The Solvolysis of 4(5)-(2-Amino-3-bromopropyl)imidazole

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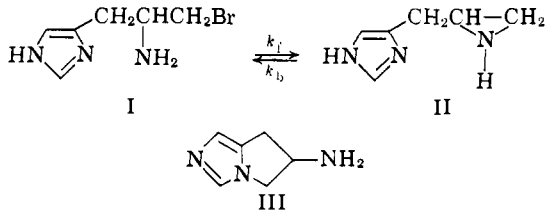
Abstract: The rate of formation of the aziridine II from the title compound I has been measured in aqueous solution at 25° as a function of pH. The same product (II) is formed at high pH and at low pH, but the rate of the reaction at pH 4.8 is six times faster than that predicted on the basis of the macroscopic pK_a values of I. Consideration of the microscopic pK_a values of I allows a good fit with the experimental data though the microscopic constants cannot be determined uniquely from the kinetic data alone. At the lower values of pH, the reverse reaction of bromide ion with II to give I becomes important.

In connection with the synthesis of some possible enzyme models related to ribonuclease, it became necessary to study the behavior, in alkaline aqueous solution, of 4(5)-(2-amino-3-bromopropyl)imidazole (I). This compound has been reported[1] in the patent literature, and was said to have a protracted depressing effect on blood pressure. However, it can be calculated from available data on related 2-bromoethylamines[2] that the half-life of compound I under physiological conditions may easily be as low as 5 min; the expected product would be either 4(5)-(2-aziridinylmethyl)imidazole (II), or possibly III, the result of nucleophilic attack by the imidazole.[3] The former product combines in one molecule two biologically interesting structural features: a marked resemblance to histamine, and an aziridine ring. Substituted aziridines are frequently mutagenic, and have been investigated as possible agents for cancer chemotherapy.[4]



I II

III

Both the formation[2,5] and ring-opening reactions[5b,6] of aziridines have been previously investigated in great detail. In the present paper, the behavior of I in aqueous solution at pH values between 4.8 and 10.8 is described.

Experimental Section

Materials. 4(5)-(2-Amino-3-bromopropyl)imidazole (I). The preparation of I was based on that of Schnider.[1] L-Histidinol bishydrochloride (Cyclo Chemical lot K-4334, 2.14 g; 0.01 moles) was refluxed for 4 days with 50 ml of aqueous hydrobromic acid (48%) containing two drops of 47% hydriodic acid. Evaporation of the reaction mixture gave crystals which were reprecipitated from methanol solution by the addition of ether. One recrystallization from methanol–ether gave 1.9 g (5.2 mmoles) of L-4(5)-(2-amino-3-bromopropyl)imidazole bishydrobromide, mp 211–213° dec (lit.[1] 210–212° dec), $[\alpha]^{23}D$ +16.1° (*c* 5, water) (lit.[1] $[\alpha]^{23}D$ +16.8° (*c* 5)); nmr spectrum: δ 3.33 (d, 2, J = 7 cps, CH_2–Im), 3.6–4.2 (m, 3, CH and CH_2Br), 7.54 (s, 1, ring H-5), 8.75 (s?, 1, ring H-2).

Anal. Calcd for $C_6H_{12}Br_3N_3$: C, 19.69; H, 3.31; Br, 65.52; N, 11.48. Found: C, 19.87; H, 3.51; Br, 65.39; N, 11.61.

The bisperchlorate of I was prepared in solution from the bishydrobromide by treatment of a solution of a known quantity of the latter with the calculated volume of aqueous silver perchlorate. The mixture, now containing precipitated silver bromide, was made up to a known volume and filtered in the dark through a medium porosity glass frit. The filtrate was tested for the absence of excess silver or halide ions and was used immediately for kinetic studies.

Standard potassium hydroxide (0.100 *N*, Fisher lot 753631, carbonate free) was restandardized against potassium hydrogen phthalate; the amount of carbonate was shown by titration to be negligible. Standard hydrochloric acid (0.100 *N*, Fisher lot 753556) and standard hydrobromic acid (0.100 *N*) were then checked against the potassium hydroxide. Aqueous silver perchlorate (0.265 *M*) was prepared from perchloric acid and freshly precipitated silver oxide. The solution was standardized against sodium chloride (0.1000 *M*), using potassium chromate[7] as an indicator, and kept in the dark. Tetraethylammonium perchlorate was the Eastman White Label product; all other materials used were analytical reagent grade.

