Distillation of the acetic anhydride mother liquors yielded 4.6 g. (72%) of acetylmorpholine, b. p. 230–240°.

N-Butyroxymethyl-N′-acetylurea.—Morpholinomethylurea (0.05 mole) and butyric anhydride (0.31 mole) were heated on the steam-bath for two and one-half hours. A clear solution formed within five minutes and on cooling yielded 8.5 g. (84%) of plate-like crystals which, crystallized from ethyl acetate, melted at 116.8–117°.

Anal. Calcd. for $C_8H_{14}N_2O_4$: N, 13.85; saponification equivalent, 101. Found: N, 13.80; saponification equivalent, 100.

Picrates of Morpholinomethyl Derivatives.—Mono- and bis-(morpholinomethyl)-urea formed unstable picrates (m. p. 140–160°) from alcohol and water solutions. In contrast to these results stable picrates were prepared from glacial acetic acid solutions in a yield expected for a one to one molecular combination from mono- and bis-(morpholinomethyl)-urea. They melted at 162.0–163.3° and 163–164°.

Morpholinomethyl derivatives of alkylureas did not form picrates in glacial acetic acid solutions. In alcohol and water solutions unstable picrates were obtained which on purifying yielded morpholine picrate (m. p. 148.8–149.2°).

Stable picrates of morpholinomethyl derivatives of acetylurea (m. p. 195°), phenylurea (156–158°), phthalimide (205°), and succinimide (188–189°) were prepared in either water, alcohol or glacial acetic acid solutions.

Summary

Mono- and bis-(morpholinomethyl)-urea were prepared and described. These compounds were cleaved by hydrolysis, reduction, and acetic anhydride to yield morpholine, methylmorpholine and acetylmorpholine.

Morpholinomethyl derivatives of 18 substituted ureas were prepared and characterized; N-morpholinomethyl-N′-acetylurea underwent reaction with acetic anhydride, yielding N-acetoxymethyl-N′-acetylurea and acetylmorpholine. Butyric anhydride gave the analogous butyroxymethyl derivative.

The morpholinomethyl derivatives of thiourea, succinimide, phthalimide, benzene- and *p*-toluenesulfonamide were prepared and characterized.

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[CONTRIBUTION FROM THE INSTITUTE OF EXPERIMENTAL BIOLOGY, UNIVERSITY OF CALIFORNIA]

Kinetics of Reactions between Iodine and Histidine

BY CHOH HAO LI

Imidazole compounds react readily with iodine.[1] The imidazole containing amino acid, histidine, however, has not hitherto been iodinated. Bauer and Strauss[2] were not able to halogenate the histidine with ICl, but their results[3] with globin indicate the possibility of iodohistidine formation. On the other hand, Pauly[1b] succeeded in preparing benzoyldiiodo-histidine and tetraiodo-histidine anhydride. The fact that histidine cannot be iodinated in the free state but both its anhydride and its benzoyl derivative smoothly take up one mole of iodine (on the two C atoms of the imidazole ring) in a slightly alkaline solution has recently been confirmed by Bauer, Strauss and Maschmann.[4]

When histidine and iodine are allowed to react in a nearly neutral solution, the disappearance of iodine follows the rate law

$$dx/dt = (a - x)(b - 2x) \qquad (1)$$

where a and b are the initial concentrations of histidine and titratable iodine, respectively; k_2 is the specific rate constant and x is the concentration of diiodo-histidine which is formed according to the reaction: histidine + 2 I_2 → di-iodohistidine + 2HI. The rate law (1) is identical with that found in the formation of diiodotyrosine.[5] Table I gives the values of k_2 in a typical run for successive time intervals by means of the integrated form of equation (1). The concentrations are in moles per liter and time in minutes. The bimolecular rate law is further verified when one varies the initial concentrations of iodine and histidine. The specific rate is not changed by the addition of neutral salts.

