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THE SUBSTITUTION REACTION OF HISTIDINE AND SOME OTHER IMIDAZOLE DERIVATIVES WITH IODINE[1]

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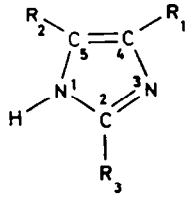
INTRODUCTION

DURING an exploratory investigation into the iodination of histidine and some related compounds[2] carried out several years ago in connection with a study on the mode of action of chymotrypsin[3–5] it was found that this reaction shows many curious features. The results of a more thorough investigation into the reactivity of various imidazole derivatives towards iodine are presented in this paper.

The first kinetic study of the iodination of histidine was reported by Li[6] who presumed diiodohistidine to be the final product of reaction. According to Roche,[7,8] monoiodohistidine is an intermediate in substantial concentration in ammoniacal medium, while diiodohistidine consumes iodine under these conditions.

Brouwer *et al.*[2] observed that in neutral medium diiodohistidine reacts with iodine twice as fast as histidine. Phosphate was found to accelerate the reaction considerably, a phenomenon reported before by Li.[6] A comparative investigation with some imidazole derivatives showed that this effect of phosphate is rather specific and, furthermore, that imidazole and 2-methyl-imidazole consume far less iodine than histidine. It was suggested that the different behaviour of histidine might be caused by intramolecular interaction.

Data on the reaction of imidazole with iodine have been given by Ridd,[9] Grimison[10,11] and recently by Vaughan *et al.*[12,13] The kinetic equations derived by



[1] A detailed report of this investigation is given in L. Schutte, Thesis, Leiden (1964).
[2] D. M. Brouwer, M. J. van der Vlugt and E. Havinga, *Proc. Kon. Acad. Wet.* **B62**, 93 (1959).
[3] D. M. Brouwer, Thesis, Leiden (1957).
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[6] C. H. Li, *J. Amer. Chem. Soc.* **66**, 225 (1944).
[7] J. Roche, S. Lissitzky, O. Michel and R. Michel, *Biochim. Biophys. Acta* **7**, 439 (1951).
[8] J. Roche, S. Lissitzky, O. Michel and R. Michel, *Ann. Pharm. Fr.* **9**, 163 (1951).
[9] J. H. Ridd, *J. Chem. Soc.* 1238 (1955).
[10] A. Grimison and J. H. Ridd, *Proc. Chem. Soc.* 256 (1958).
[11] A. Grimison and J. H. Ridd, *J. Chem. Soc.* 3019 (1959).
[12] J. D. Vaughan and V. L. Vaughan, *Symposium Am. Chem. Soc. Div. Org. Chem.* 15Q, 29 (1964).
[13] J. D. Vaughan, D. G. Lambert and V. L. Vaughan, *J. Amer. Chem. Soc.* **86**, 2857 (1964).

these authors show a suggestive resemblance to those of the iodination of phenols.[14,15]

The present study deals with the kinetics of the substitution by iodine of the following imidazole derivatives (the abbreviations will be used throughout this paper):

1. Im	= Imidazole	$R_1 = R_2 = R_3 = H$		
2. ILA	= Imidazole lactic acid	$R_1 = -CH_2-CHOH-COOH$	$R_2 = R_3 = H$
3. BH	= Benzoylhistidine	$R_1 = -CH_2-CH(NHCOC_6H_5)-COOH$	$R_2 = R_3 = H$
4. Nor	= Norhistidine	$R_1 = -CHNH_2-COOH$	$R_2 = R_3 = H$
5. His	= Histidine	$R_1 = -CH_2-CHNH_2-COOH$	$R_2 = R_3 = H$
6. Ham	= Histamine	$R_1 = -CH_2-CH_2NH_2$	$R_2 = R_3 = H$
7. Hes	= Histidine methylester	$R_1 = -CH_2-CHNH_2-COOCH_3$	$R_2 = R_3 = H$
8. Homo	= Homohistidine	$R_1 = -CH_2-CH_2-CHNH_2-COOH$	$R_2 = R_3 = H$
9. MIH	= Monoiodohistidine	$R_1 = -CH_2-CHNH_2-COOH$	$R_2 = I\ R_3 = H$
10. DIH	= Diiodohistidine	$R_1 = -CH_2-CHNH_2-COOH$	$R_2 = R_3 = I$

In order to obtain information essential to the mechanistic aspects of the reactions, pK values of the dissociating groups of the compounds have been determined. Besides, the position of the iodine atom in monoiodohistidine has been established by NMR measurements.