The water used in the preparation of solutions was obtained by passage of distilled water through a mixed-bed ion-exchange column (Barnstead Red Dot) and had a specific resistance of greater than 3 Mohms as measured with a Barnstead PM 4 meter.

Apparatus. Potentiometric titrations and the majority of the kinetic runs were made in a Radiometer TTA31 microtitration assembly, using a thermostated vessel (Radiometer V 521/526). The temperature in the test solution was kept constant at 25° by circulation of water from a Haake Model F thermostat, and was measured in the solution by means of a calibrated thermistor. Approximately 5 ml of water was placed in the space between the outer thermostated vessel and the inner titration vessel as a heat-transfer fluid; in its absence serious error could be introduced by the long time necessary to reach thermal equilibrium (half-time about 5 min). The temperature in the test solution was found to be constant to within ±0.005° (short term) and to within ±0.02° (long term).

The kinetic runs were controlled by a Radiometer titrator Model TTT1c, and the addition of reagents from the syringe buret unit was recorded on a Radiometer titrigraph Model SBR2c. The volume and time axes were calibrated. For the determination of pK_a values, the titrator was used in conjunction with a scale expander, Model PHA 630T; the addition of reagents was now made manually from a calibrated syringe microburet.

Atmospheric carbon dioxide was excluded from the titration vessel by a stream of nitrogen gas that had been first passed through a column of soda lime and then through water. The filling of syringe reservoirs was performed in a glove box in an atmosphere of

(1) O. Schnider, U. S. Patent 2,677,692 (1954).
(2) (a) H. Freundlich and H. Kroepelin, *Z. Physik. Chem.*, **122**, 39 (1926); (b) B. Hansen, *Acta Chem. Scand.*, **16**, 1945 (1962).
(3) C. Pasini and S. Coda, *Gazz. Chim. Ital.*, **87**, 1440 (1957).
(4) See, for example, L. H. Schmidt, R. Fradkin, R. Sullivan, and A. Flowers, *Cancer Chemotherapy Rept., Suppl.*, **2**, 1 (1965).
(5) (a) C. C. Howard and W. Marckwald, *Ber.*, **32**, 2036 (1899). (b) H. Freundlich and G. Salomon, *Z. Physik. Chem.*, **166A**, 616 (1933), and earlier papers; P. D. Bartlett, S. D. Ross, and C. G. Swain, *J. Am. Chem. Soc.* 71, 1415 (1949), and earlier papers; B. Cohen, E. R. Van Artsdalen, and J. Harris, *ibid.*, **74**, 1875, 1878 (1952); W. E. Hanby, G. S. Hartley, E. O. Powell, and H. N. Rydon, *J. Chem. Soc.*, 519 (1947).
(6) J. E. Earley, C. E. O'Rourke, L. B. Clapp, J. O. Edwards, and B. C. Lawes, *J. Am. Chem. Soc.*, **80**, 3458 (1958).
(7) A. I. Vogel, "Quantitative Inorganic Analysis," 2nd ed, Longmans, Green and Co., London, 1955, p 251.

carbon dioxide free nitrogen. The titration assembly was equipped with a Radiometer G2222B glass electrode and a K4001 calomel reference electrode. A rapid response of the glass electrode to a change in pH was essential, and three electrodes were discarded before a satisfactory one was found; optimum performance was obtained after about 1 week of use. The sensitivity of the glass electrode was checked[8,9] periodically at 25° between 0.05 *M* potassium hydrogen phthalate (pH 4.008) and 0.05 *M* borax (pH 9.185), and linearity by using as a third point pH 7.0 buffer (Corning). Any deviation from the theoretical sensitivity was corrected for by adjustment of the temperature compensator; when the difference between the true solution temperature and the setting on the compensator exceeded 3°, the electrode was discarded. No correction was made for liquid junction potentials. Stirring, by means of a magnetic disk, was continued during the calibration of the electrodes. A few kinetic runs were made in a Cary 15 spectrophotometer equipped with a thermostated cell holder.

