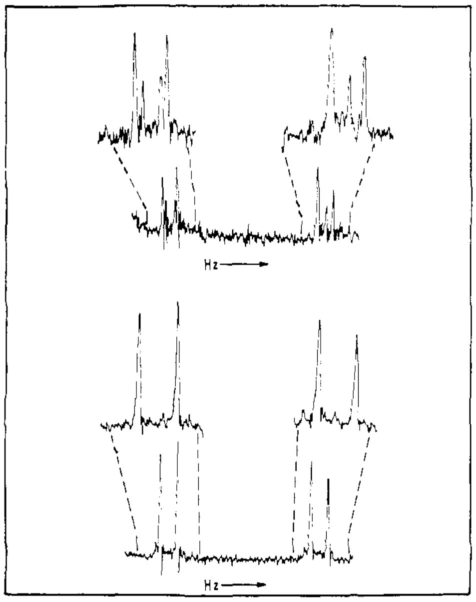


Figure 6. Top, nmr spectra of aspartic acid synthesized by heating a solution of sodium fumarate and ND_3 at $\text{pD}_{116.3°}$ 8; bottom, nmr spectra of enzymatically prepared *erythro*-3-deuterio-L-aspartic acid.

The nmr spectra of the aspartic acid synthesized at 116.3° by heating ND_3 and fumarate in D_2O at pD 8 is shown in Figure 6. Also shown is the nmr spectra of *erythro*-3-deuterio-L-aspartic acid, which was prepared enzymatically. The enzymatically synthesized aspartic acid has been shown to have the *erythro* configuration due to a *trans* addition of ammonia to fumaric acid.[27, 28]

The spectra in Figure 6 indicate that the nonenzymatically prepared aspartic acid has four peaks in the low-field portion of the nmr spectra (methine proton) while the enzymatically prepared aspartic acid has two. The coupling constants calculated from these peaks are 7.4 ± 0.3 cps and 4.2 ± 0.2 cps for the nonenzymatically synthesized aspartic acid, and 8.3 ± 0.2 cps for the enzymatically synthesized aspartic acid. The coupling constants for the *erythro* and *threo* isomers of 3-deuteriomalic acid are 7.1 and 4.3 cps, respectively.[28] These data suggest that the aspartic acid prepared in the nonenzymatic reaction is a mixture of the *erythro* and *threo* isomers of 3-deuterio-DL-aspartic acid. Based on the coupling constants determined for the enzymatically prepared aspartic acid, and those for the *erythro* and *threo* isomers of 3-deuteriomalic acid, the two outer peaks in the low-field portion of the spectra of the nonenzymatically synthesized aspartic acid can be assigned to the *erythro* isomer, and the inner peaks to the *threo* isomer. The areas under the *erythro* and *threo* peaks show that the addition is approximately 60% *trans* and 40% *cis*.

---

[26] M. Friedman, J. F. Cakins, and J. S. Wall, *J. Amer. Chem. Soc.*, **87**, 3672 (1965).

[27] A. I. Krasna, *J. Biol. Chem.*, **233**, 1010 (1958); S. Englard, *ibid.*, **233**, 1003 (1958).

[28] O. Gawron and T. P. Fondy, *J. Amer. 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 possibility can be ruled out that the nonenzymatic addition is stereoselective and that the product rapidly isomerized during the course of the reaction. The *erythro* and *threo* isomers can be interconverted by carbanion formation at either the α or β carbons. Carbanion formation at the β carbon would lead to the 3-dideuterioaspartic acid which has only a single peak in the methine region. This would not affect the areas under the *erythro* and *threo* peaks.

The rate of carbanion formation at the α carbon is equal to the rate of racemization of aspartic acid. At pH 8 and 116.3°, the first-order constant for racemization[29] is 1.2×10^{-6} sec^{-1}. The maximum amount of *erythro–threo* interconversion by α-carbon carbanion formation would therefore be $\sim 3\%$ since the aspartic acid was synthesized by heating at 116.3° the ND_3–fumarate solution for 6 hr.