In a following paper a study of the oxidation reaction of histidine with an excess of iodine will be reported.[1,16]

EXPERIMENTAL

Materials. Im (Fluka, purum, mp: 88–90°, lit. 90°), DL-His·HCl·2H_2O (S.A.F. Hoffmann-LaRoche & Co. Ltd), L-His (idem) and DL-His·2HCl (idem) were used without further purification.

ILA (mp: 201–202°, lit.[17] 203–204°) and BH (mp: 245°, lit.[18] 247°) were prepared according to Celander[17] and Gerngross,[18] respectively.

Nor was prepared as described by Stewart.[19] This method was modified in that the Strecker synthesis proceeded via the hydantoin (mp: 223–225° d, lit.[19] 220–254° d). Homo was prepared by Bloemhoff[20] (mp: 239°). Independently, Schneider[21] synthesized Homo·HCl following the same reaction path.

The preparation of Hes was carried out following the method of Boisonnas c.s.[22] (mp: 196–197°, lit.[23] 196°).

MIH was prepared according to Brunings.[24] The free base was obtained by dissolving the product in dilute ammonia. After treatment with charcoal and hyflosupercell air was transmitted at 50° under diminished pressure, until a white product appeared (mp: 208–210° d). (Found: C, 25·6; H, 2·9; N, 14·9. calc. for $C_6H_8N_3O_2I$: C, 25·6; H, 3·0; N, 15·0%.)

DIH was also prepared by the procedure of Brunings.[24] The crude product was washed with alcohol and crystallized several times from water. The resulting crystals were dissolved in hot water. LiOH solution was added until pH 5·45 was reached. The water was distilled off and the residue was washed with MeOH. The DIH thus obtained was not perfectly pure, but the impurities did not seem to affect the rate of iodination (mp: 180–182°). (Found: C, 18·3; H, 2·6; N, 9·6. calc for $C_6H_7N_3O_2I_2$: C, 17·7; H, 1·7; N, 10·3%.)

The purity of the compounds was checked by potentiometric titration.

[14] E. Berliner, *J. Amer. Chem. Soc.* **73**, 4307 (1951).
[15] E. Grovenstein and N. S. Apprahamian, *J. Amer. Chem. Soc.* **84**, 212 (1962).
[16] L. Schutte and E. Havinga, to be published.
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[18] O. Gerngross, *Z. Physiol. Chem.* **108**, 50 (1919).
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[24] K. J. Brunings, *J. Amer. Chem. Soc.* **69**, 205 (1947).

p*K Determinations.* The p*K* values of imidazole and its derivatives were determined at room temp and at an ionic strength of 0·5 employing a Radiometer Titrator TTTlb in conjunction with a Titrigraph SBR2c and a glass-calomel electrode GK.2025B. 0·02 mmol of imidazole derivative was dissolved in 10 ml of 0·5M KCl solution. The pH was lowered by addition of a few drops of HCl solution. The titrations were carried out with 0·2N NaOH from a 0·5 ml burette.

NMR *spectra.* Both L-His and DL-MIH, in concentrations of about 1 mole/l, were dissolved in D_2O saturated with Na_2CO_3. The NMR spectra of the freshly prepared solutions were obtained by means of a Varian-A-60-spectrometer (internal reference H_2O).

Kinetic measurements. Standard solutions of boric acid (Merck), borax (idem), KH_2PO_4 (BDH) and Na_2HPO_4 (Merck) were prepared and all adjusted to a constant ionic strength of 0·5 by addition of KCl (Merck). A 0·5M KI solution (Merck) was prepared as well. For each kinetic experiment a borate- or phosphate buffer of the desired pH was composed.

In an erlenmeyer a weighed amount of imidazole derivative ($\sim 10^{-4}$ mole) was dissolved in 2 ml of the KI solution and 7·6 ml of the buffer. Three other batches were prepared in which were varied either the KI concentration, or the buffer concentration—the difference in volume made up by a 0·5M KCl solution—or the weighed quantity of the imidazole derivative. After the solution had been thermostated at 25°, 0·4 ml of I_2 (10^{-6} mole) with a threefold excess of KI were added. The rate of disappearance of the I_2 in these solutions was determined at 3505 Å with a Cary spectrophotometer, type 14, in 1 cm quartz cuvettes. The pH of the solutions was measured with a Radiometer Titrator TTTlb with a scale expander PHA 630Ta and a glass-calomel electrode, GK. 2025B. Standard was a Titrisol Buffer pH 7.