A-grade volumetric apparatus was used in the preparation of all solutions. Nuclear magnetic resonance spectra were determined in deuterium oxide with a Varian Model A-60 spectrometer, using external tetramethylsilane and benzene for field calibration. Chemical shifts are given in ppm downfield from external TMS.

Kinetic Methods. In all cases, reactions were followed to completion (>ten half-lives) and gave good, steady infinity readings. Repeat determinations gave rate constants that agreed to better than ±5%.

Reaction of I to give II. A solution (2.0 ml) 0.01 *M* in I, and containing other salts as noted in Table III, was transferred by means of a pipet to the titration vessel. The solution pH, initially about 3.8, was raised to that required for a particular run by the rapid addition of base from the manual syringe microburet, and the pH was then held constant at the predetermined value by the pH-Stat and associated syringe buret unit.[2b] A rapid response in all parts of the system was essential for a smooth start to the run. The variation in pH throughout a run did not exceed 0.03 unit. Except at pH 7.1 when only a single run was made, the rates were run in duplicate or in triplicate. The specific rate for the uptake of base (or of acid in other cases, see below) was calculated in the usual way[10] from the slope of the line obtained by plotting ln $(B_{inf} - B_t)$ *vs.* time, where B_{inf} is the uptake of base after more than ten half-lives, and B_t that at time t. Values of base uptake extending over at least three half-lives were used in the calculation of rate constants; strictly first-order kinetics were observed at all values of pH except as noted below.

Reaction of II with Thiosulfate.[2b,6] The pH of a solution containing II (or a known mixture of I and II) was adjusted to 3.6 by the addition of hydrochloric acid, and 0.1 or 0.2 ml of 1.0 *M* sodium thiosulfate was added rapidly from a syringe. The addition caused the pH of the solution to rise at once to about 3.9, at which value it was then maintained during the ensuing reaction by the automatic addition of hydrochloric acid from the syringe buret.[2b] Thiosulfate did not react significantly with tetraethylammonium perchlorate nor with I under these conditions. The thiosulfate ester was stable at pH 9–10, as has been found previously for related compounds.[2b]

Spectrophotometric Rate. A solution (8 μl) of 10 mg of the bromide I in 1 ml of water was injected by means of a microliter syringe (Hamilton) into 3.0 ml of 0.05 *M* borate buffer (pH 9.18) contained in a 1-cm path length silica cell. The cell was stoppered, shaken, and then replaced in the thermostated cell compartment of a Cary 15 spectrophotometer. The reaction was followed to completion at 210 mμ (shoulder) using the 0 to 0.1 slidewire.

pK_a Determination. "Mixed" (concentration-dependent) pK_a' values for the first and second macroscopic acid dissociation steps of I and of II were obtained by potentiometric titration as follows; no activity corrections were performed.

(Aminobromopropyl)imidazole. A solution 0.01 *M* in I bishydrobromide and containing potassium chloride as noted in Table I was titrated with standard potassium hydroxide. Complete neutralization requires 2 equiv of base, but for amounts of base added over about 1 equiv, the rate of decomposition of I was too rapid to permit more than one reading per experiment. Accordingly, the pH of the solution was quickly measured after the rapid addition of 1.25, and with a fresh portion of solution, 1.50 equiv of base. For amounts of base added well below 1 equiv the titration figures were obtained in the conventional[8] way. The pK_a' values were calculated assuming nonoverlapping constants. In addition, the method of Noyes[11] for overlapping constants was applied to the pair of readings obtained at 0.5 and 1.5 equiv of base added. The result did not differ significantly from that obtained in the first calculation. The results are given in Table I; by comparison with similar compounds,[12] the higher pK_a' would be assigned to the amino group.

Table I. The pK_a' Values of I and II in Aqueous Solution at 25°

Compd	Molarity of added KCl	μ[b]	pK_1'	pK_2'
I	0.0	0.03	5.55[a]	7.95[a,c]
I	0.1	0.13	5.67[a]	7.95[a]
II	0.0	0.04	5.58 ± 0.01	7.61 ± 0.05

[a] No spread is given for these values because of the limited number of readings taken. [b] At the start of the titration. [c] The values of pK_2 calculated from the readings at 1.25 and at 1.50 equiv of base added were identical. The agreement is fortuitously good but is also reassuring, since the amount of decomposition of I is obviously less in the first case than in the second, and is therefore probably not very great in either case.