These results indicate that whereas the aspartase-catalyzed reaction is stereoselective giving complete *trans* addition, the nonenzymatic reaction is essentially nonstereoselective, giving about equal amounts of both the *cis* and *trans* addition products. The nonstereoselectivity of the nonenzymatic reaction is consistent with a planar carbanion intermediate which has a finite lifetime. Since the probability of proton addition to either the top or bottom side of a planar carbanion would be about the same, equal amounts of *cis* and *trans* addition products would be produced. A concerted addition would probably give more stereoselectivity. If the enzymatic reaction also involves a carbanion intermediate, the stereoselectivity may result from the carbanion, which would be bound to the enzyme, being shielded on one side by the enzyme. Alternative explanations are that the enzymatic reaction is concerted or that the carbanion is not planar.

The carbanion mechanisms are supported by the fact that at each pH studied, the rate of exchange of the aspartate methylene protons is greater than the rate of deamination (Table III). This indicates that the rate of protonation of the carbanion intermediate to give aspartate is greater than the rate at which ammonia leaves the carbanion to give fumarate. Further support is given to the carbanion mechanism from measurements of the kinetic isotope effect for the deamination of the enzymatically prepared 3-deuterio-L-aspartic acid at 100°. Heating solutions of aspartic acid and 3-deuterioaspartic acid at pH 7.7 for 257 hr gave $k_{\mathrm{deam(asp)}}/k_{\mathrm{deam(3\text{-}deuterio\text{-}asp)}} = 1.68$ while heating for 659 hr gave $k_{\mathrm{deam(asp)}}/k_{\mathrm{deam(3\text{-}deuterio\text{-}asp)}} = 1.34$. This small kinetic isotope effect and the decrease in $k_{\mathrm{deam(asp)}}/k_{\mathrm{deam(3\text{-}deuterio\text{-}asp)}}$ with time indicates that the 3-deuterio aspartic acid has exchanged most of its deuterium for hydrogen before the amount of deamination has become appreciable. These results suggest that the formation of the carbanion is rapid compared to the elimination of ammonia, which is the rate limiting step.

These stereochemical, methylene proton exchange, and kinetic isotope effect results are consistent with the AdN2 mechanism[1b] shown in eq 10 for addition of NH_3 to fum^{0-}. The mechanism for the deamination of aspartic acid may therefore be classified as ElcB. Aspartic acid satisfies the classical structural requirements[30] for the ElcB mechanism since it has substituents which can stabilize the intermediate carbanion and ammonia is a poor leaving group. The fact that the deamination of aspartic acid is not catalyzed by general base is also consistent with the ElcB mechanism.[31]

The rate of protonation of the carbanion intermediate relative to the rate of leaving of ammonia was evaluated for asp^{0+-} and asp^{-+-} from the data in Table III. In the preceding section it was shown that eq 8 describes the rate of amination of fumaric acid between pH 1 and 13. The corresponding rate equation for the deamination of aspartic acid between pH 6 and 13 is

$$\mathrm{rate_{deam}} = k_{\mathrm{deam}}{}^{0+-}(\mathrm{asp}^{0+-})(\mathrm{OH}^-) + k_{\mathrm{deam}}{}^{-+-}(\mathrm{asp}^{-+-})(\mathrm{OH}^-) \quad (11)$$

where the k's are the rate constants for the deamination of the indicated ionic forms of aspartic acid. Equation 11 can be rewritten as

$$k_{\mathrm{deam}} = \frac{k_{\mathrm{deam}}{}^{0+-}K_{\mathrm{w}}}{[\mathrm{H}^+]\left[1 + \frac{\mathrm{H}^+}{K_1} + \frac{K_2}{\mathrm{H}^+} + \frac{K_2K_3}{(\mathrm{H}^+)^2}\right]} + \frac{k_{\mathrm{deam}}{}^{-+-}K_{\mathrm{w}}}{[\mathrm{H}^+]\left[1 + \frac{(\mathrm{H}^+)^2}{K_1K_2} + \frac{\mathrm{H}^+}{K_2} + \frac{K_3}{\mathrm{H}^+}\right]} \quad (12)$$

where the K's are the various ionization constants of aspartic acid. Curve fitting[32] this equation to the k_{deam} values in Table III gives $k_{\mathrm{deam}}{}^{0+-} = 8.2 \times 10^1$ sec^{-1} M^{-1} and $k_{\mathrm{deam}}{}^{-+-} = 1.1 \times 10^{-2}$ sec^{-1} M^{-1}.