TABLE I

A TYPICAL KINETIC EXPERIMENT AT 25°

$b = 2a = 5.26 \times 10^{-3}m$, $(I^-) = 3.15 \times 10^{-2}m$, pH 7.21 citrate–phosphate buffer ($HPO_4^=$) = $3.50 \times 10^{-2}m$.

t, min.	(b) 10^3	(x) 10^3	k_2
0	(5.26)		
5.0	4.50	(0.38)	(6.4)
12.5	3.72	.77	6.2
18.0	3.30	.98	6.2
30.0	2.65	1.30	6.1
50.0	2.00	1.63	6.3
			Av. 6.2

The Rate as a Function of *p*H.—The dependence of specific rate on pH of the environment is similar to that found for the iodine-phenol reaction,[5] *i. e.*, the reaction becomes slower as the acidity of the solution increases. Data, summarized in Table II, give a relationship

$$k_2 = 0.10 + (5 \times 10^{-8}/(H^+)) \qquad (2)$$

in citrate buffers containing $(I^-) = 3.15 \times 10^{-2}$.

(1) (a) H. Pauly and K. Gundermann, *Ber.*, **41**, 3999 (1909); (b) H. Pauly, *ibid.*, **43**, 2243 (1910); (c) H. Pauly and E. Arauner, *J. prakt. Chem.*, **118**, 33 (1928).

(2) H. Bauer and E. Strauss, *Ber.*, **69B**, 245 (1936).

(3) H. Bauer and E. Strauss, *Biochem. Z.*, **284**, 197, 231 (1936).

(4) H. Bauer, E. Strauss and E. Maschmann, *Ber.*, **68B**, 1108 (1935).

(5) C. H. Li, THIS JOURNAL, **64**, 1147 (1942).

TABLE II

EFFECT OF *p*H ON THE RATE AT 25°

Citrate buffer (I^-) = $3.15 \times 10^{-2}m$

*p*H	k_2	k_2 calcd.
6.00	0.05	0.06
6.56	.25	.28
6.94	.50	.54
7.07	.72	.70
7.21	1.00	.90

The Rate as a Function of (I^-).—In a constant *p*H solution, the rate decreases as the (I^-) increases. This is evident from the results shown in Table III. The last column of Table III is calculated by the equation

$$k_2 = \frac{4.0 \times 10^{-3}}{(I^-)} + \frac{6.0 \times 10^{-4}}{(I^-)^2} \qquad (3)$$

The agreement between the observed and calculated k_2 appears satisfactory.

TABLE III

EFFECT OF (I^-) ON THE RATE AT 25°

Citrate buffer, *p*H 7.07

(I^-) 10^2	k_2	k_2 calcd.
3.15	0.72	0.73
3.80	.52	.52
5.00	.33	.32
7.10	.19	.17
10.00	.10	.10

From the preceding studies of diiodo-tyrosine formation,[5] it may be assumed that free iodine and hypoiodous acid react with the histidine. The rate law becomes

$$\frac{-\,d(\text{histidine})}{dt} = k_2'(\text{histidine})(I_2) + k_2''(\text{histidine})(HOI) \qquad (4)$$

Since the concentrations of iodine and hypoiodous acid are determined by the equilibria (5) and (6)

$$I_2 + I^- \rightleftarrows I_3^- \qquad (5)$$

$$I_2 + H_2O \rightleftarrows HOI + H^+ + I^- \qquad (6)$$

it follows that

$$-\frac{d(\text{histidine})}{dt} = \left[\frac{k_2'}{K_5(I^-)} + \frac{k_2''K_6}{K_5}\frac{1}{(H^+)(I^-)^2}\right](\text{histidine})(I_3^-) \qquad (7)$$

therefore

$$k_2 = \frac{k_2'}{K_5(I^-)} + \frac{k_2''K_6}{K_5}\frac{1}{(H^+)(I^-)^2} \qquad (8)$$

Thus, in a constant *p*H with variations of (I^-) the specific rate may be represented by the expression

$$k_2 = \frac{A}{(I^-)} + \frac{B}{(I^-)^2} \qquad (9)$$

where $A = k_2'/K_5$ and $B = k_2''K_6/K_5(H^+)$. This expression is in accordance with the empirical equation (3). By comparison of equations (3) and (9) together with the equilibrium constants,[6,7] $K_5 = 715$, $K_6 = 3 \times 10^{-13}$ at 25°, we have obtained $k_2' = 2.9$ and $k_2'' = 1.2 \times 10^5$.