Compound II. The aziridine II was not isolated, but was produced in solution from I. A solution 0.01 *M* in I bishydrobromide (2.0 ml) was held at pH 9.4 until reaction was complete (30 min). The take-up of base was 99% of the theoretical amount, allowing for the difference in pK_2 between I and II. The pH of the solution was then lowered to 3.80 by the addition of hydrochloric acid (0.1 *M*). This value was chosen after preliminary runs were made to determine at what pH the aziridine would be essentially fully protonated. The volume of the solution at this stage was 3.0 ml. Base (2.0 equiv) was then added in 20 equal increments, and the pH was measured after each addition. The aziridine was stable to this treatment for the length of time required to make the measurements; this was shown by the absence of a downward drift in the pH during reading, toward the end of the titration, and by the quantitative reaction of the material with thiosulfate at pH 3.9 (to which pH the solution was returned at the end of the titration). The pK_a' values were calculated by the method of Noyes,[11] using pairs of readings taken symmetrically about the midpoint and with allowance being made for dilution by the addition of the various reagents; the calculations were performed on a CDC 1604 digital computer. The results are given in Table I; all readings between 0.2 and 1.8 equiv of added base were used in calculating the spread.

Product of the Reaction of I at pH 9–10. The reaction of I to give II at constant pH followed first-order kinetics for at least three half-lives and gave about the same first-order rate constant by spectrophotometry at $^1/_{100}$th the concentration used in the pH-Stat method. The rate of take-up of base at constant pH as a function of pH in the region 7–10 followed that expected for the involvement of the amino group of I, and at pH 10 was within a factor of ten of the rate of formation of aziridine from the free base form of 2-bromoethylamine.[2b]

A solution of I in deuterium oxide was treated with 10 *N* sodium hydroxide at pH 10 until reaction was complete. The nmr spectrum of the solution was taken, then an excess of deuterium chloride was added and the spectrum again taken. The free base form showed: δ 1.45 and 1.9 (broad s each, 1 each, CH_2N), 2.05–2.50 (broad m, 1, aziridinyl CH), 2.67 (d, 2, J = 6 cps, CH_2–Im), 6.97 (s, 1, H-5), 7.68 (s, 1, H-2). The dication form showed: δ 2.8–3.2 (m, 2, CH_2N), 3.2–3.6 (m, aziridine CH), 3.37 (s, CH_2–Im), 7.54 (s, 1, H-5), 8.75 (s, 1, H-2).

Comparison of the spectra of the dication forms of I and of II showed that the chemical shifts of the imidazole ring protons H-2 and H-5 and the side-chain CH_2–Im did not change significantly on going from I to II. However, the resonances of the side-chain CH and CH_2(Br) protons appeared at 40–50 cps higher field in the product. In addition the free base form of II showed CH_2(az) resonances with the chemical shift expected for an aziridine CH_2.[13]

(8) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen and Co., London, 1962, p 19.
(9) R. G. Bates, "Determination of pH," John Wiley and Sons, Inc., New York, N. Y., 1964, pp 62–94.
(10) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N Y., 1961, p 38.
(11) Reference 8, p 52.
(12) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution," Butterworth and Co. (Publishers), Ltd., London, 1965.

The known[2b,6] rapid reaction of the aziridinium cation with thiosulfate dianion was used to estimate the percentage of II formed from I. Complete reaction of 1 equiv of the aziridine II with excess thiosulfate at constant pH 3.9 would be expected to give a take-up of a maximum of 1.02 equiv of hydrochloric acid.[14]

It was found consistently that the reaction required from 1.01 to 1.03 equiv of acid, and that second-order kinetics were followed to at least 94% reaction. The specific rate of the reaction (see Table II) was about that expected for an aziridine. Thus it seemed likely that the reaction of I in water at a concentration of 0.01 *M* at pH 9–10 gave at least 99% of the aziridine II.

Table II. The Reaction of II with Bromide and with Thiosulfate at 25°

Nucleophile (*M*)	pH	Ionic strength	10^4k, M^{-1} sec^{-1}
Br^- (0.094)	3.0	0.1	4.1[a,d]
Br^- (0.0625)	3.0	0.1[b]	4.6[a]
Br^- (0.094)	3.5	0.1	4.4[a]
Br^- (0.119)	4.8	0.13	3.2[c,d]
$S_2O_3^{2-}$ (0.032)	3.9	0.20	1360

[a] Initial rates. [b] With added tetraethylammonium perchlorate. [c] From rate of approach to equilibrium starting with I. [d] The accuracy of these figures is about ±10%.