The rate of exchange results in Table III can be interpreted by the rate expression

$$\mathrm{rate_{exch}} = k_4{}^{0+-}(\mathrm{asp}^{0+-})(\mathrm{OH}^-) + k_4{}^{-+-}(\mathrm{asp}^{-+-})(\mathrm{OH}^-) + k_4{}^{-0-}(\mathrm{asp}^{-0-})(\mathrm{OH}^-) \quad (13)$$

The k's are the rate constants for the various reactions. Equation 13 can be rewritten as

$$k_{\mathrm{exch}} = \frac{k_4{}^{0+-}K_{\mathrm{w}}}{[\mathrm{H}^+]\left[1 + \frac{\mathrm{H}^+}{K_1} + \frac{K_2}{\mathrm{H}^+} + \frac{K_2K_3}{(\mathrm{H}^+)^2}\right]} + \frac{k_4{}^{-+-}K_{\mathrm{w}}}{[\mathrm{H}^+]\left[1 + \frac{(\mathrm{H}^+)^2}{K_1K_2} + \frac{\mathrm{H}^+}{K_2} + \frac{K_3}{\mathrm{H}^+}\right]} + \frac{k_4{}^{-0-}K_{\mathrm{w}}}{[\mathrm{H}^+]\left[1 + \frac{\mathrm{H}^+}{K_3} + \frac{(\mathrm{H}^+)^2}{K_2K_3} + \frac{(\mathrm{H}^+)^3}{K_1K_2K_3}\right]} \quad (14)$$

The values $k_4{}^{0+-} = 1.2 \times 10^3$ sec^{-1} M^{-1}, $k_4{}^{-+-} = 3.6 \times 10^{-2}$ sec^{-1} M^{-1}, $k_4{}^{-0-} = 2.6 \times 10^{-5}$ sec^{-1} M^{-1} were obtained by curve fitting the above equation to the observed exchange rates. Figure 7 shows the experi-

---

(29) The racemization rate was determined by measuring the decrease in optical rotation of the buffered solutions. The solutions were diluted in 6 M HCl and the measurements made at 375 mμ on a Durrum-Jasco UV-5 spectropolarimeter.

(30) J. Hine, R. Weisboeck, and O. B. Ramsay, *J. Amer. Chem. Soc.*, **83**, 1222 (1961).

(31) D. L. McLennan, *Quart. Rev.* (London), **21**, 490 (1967).

(32) The pK_3 of aspartic acid in D_2O was estimated from values of pK_a of ammonia[33] in H_2O and D_2O and pK_1 and pK_2 of aspartic acid in D_2O were estimated from the ratios[34] $K_{1(\mathrm{H_2O})}/K_{1(\mathrm{D_2O})}$ for glycine and glutamic acid, respectively. The estimated values at 116.3° are $\mathrm{p}K_{1(\mathrm{D_2O})} = 2.59$, $\mathrm{p}K_{2(\mathrm{D_2O})} = 4.49$, and $\mathrm{p}K_{3(\mathrm{D_2O})} = 8.86$. A value of 12.86 was used[35] for $\mathrm{p}K_{\mathrm{D_2O}}$ at 116.3°.

(33) G. Schwarzenback, A. Epprecht, and H. Erlenmeyer, *Helv. Chim. Acta*, **19**, 1292 (1936).

(34) H. H. Hyman, A. Kaganove, and J. J. Katz, *J. Phys. Chem.*, **64**, 1653 (1960).

(35) R. W. Kingerley and V. K. LaMer, *J. Amer. Chem. Soc.*, **63**, 3256 (1941).

mentally measured values of k_{deam} and k_{exch} and the curves calculated from eq 12 and 14; also shown are the rates of the exchange and deamination reactions of the various ionic forms of aspartic acid as a function of pH.

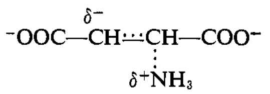
Assuming the carbanion intermediate to be in a steady state, the relationship between the four rate constants shown in eq 10 and $k_{\mathrm{deam}}{}^{0+-}$ is

$$k_{\mathrm{deam}}{}^{0+-} = \frac{k_2 k_4{}^{0+-}}{k_2 + k_3} = \frac{k_4{}^{0+-}}{1 + (k_3/k_2)_{\mathrm{asp}}{}^{0+-}} \qquad (15)$$

The carbanion mechanism for NH_3 addition to fum^{2-} gives a similar relationship for $k_{\mathrm{deam}}{}^{-+-}$. The calculated ratios at 116° are $(k_3/k_2)_{\mathrm{asp}^{0+-}} = 14$ and $(k_3/k_2)_{\mathrm{asp}^{-+-}} = 2.5$.