Except for His, Ham, Hes and MIH, the I_2 disappearance follows first order kinetics up to 90% conversion. With His, Ham, Hes and MIH a zero order reaction in iodine competes with the first order reaction.

RESULTS

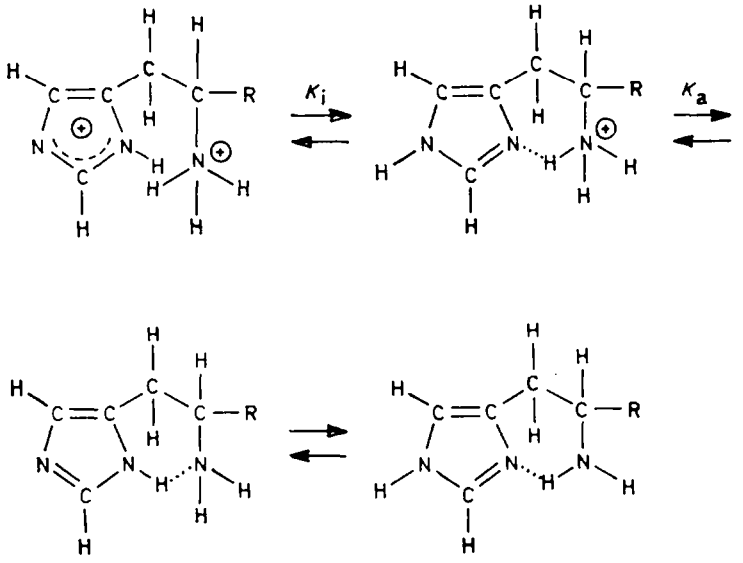
p*K Values.* The p*K* values at an ionic strength of 0·5 of the imidazole group (pK_i) and of the ammonium group in the side chain (pK_a) are presented in Table 1.

TABLE 1. THE p*K* VALUES OF THE IMIDAZOLIUM GROUPS (pK_i) AND OF THE AMMONIUM GROUPS (pK_a) OF SOME IMIDAZOLE DERIVATIVES AT AN IONIC STRENGTH OF 0·5

	pK_i	pK_a
Imidazole	7·0[0]	—
Imidazole lactic acid	7·2[2]	—
Benzoylhistidine	7·0[0]	—
Norhistidine	4·6[9]	8·8[7]
Histidine	6·1[5]	9·0[2]
Histamine	6·3[8]	9·9[2]
Histidine methylester	5·4[0]	7·3[6]
Homohistidine	7·0[2]	9·2[8]
Monoiodohistidine	4·2[5]	8·6[0]
Diiodohistidine	3·2[9]	8·1[0]

From IR absorption spectra it is known that in imidazole derivatives bearing a side chain with a functional group intramolecular hydrogen bonds can be formed.[25] Brunings[24] infers from the p*K* values of His, MIH and DIH the existence of such an interaction in solution. The lower value of pK_i of His, Ham and Hes as compared with the p*K* of imidazole and of pK_a as compared with the p*K* values of alanine (9·8), ethylamine (10·8) and alanine methylester (7·9) may also be explained by

[25] A. Lukton, *Nature, Lond.* **192,** 422 (1961).



Scheme 1. The occurrence of hydrogen bonds in histidine derivatives.

the occurrence of intramolecular hydrogen bonds between the imidazole ring and the ammonium group in the side chain (Fig. 1).

The low pK values of Nor, however, are more likely caused by an inductive effect of the nearby ammonium group and the imidazole ring, respectively. The pK values of Homo seem to be less affected. Intramolecular hydrogen bonding may be expected to be less important as this would lead to a seven membered ring.