Product of the Reaction of I at pH 4.8–5.7. A solution of I bishydrobromide (0.01 *M*) in 0.1 *M* tetraethylammonium perchlorate was kept at pH 5.68 (25°) until reaction was complete (15 hr); base take-up was 95% of the theoretical amount (see below). This product reacted with thiosulfate (at pH 4) at the same rate as did the product that was formed from I at pH 9.4; the amount of aziridine that had been formed accounted for more than 98% of the take-up of base. After the thiosulfate reaction was complete, the amount of I present in the solution (5%) was estimated by raising the pH to 9.4 and following the rate of addition of base at constant pH. (The validity of this method in the presence of thiosulfate was checked on a known mixture of I with II.)

An equivalent experiment was performed at pH 4.8, except that potassium bromide was used in place of the tetraethylammonium perchlorate. Reaction was complete at 20 hr; base take-up was 33% of the theoretical amount.

Aziridine formation accounted for 100% of the take-up of base, and the amount of I (or its equivalent) still present in the solution was again found to fully account for the difference between the actual base take-up (33%) and the theoretical amount (see below).

The Reaction of II with Bromide. A solution of I bishydrobromide in water (2 ml of 0.01 *M*) was held at pH 9.4 until reaction was complete. Potassium bromide (0.2 ml of 1 *M*) was added, and the pH of the solution was lowered to 3.0 by the addition of hydrobromic acid. The reaction was followed by the addition of acid at constant pH and was allowed to go to completion (40 hr). At the end of this time, the pH of the solution was returned to 9.3, and the ensuing reaction was now followed by the addition of base. First-order kinetics were followed for more than three half-lives; at least 90% of the aziridine had reacted to form a compound that liberated acid at pH 9.3 at precisely the same rate as did I. In separate experiments the reaction of II with bromide was found to be approximately first order in bromide ion, and had about the same rate at pH 3.5 as at pH 3.0. The specific rate for this reaction (see Table II) was about 20 times that found[6] for the reaction of bromide with 2-ethylaziridine, and as found[6] previously, bromide was about $^1/_{300}$th as effective a nucleophile as thiosulfate.

The Rate of Reaction of I at pH 4.8. As described above, the reaction of I at pH 4.8 appeared to reach completion at only 33% of the theoretical take-up of base. Evidently the rate of formation of II from I was sufficiently slow at pH 4.8 that the reverse reaction of bromide with II had become kinetically significant, and this was confirmed by an independent measurement of this reverse reaction. This type of equilibrium situation has been found[5] in previous work in this field. The amount of hydrolysis or of piperazine formation[5b] was negligible under these conditions. Thus the rate of uptake of base at pH 4.8 was a measure of the rate of approach to equilibrium and not simply that of the forward reaction of I to give II. The true rate constant for the forward reaction (k_f) was obtained in two ways.

(1) An excess of bromide ion was added to a solution of I and the rate of approach to equilibrium was measured. The reverse reaction now followed pseudo-first-order kinetics[16] and the rate of approach to equilibrium (k_e) was the sum[17] of the rates of the forward and back reactions ($k_f + k_b$). The ratio of the same rate constants (k_b/k_f) was given by the ratio (R) of the concentrations of I and of II present at equilibrium. Then $k_f = k_e/(1 + R)$.

(2) The reaction was carried out on the bisperchlorate salt of I using tetraethylammonium perchlorate as a swamp electrolyte. Since the initial concentration of bromide ion was zero, and the reverse reaction was bimolecular, the particular values of the rate constants involved in this system allowed k_f to be obtained with reasonable precision under these conditions from the initial rate of take-up of base, using as B_{inf} the *theoretical* base uptake for complete reaction. The "theoretical" value was checked by carrying out a reaction at pH 9.4 on a fresh portion of the same solution. The value of k_f obtained in this way agreed well with that calculated by the first method. This procedure was also used to obtain k_f at pH 5.3 and 5.7. At higher pH values the presence of 0.02 *M* bromide ion did not affect significantly the measurement of k_f.