For an ElcB mechanism[31] $k_3 > k_2$ and this is the case for the deamination of asp^{0+-}. For the deamination of asp^{-+-}, however, k_3 is only 2.5 greater than k_2. This indicates that for the deamination of asp^{-+-} the mechanism probably has less ElcB character than for the deamination of asp^{0+-}.

Since the mechanism for the reversible dehydration of malic acid in basic solutions would also be classified as ElcB, the ratio $(k_3/k_2)_{\mathrm{asp}^{-+-}}$ may be compared to the similar ratio for the dehydration of mal^{2-}; $(k_3/k_2)_{\mathrm{mal}^{2-}}$ calculated from the data in Table III is 15 at 116.3°. The ratio $(k_3/k_2)_{\mathrm{asp}^{-+-}}/(k_3/k_2)_{\mathrm{mal}^{2-}}$ is a measure of the relative leaving rate of NH_3 compared to OH^- provided the rate protonation of the asp^{-+-} and mal^{2-} carbanions are about equal. On this basis the leaving rate of NH_3 is about 6 times greater than the leaving rate of OH^-. It might be expected that rate of NH_3 elimination would be several orders of magnitude greater than that for OH^- elimination. The Brønsted relationship, using α of 0.5 and the ionization of ammonium ion and water as model reactions, gives an estimate of about 10^3 for the leaving rate of ammonia relative to hydroxide ion. However, this estimate is too large since it does not take into account differences in solvation of the $-NH_3^+$ and $-OH$ groups in the intermediate carbanions. The $-NH_3^+$ group is probably solvated by water molecules to a much greater extent than the neutral $-OH$ group. The transition state for the elimination of NH_3 from the asp^{-+-} carbanion would be



$$\overset{\delta-}{{}^{-}\mathrm{OOC{-}CH}}\cdots\underset{\delta+\mathrm{NH_3}}{\mathrm{CH}}\mathrm{{-}COO^{-}}$$

There would be loosening of the bound water molecules around the $-NH_3^+$ group in going from the carbanion to the transition state since the positive charge of the $-NH_3^+$ group has been partly neutralized. This desolvation would increase the free energy of activation for the elimination of NH_3 from the asp^{-+-} carbanion. Conversely, due to the generation of negative charge on the $-OH$ group in the transition state, the free energy of activation for the elimination of OH^- from the mal^{2-} carbanion would be reduced. These differences in solvation would decrease the leaving tendency of ammonia and increase the leaving tendency of hydroxide ion relative to that expected without solvation effects. Studies of the deamination of asp^{-+-} and the dehydration of mal^{2-} in nonaqueous solvents would probably give a value for the leaving rate of NH_3 relative to OH^- closer to the 10^3 value estimated from the Brønsted relationship.

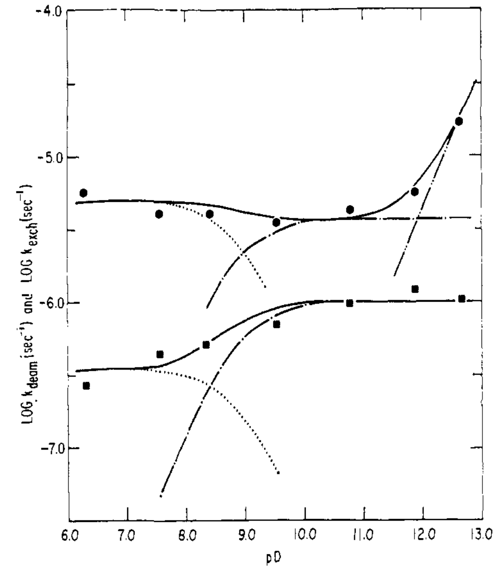


Figure 7. Rate of exchange of the methylene protons of aspartic acid (●) and rate of deamination of aspartic acid (■) as a function of pD at 116.3°: · · · · ·, rate of exchange and deamination of asp^{0+-}; —·—, rate of exchange and deamination of asp^{-+-}; —· · —, rate of exchange of asp^{-0-}.