When the reaction occurs in a constant (I^-) with variations of (H^+), equation (8) may be written as

$$k_2 = C + (D/(H^+)) \qquad (10)$$

where $C = k_2'/K_5\,(I^-)$ and $D = k_2''\,K_6/K_5\,(I^-)^2$. This is identical with the empirical equation (2). From equations (2), (10), and equilibrium constants K_5, K_6, the specific rate k_2' and k_2'' are computed to be 2.3 and 1.18×10^5, respectively. These values agree very well with those obtained with a different set of data as described.

Therefore, the reaction of iodine and histidine in citrate buffer follows the course expressed by

$$-\,d(\text{histidine})/dt = 2.5(\text{histidine})(I_2) + 1.2 \times 10^5(\text{histidine})(HOI) \qquad (11)$$

It is evident that hypoiodous acid is far more reactive as an iodinating agent than free iodine. This conclusion is consistent with that obtained in the study of the iodination of phenols.[5]

Catalytic Effect of ($HPO_4^=$) on the Rate.—When a phosphate buffer instead of the citrate buffer of the same *p*H was used, the specific rate was greatly increased. Since the rate is not accelerated by the addition of neutral salt, the effect of phosphate buffer must be specific.

When a phosphate buffer of the same *p*H was added gradually to the citrate buffer, the rate appeared to increase according to the concentration of ($HPO_4^=$). The data in Table IV bear out these conclusions. A similar catalytic effect of phosphate buffer was observed in the kinetic studies of diiodo-tyrosine formation.[8]

TABLE IV

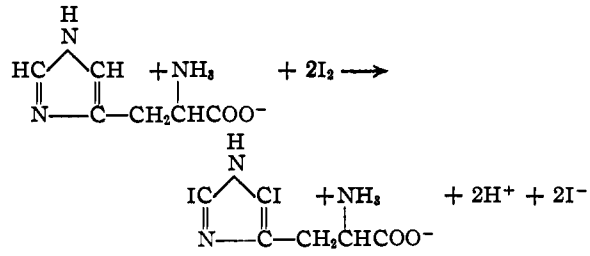
EFFECT OF ($HPO_4^=$) ON THE RATE AT 25°, (I^-) = 3.15×10^2

*p*H of buffer	($HPO_4^=$) 10^2	k_2
7.21	0.00	1.0
	1.75	3.2
	3.50	6.2
	5.25	8.4
	7.00	11.1
6.56	0.00	0.25
	1.10	.55
	2.20	1.0
	3.30	1.2
	4.40	1.6

Discussion

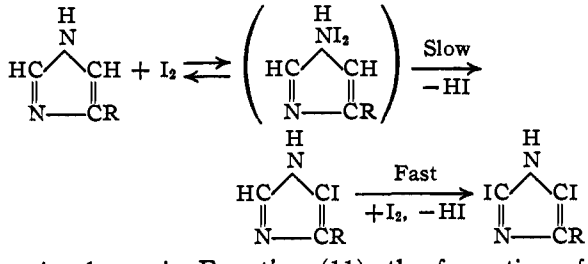
The fact that the kinetic data herein reported are analogous to those secured in the iodination of tyrosine indicates that iodine reacts with the imidazole ring of the histidine molecule to form diiodo-histidine

(6) W. C. Bray and Mackay, THIS JOURNAL, **32**, 914 (1910).
(7) W. C. Bray, *ibid.*, **32**, 932 (1910).
(8) See footnote 8a on ref. 5 and the following paper.

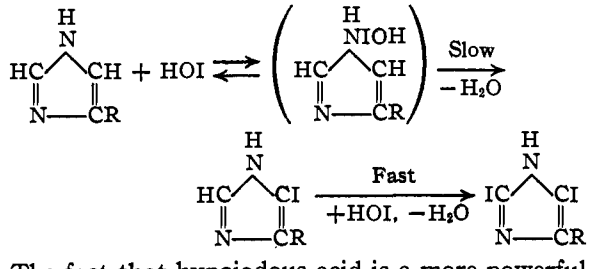


Preliminary experiments in which diiodo-histidine was isolated, indicated that this is indeed the case. Bauer and Strauss[3] have postulated that one iodine atom is attached to the amino group of the imidazole ring. Our experiments do not support this assumption.