Discussion

The rate constants for the forward reaction, k_f, at various values of pH are given in Table III. The rate at high pH was fairly insensitive to a change in the ionic strength; this has been previously noted[2b] for the

Table III. Rate Constants for the Reaction of (Aminobromopropyl)imidazole (I) at 25° at Various Values of pH

pH	Ionic strength[a]	10^5k_f, sec^{-1}
10.85	0.10	483
10.30	0.10	447
9.80	0.10	450
9.60	0.02[b]	420[b]
9.36	0.10	427
9.36	0.10[c]	430[c]
9.36	0.02[b]	400[b]
9.2	0.15	480[d]
8.40	0.10	310
7.90	0.10	203
7.48	0.11	118
7.10	0.11	63.0
6.63	0.11	32.3
6.10	0.11	16.0
5.67	0.12	9.83[c,e]
5.30	0.12	5.37[c,e]
4.80	0.13	1.98[c,e]
4.80	0.13	1.93[f]

[a] At pH values above 6.0 either potassium chloride or tetraethylammonium perchlorate was used to adjust the ionic strength; the rates were the same in either. [b] No added salt. [c] Using the bisperchlorate salt of I and tetraethylammonium perchlorate. [d] Rate determined spectrophotometrically. [e] From initial rate. [f] From rate of approach to equilibrium.

(13) N. S. Bhacca, D. P. Hollis, L. F. Johnson, and E. A. Pier, "NMR Spectra Catalogue," Vol. 2, Varian Associates, Palo Alto, Calif., 1963, Spectrum No. 372.

(14) At the pH of the reaction (3.9), II exists to the extent of 2% as the monocation. If the thiosulfate (pK_a^T's about 0.6 and 1.5)[15] is considered to be negligibly (0.4%) protonated at this pH and remains unprotonated in the product, then the ratio of the number of equivalents of acid required to maintain a constant pH to the number of equivalents of II undergoing reaction is $(1.02 - F)$ where F is the fraction of the product that is monoprotonated at pH 3.9. The pK_a' values for this product were not determined but a lower limit for the amount of II present can obviously be calculated by setting $F = 0.0$.

(15) L. G. Sillén and A. E. Martell, Ed., "Stability Constants of Metal–Ion Complexes," Special Publication No. 17, The Chemical Society, London, 1964, p 224.

(16) During the approach to equilibrium, the change in bromide ion concentration and the change in ionic strength (due to dilution and reaction) were each about 0.5%.

(17) Reference 10, p 186.

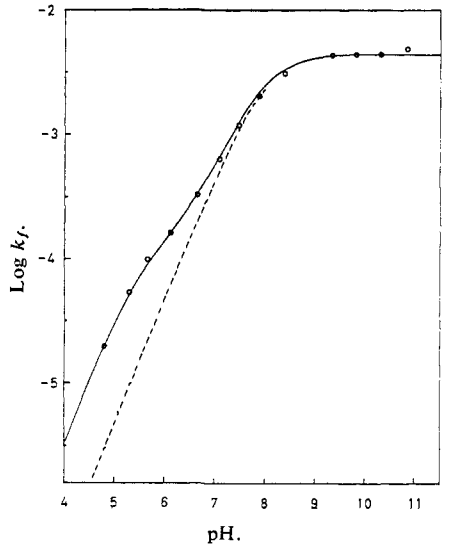


Figure 1. pH-rate profile for the reaction of I at 25° and ionic strength 0.12 to 0.13. The solid line is calculated from eq 2 with $x = 7.8 \times 10^{-8}$ M; the broken line is calculated from the equation $k'K'_a/(K_a' + a_H)$ with $k' = 4.5 \times 10^{-3}$ and $pK_a' = 7.95$. The experimental points are denoted by circles. Rate constants are expressed in sec^{-1}.

cyclization of 2-bromoethylamine. The reaction gave the same product both at high and at low pH, and involved intramolecular nucleophilic attack by the free base form of the neighboring amino group. There was no evidence for the formation of III. Thus it may be expected that the rate as a function of pH should follow[18] the equation: $k_f = k'K_a'/(K_a' + a_H)$, where k' is the specific rate for the free base form of I, K_a' is the acid dissociation constant of the amino group of I, and a_H is the activity of hydrogen ions in the solution as measured with the glass electrode. The values of k_f predicted from this equation are plotted in Figure 1. However, it can be seen that at low pH the experimental values of k_f deviate considerably from the calculated ones: at pH 4.8 they differ by a factor of 6.2. This behavior can be completely accounted for if the microscopic dissociation constants of I are considered.