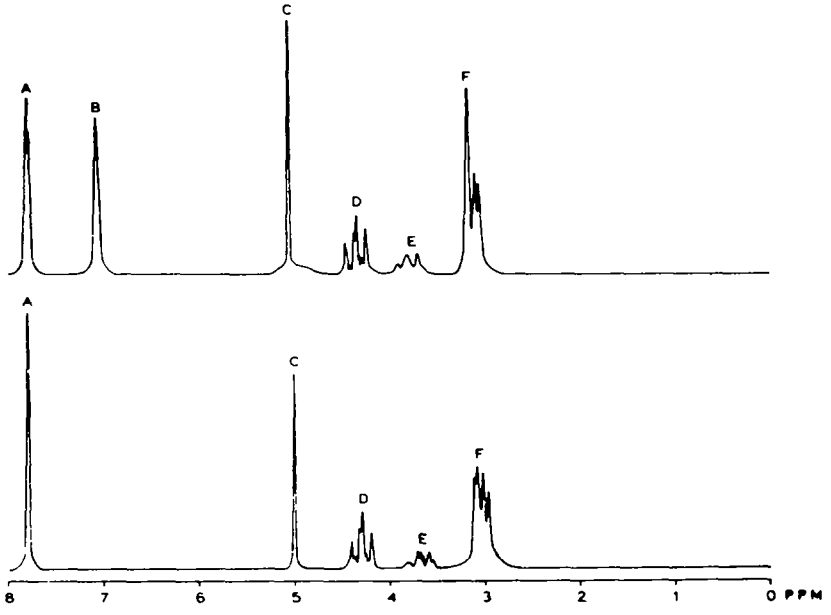


FIG. 1. NMR spectra of His (top) and of MIH (below) in an alkaline D_2O solution. A is due to: H-2, B: H-4, C: H_2O, D: α-CH, E: NH_2 and F: β-CH_2.

Determination of the position in histidine where the first substitution by iodine occurs. A comparison of the NMR spectrum of the MIH synthesized with that of His shows the complete disappearance of the imidazole-4-(or 5-)H signal (Fig. 1). Therefore, it seems reasonable to assume that under the conditions applied iodine attacks initially on the 4-(or 5-)position of the ring of histidine as in the case of imidazole.[10] The NMR spectra of imidazole and of 4-(or 5-)methyl imidazole have been reported.[26,27]

Rate laws. The experimental data of the iodinations of the various imidazole derivatives can be described by the following kinetic equations:

1. $$-\frac{d[I_3^-]}{dt} = k_1 \frac{[Im_0][I_3^-]}{[H^+]([I^-]^2 + a[I^-])} + k_2 \frac{[Im_0][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

2. $$-\frac{d[I_3^-]}{dt} = k_1 \frac{[ILA][I_3^-]}{[H^+]([I^-]^2 + a[I^-])} + k_2 \frac{[ILA][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

3. $$-\frac{d[I_3^-]}{dt} = k_1 \frac{[BH^-][I_3^-]}{[H^+]([I^-]^2 + a[I^-])} + k_2 \frac{[BH^-][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

4. $$-\frac{d[I_3^-]}{dt} = k_1' \frac{[Nor^-][I_3^-]}{[H^+]([I^-]^2 + a[I^-])} + k_2 \frac{[Nor_0][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

5. $$-\frac{d[I_3^-]}{dt} = k_0 \frac{[His_0]}{[H^+]} + k_1' \frac{[His^-][I_3^-]}{[H^+]([I^-]^2 + a[I^-])} + k_2 \frac{[His_0][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

6. $$-\frac{d[I_3^-]}{dt} = k_0 \frac{[Ham^+]}{[H^+]} + k_1' \frac{[Ham_0][I_3^-]}{[H^+][I^-]^2} + k_2 \frac{[Ham^+][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

7. $$-\frac{d[I_3^-]}{dt} = k_0 \frac{[Hes^+]}{[H^+]} + k_1' \frac{[Hes_0][I_3^-]}{[H^+]([I^-]^2 + a[I^-])} + k_2' \frac{[Hes_0][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

8. $$-\frac{d[I_3^-]}{dt} = k_1 \frac{[Homo_0][I_3^-]}{[H^+][I^-]^2} + k_1' \frac{[Homo^-][I_3^-]}{[H^+][I^-]^2}$$

9. $$-\frac{d[I_3^-]}{dt} = k_0 \frac{[MIH_0]}{[H^+]} + k_1' \frac{[MIH^-][I_3^-]}{[H^+][I^-]^2} + k_2 \frac{[MIH_0][HPO_4^{2-}][I_3^-]}{[H^+][I^-]^2}$$

10. $$-\frac{d[I_3^-]}{dt} = k_1 \frac{[DIH^-][I_3^-]}{[I^-]^2} + k_1' \frac{[DIH^{2-}][I_3^-]}{[I^-]^2}$$

The numerical values of the constants in these equations are summarized in Table 2.

The concentration of the species with a neutral imidazole nucleus ([X]) in Im, BH and ILA was calculated from the expression: $[X] = (X_t)/\{10^{pK_t - pH} + 1\}$.