The kinetic data here reported show that the rate-determining step requires a reaction of the first order with respect to both histidine and iodine although the net reactions use up two moles of iodine. Since most aromatic substitution reactions are usually explained by the rearrangement mechanism,[5,9] a similar scheme may be applied to the present case

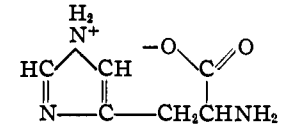


As shown in Equation (11), the formation of diiodo-histidine is also achieved through another path in which the hypoiodous acid is the iodinating agent



The fact that hypoiodous acid is a more powerful iodinating agent than iodine may be partly attributed to the greater tendency to form *undissociated* water molecule as compared with the formation of ionizing hydrogen iodide when free iodine acts as an iodinating agent.

In acid solution the occurrence of $>NH_2^+$ ion prevents the formation of the $>NHI_2$ or $>NHIOH$ complex and in turn the rate becomes slower. A similar explanation may be offered of the ease of isolation of the tetraiodo-histidine anhydride as observed by Pauly and others. In free histidine, it is probable that there exists a dipolar ion



which is absent in histidine anhydride.

The iodination of tyrosine is much faster than that of histidine and this may be partly due to the occurrence of such a dipolar ion in the histidine molecule. Table V summarizes the specific rates of diiodo-histidine and diiodo-tyrosine formation under comparative conditions. At *p*H 6.0, for

TABLE V

COMPARISON OF THE RATE OF DIIODO-HISTIDINE AND DIIODO-TYROSINE FORMATION AT 25°

*p*H	Buffers	$(I^-)\ 10^2$	k_2 Tyrosine	k_2 Histidine
7.21	Citrate	3.15	27.0	1.0
7.21	Phosphate[a]	3.15	(310)	16.0
7.00	Phosphate[b]	10.00	7.4	0.34
6.00	Citrate	3.15	3.9	.05

[a] $(HPO_4^-) = 0.125\ m$. [b] $(HPO_4^-) = 3.06 \times 10^{-2}\ m$ containing 0.90 *m* $NaNO_3$.

instance, k_2 for histidine is about one hundredth of that for tyrosine. It is of interest to note that in the iodination of certain proteins, such as insulin[10] and pituitary lactogenic hormone,[11] the I_2 reacts only with the tyrosine residue although the protein contains both tyrosine and histidine. If the iodine is in excess and the solution is allowed to react longer, the histidine residue in the protein may take up iodine as well as the phenolic groups of the molecule.

Experimental

The crystalline l-histidine monohydrochloride (Eastman Kodak Co.) was used without further purification. Citrate and phosphate buffers were made according to Clark.[12] A weighed amount of histidine in 20 cc. of buffer was mixed with 20 cc. of iodine buffer solution containing the known concentration of potassium iodide in a glass-stoppered flask at 25.0 ± 0.02° thermostat, after both histidine and iodine solutions had attained the temperature of the thermostat. The time of mixing was taken as 0 time. The disappearance of iodine during the reaction was followed at known time intervals by pipetting 5 cc. of solution into 10 cc. of 1.0 *m* hydrochloric acid, which stopped the reaction. The excess of iodine was titrated with 0.010 *N* thiosulfate solution using starch solution as indicator.

Summary

The reaction between iodine and histidine to form diiodo-histidine is evident from the kinetic data; the kinetics of its formation is similar to that found in diiodo-tyrosine formation. At 25° in citrate buffer the rate of diiodo-histidine formation follows the expression: rate = 2.5 (histidine) $(I_2) + 1.2 \times 10^5$ (histidine) (HOI).

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(9) L. F. Fieser, in H. Gilman, "Organic Chemistry," II, 2nd Edition, 1943, pp. 174–202.

(10) C. R. Harrington and A. Neuberger, *Biochem. J.*, 30, 810 (1936).

(11) C. H. Li, W. R. Lyons and H. M. Evans, *J. Biol. Chem.*, 43, 139 (1941).

(12) W. M. Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Maryland, 1922.