The ionization scheme for I is shown in Figure 2.[19] The two forms with a nonprotonated amino group are HL and L, and both forms contribute to the rate of formation of II. Thus if L_t is the total amount of I present, then

$$\text{rate} = k_f(L_t) = k_L(L) + k_{HL}(HL)$$

and thus from the equilibria in Figure 2

$$k_f = \frac{k_L}{1 + \frac{a_H}{K_3} + \frac{a_H}{K_4} + \frac{(a_H)^2}{K_1K_3}} + \frac{k_{HL}}{1 + \frac{K_4}{a_H} + \frac{K_1}{K_2} + \frac{a_H}{K_2}}$$

The macroscopic constants K_1' and K_2' and the four microscopic constants are related[20] by the three equations $K_1' = K_1 + K_2$, $K_2' = K_3K_4/(K_3 + K_4)$, and

(18) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, Inc., New York, N. Y., 1966, pp 11–14.

(19) Strictly, the position of the proton on one or other of the ring nitrogens gives rise to two tautomeric forms for each of L and LH. The tautomeric constants involved are not known and cannot be estimated from the data presented in this paper. For simplicity, therefore, L and LH are each regarded as consisting of an equilibrium mixture of the two forms (see Figure 2).

(20) E. Q. Adams, *J. Am. Ch m. Soc.*, **38**, 1503 (1916).

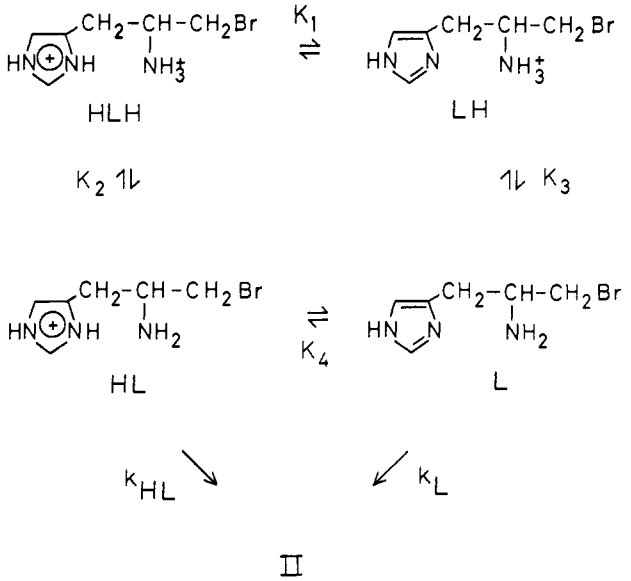


Figure 2. The microscopic dissociation equilibria of I.[19] Forms containing the imidazole anion are not considered.

$K_1K_3 = K_2K_4$ so that, as is well known,[20] one of the microscopic constants must be treated as an independent variable in the absence of further information.

The microscopic constant K_2 was arbitrarily chosen as the independent parameter, and k_f was then expressed in terms of the known quantities a_H, K_1', K_2', and k_L (from the limiting rate at high pH), and the unknowns k_{HL} and K_2

$$k_f = \frac{(k_LK_1'K_2' + k_{HL}K_2a_H)}{(K_1'K_2' + a_HK_1' + (a_H)^2)} \quad (1)$$

The two independent variables appear only as the product $(k_{HL}K_2)$ and thus raising one of them by the same factor that the other is lowered would leave the predicted curve of k_f *vs.* pH unchanged. More importantly, variation of k_{HL} does not produce a different curve from that obtained by making instead an equivalent change in K_2, so that it was not possible to estimate these variables independently. Rather than plotting a linear form of (1), a best fit of the experimental values of k_f between pH 4.8 and 10.85 was obtained by effectively varying the value of the product $k_{HL}K_2$ and comparing the values of k_f calculated from eq 1 with the experimental figures. The calculations were carried out on a digital computer. In practice eq 1 was expressed in the form

$$k_f = \frac{k_L(K_1'K_2' + xa_H)}{(K_1'K_2' + a_HK_1' + (a_H)^2)} \quad (2)$$

and x $(= K_2k_{HL}/k_L)$ was varied until a minimum was found in $\Sigma|k_f(\text{exptl}) - k_f(\text{calcd})|$ where the sum was taken over all the values of pH. The best fit was obtained with $x = 7.8 \times 10^{-8}$ M, and a variation of $\pm 5\%$ in x gave a perceptibly worse fit.