Figure 7 shows that for pD values greater than 12, the exchange rate of the methylene protons of aspartate becomes approximately first order in OD^-. This is due to the exchange of the methylene protons of asp^{-0-}. However, the carbanion of asp^{-0-} does not deaminate since NH_2^- is a very poor leaving group.

The Addition of Ammonia to Fumaric Acid for pH Values Less Than 1. The values of k_{addn} calculated from eq 9 and $k_{00}{}^{\mathrm{NH_3}}$, $k_{0-}{}^{\mathrm{NH_3}}$, and $k_{2-}{}^{\mathrm{NH_3}}$ are in close agreement with the observed k_{addn} except for pH values less than 1 at 116.3 and 135.3°. At each of these temperatures, k_{addn}(obsd) is always greater than k_{addn}(calcd) at pH <1. This difference was calculated at 135° and the results are shown in Table VI. The pH 0.70 calculation was omitted because the difference between $k_{\mathrm{addn(obsd)}}$ and $k_{\mathrm{addn(calcd)}}$ was less than the uncertainties in the rate constants. The results in Table VI indicate that the difference is independent of pH. It has been shown by Rozelle and Alberty[12] that the hydration of fumaric acid is acid catalyzed for pH's less than 1. An analogous reaction for the amination of fumaric acid would be

Table VI. Values of k_{addn} (obsd) − k_{addn} (calcd) at 135.3°

pH	k_{addn} (obsd)	k_{addn} (calcd)	k_{addn} (obsd) − k_{addn} (calcd)
−0.95	1.10×10^{-8}	2.41×10^{-9}	8.63×10^{-9}
−0.71	1.42×10^{-8}	4.43×10^{-9}	9.79×10^{-9}
−0.39	1.78×10^{-8}	9.30×10^{-9}	8.52×10^{-9}
−0.08	2.82×10^{-8}	1.90×10^{-8}	9.27×10^{-9}
0.30	5.33×10^{-8}	4.52×10^{-8}	8.14×10^{-9}

$$\mathrm{fum}^{00} + NH_3 + H_3{}^{+}O \rightleftharpoons \mathrm{asp}^{0+0} + H_2O \qquad (16)$$

The rate expression for this amination reaction in acid solutions is

$$\frac{d(\text{aspartic acid})}{dt} = k_{\text{addn}}^{\text{H}^+}(\text{fum}^{00})(\text{NH}_3)(\text{H}^+) \cong \frac{k_{\text{addn}}^{\text{H}^+}(\text{fum})_{\text{T}}(\text{NH}_3)_{\text{T}}(\text{H}^+)}{\left[1 + \frac{\text{H}^+}{K_{\text{a}}}\right]} \cong k_{\text{addn}}^{\text{H}^+}K_{\text{a}}(\text{fum}^{00})(\text{NH}_4^+) \qquad (17)$$

This equation indicates that in acid solutions, the rate of amination of fumaric acid by the reaction given in eq 16 will be independent of pH. This is in agreement with the values of $k_{\text{addn}}(\text{obsd}) - k_{\text{addn}}(\text{calcd})$ listed in Table VI. The reaction becomes important relative to the other amination reactions only for pH values less than 1.

Equation 16 gives

$$\frac{d(\text{fumaric acid})}{dt} = \frac{d(\text{ammonia})}{dt} = k_{\text{deam}}^{\text{H}^+}(\text{asp}^{0+0})(\text{H}_2\text{O})$$

for the deamination rate of aspartic acid. This rate expression is independent of pH in acid solutions which is in agreement with the observed deamination rates of aspartic acid at 135° for pH values less than 1.

Since $k_{\text{addn}}(\text{obsd})$ was calculated from k_{deam} and $K_{\text{DL}}^{\text{app}}$, the differences between $k_{\text{addn}}(\text{obsd})$ and $k_{\text{addn}}(\text{calcd})$ may be due to changes in $K_{\text{DL}}^{\text{app}}$. The effect of strong acid and ionic strength at pH less than 3 on $K_{\text{DL}}^{\text{app}}$ has not been investigated. However, at pH values of one or less, $K_{\text{DL}}^{\text{app}}$ can be written as