An investigation[21] of the reactivity of some amino thiols toward *p*-nitrophenyl acetate required consideration of the microconstants of cysteine, and these had been previously determined.[22] However, microscopic dissociation constants do not appear to have

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been determined for any histidine derivative, so that in the present work the contributions to x of K_2 and of k_{HL}/k_L have not been separated.[23]

The experimental results are, however, satisfied by values of k_{HL}/k_L and K_2 that are chemically reasonable. As one example, if $k_{HL} = k_L$, then $pK_1 = 5.69$, $pK_2 = 7.11$, $pK_3 = 7.94$, $pK_4 = 6.51$, and (LH)/(HL) = 26. Thus it is unnecessary to invoke any special involvement of the unprotonated imidazole in the major species LH in order to explain the rate of decomposition of I at low pH values.

(23) An estimate of K_2 could obviously be obtained from the K_a of the (unknown) N,N'-dimethylimidazolium derivative of I.

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Inhibition of Neighboring-Group Participation by Chelation. The Effect of Cupric Ion on the Solvolysis of 4(5)-(2-Amino-3-bromopropyl)imidazole

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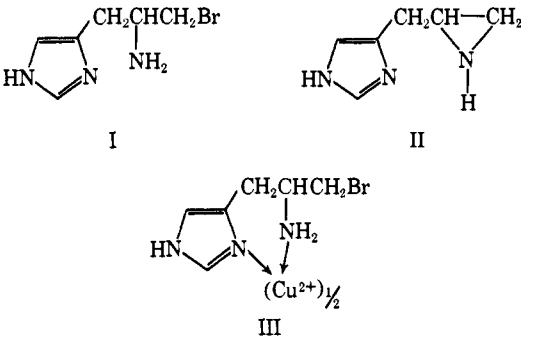
Abstract: The stability constants for complex formation between cupric ion and I and II were measured at 25° and the effect of cupric ion on the rate of the reaction of I to give II has been measured at pH 9.4. With a metal to ligand ratio of 0.6:1 a 50-fold decrease in the initial rate of reaction was found. With a metal-to-ligand ratio less than 0.5 complicated kinetic behavior was shown, which was quantitatively accounted for by the partitioning of the metal ion between the starting material and the product.

During a study, at present in progress, of the synthesis of some enzyme models related to ribonuclease, we wished to carry out a displacement of the bromide ion of the (aminobromopropyl)imidazole (I) by an external nucleophile at about pH 10. Unless the nucleophile were an unusually powerful one, an attempt to run this reaction would normally result[1,2] in a good yield of the substituted aziridine (II) and in little of the desired product. Standard methods of protecting one or both of the intramolecular nucleophiles (the amino and imidazole groups) could then be tried in the hope of allowing the bimolecular reaction to take precedence. However, in the case of I a rather different approach should be possible: formation of the complex of I with a metal ion (III) may be expected to slow down the unwanted intramolecular reaction on both electrostatic and steric grounds, and if the external nucleophile did not interfere with the formation of the complex, the required product may now be formed. Examination of literature values for the stability constants of complexes of histamine derivatives[3] suggested that the copper complex of I would be the most likely one to remain stable at pH 10. Homogeneous inhibition of a reaction by metal ions has been less well investigated than has catalysis, the



latter phenomenon obviously playing an important role in the action of many enzymes and enzyme models.[4] Cupric ion has been reported to inhibit the decarboxylation of nitroacetic acid[5] and the intermolecular reaction of imidazole and of glycylglycine with *p*-nitrophenyl acetate,[6] and has been used[7,8] to mask the α-amino group in some reactions of ornithine and lysine, thus allowing reaction (*e.g.*, acylation) to occur preferentially at the δ- and ϵ-amino groups, respec-

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