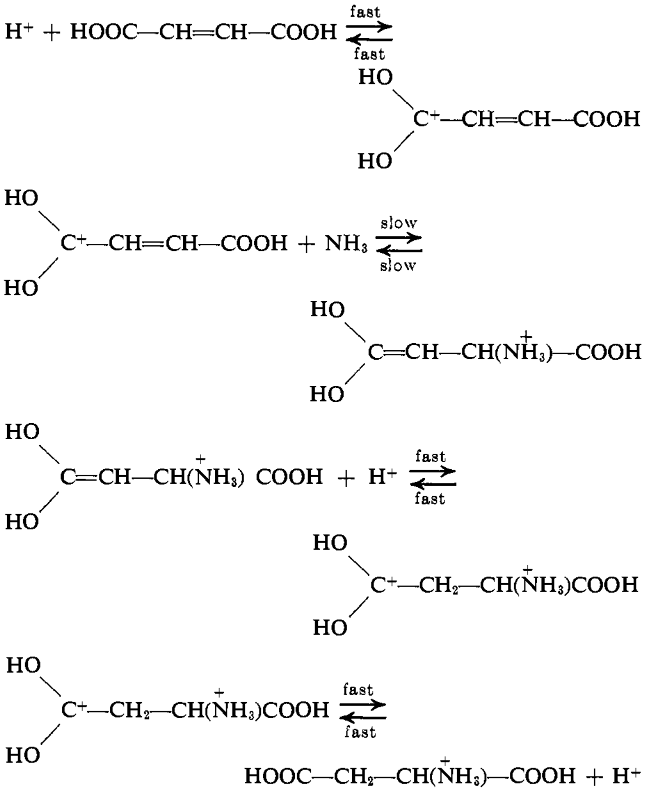
$$K_{\text{DL}}^{\text{app}} = \frac{a_{\text{fum}^{00}}a_{\text{NH}_4^+}}{a_{\text{asp}^{0+0}}} = \frac{(\text{fum}^{00})(\text{NH}_4^+)}{(\text{asp}^{0+0})}\frac{\gamma_{\text{fum}^{00}}\gamma_{\text{NH}_4^+}}{\gamma_{\text{asp}^{0+0}}}$$

The ratio of $\gamma_{\text{NH}_4^+}/\gamma_{\text{asp}^{0+0}}$ may be estimated from the activity coefficients of ammonium chloride[36] and tetrabutyl ammonium chloride.[37] In 6 *M* solutions, $\gamma^{\pm}_{\text{NH}_4^+\text{Cl}^-}/\gamma^{\pm}_{(\text{C}_4\text{H}_9)_4\text{N}^+\text{Cl}^-}$ is about 1.0. Assuming $\gamma_{\text{fum}^{00}} \cong 1$, it seems unlikely that $K_{\text{DL}}^{\text{app}}$ would have a large variation with ionic strength at pH's less than 1.

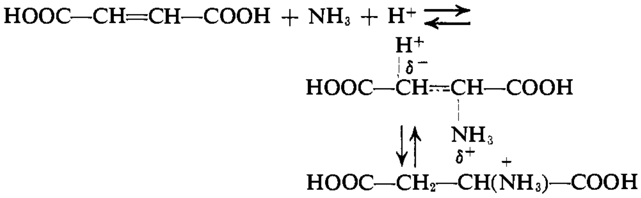
The following two mechanisms, which are both consistent with the kinetic results, are proposed.

The data do not allow distinction between these two mechanisms. Mechanism I is a 1,4 addition and is similar to the mechanisms proposed for the hydration of mesityl oxide and crotonaldehyde in acid solutions.[38]

Mechanism I



Mechanism II



Mechanism II may be viewed as a concerted carbanion mechanism. In the preceding section, carbanion intermediates were proposed for the amination reaction between pH 1 and 13. In acid solutions, the lifetime of the carbanion intermediate would be less than in neutral or basic solutions because of the high H+ concentration. The lifetime might be so short that the reaction would in effect be concerted.

Acknowledgments. We wish to thank Drs. Charles Perrin and Robert Fahey for helpful discussions. This work was supported by Grant No. GB-8056 from the National Science Foundation.

---

(36) Reference 20, p 495.

(37) S. Linderbaum and G. E. Boyd, *J. Phys. Chem.*, **68**, 911 (1964).

(38) R. P. Bell, J. Preston, and R. B. Whitney, *J. Chem. Soc.*, 1166 (1962).