In the imidazole derivatives with an ammonium group in the side chain the concentration of the species where also the ammonium group is dissociated was calculated from the expression:

$$[X] = (X_t)/\{10^{pK_t + pK_a - 2pH} + 10^{pK_a - pH} + 1\}.$$

The total charge of the species is denoted by an index. Except for DIH^{2-}—the pK of the dissociation of the imino proton of the imidazole ring in DIH was measured to be 9·45 at an ionic strength of 0·5—this index corresponds with the charge of the side chain.

[26] N. Joop and H. Zimmerman; *Z. Elektrochem.* **66**, 541 (1962).

[27] G. S. Reddy, R. T. Hobgood and J. H. Goldstein; *J. Amer. Chem. Soc.* **84**, 336 (1962).

TABLE 2. NUMERICAL VALUES OF THE REACTION CONSTANTS AT 25° AND IONIC STRENGTH 0·5. k_0 (IN mol . l^{-1} . sec^{-1}) REFERS TO THE PSEUDO ZERO ORDER REACTIONS; k_1 (IN mol^2 . l^{-2} . sec^{-1} EXCEPT FOR DIH) TO THE WATER-CATALYSED AND k_2 (IN mol . l^{-1} . sec^{-1}) TO THE PHOSPHATE-CATALYSED REACTIONS. k' REFERS TO THE REACTION OF AN IMIDAZOLE DERIVATIVE WITH AN UNPROTONATED AMMONIUM GROUP IN THE SIDE CHAIN. a IS EXPRESSED IN mol . l^{-1}

	k_0	k_1	k_1'	k_2	k_2'	a
1. Im	—		$2{\cdot}7 \cdot 10^{-13}$		$1{\cdot}8 \cdot 10^{-11}$	0·006
2. ILA	—		$1{\cdot}4 \cdot 10^{-11}$		$1{\cdot}2 \cdot 10^{-10}$	0·008
3. BH	—		$1{\cdot}9 \cdot 10^{-11}$		$1{\cdot}5 \cdot 10^{-10}$	0·007
4. Nor	—	—	$7{\cdot}4 \cdot 10^{-13}$	$2{\cdot}7 \cdot 10^{-11}$	?†	0·017
5. His	$5{\cdot}6 \cdot 10^{-14}$	—	$5{\cdot}0 \cdot 10^{-10}$	$4{\cdot}2 \cdot 10^{-10}$	?†	0·015
6. Ham	$4{\cdot}5 \cdot 10^{-14}$	—	$9{\cdot}0 \cdot 10^{-10}$	$7{\cdot}5 \cdot 10^{-10}$	?†	—
7. Hes	$12{\cdot}3 \cdot 10^{-14}$	—	$6{\cdot}9 \cdot 10^{-11}$	?†	$1{\cdot}5 \cdot 10^{-9}$	0·003
8. Homo	—	$5{\cdot}0 \cdot 10^{-10}$	$2{\cdot}0 \cdot 10^{-8}$	?†	?†	—
9. MIH	$> 10^{-13}$*	—	$1{\cdot}9 \cdot 10^{-10}$	$2{\cdot}8 \cdot 10^{-10}$	?†	—
10. DIH	—	$2{\cdot}5 \cdot 10^{-2}$	7·0	—	—	—

* This zero order reaction could be clearly observed, but the numerical value could only be estimated.

† These constants could not be evaluated, since the corresponding reaction is very slow as compared to the competing reactions.

As the pK of the equilibrium $H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$ was measured to be 6·70 at an ionic strength of 0·5, the concentration of biphosphate was calculated from the expression:

$$[HPO_4^{2-}] = (\text{Phosphate}_t) \cdot F/\{10^{6.70-pH} + 1\} \cdot 10$$

in which F is the amount (in ml) of phosphate buffer added.

As an illustrative example the data of the iodination of histidine are given below.*

In the case of the iodination of His (as for Ham and Hes) a zero order reaction in iodine competes with the first order reaction. The kinetics can be expressed in the following equation:

$$-[dI_3^-]/dt = (k_0)_{obs} + (k_E)_{obs}[I_3^-],$$

or

$$-\,d \log \frac{I_o}{I} \Big/ dt = \varepsilon(k_0)_{obs} + (k_E)_{obs} \log \frac{I_0}{I}$$

(ε is the molecular extinction of I_3^- at 3505 Å). By plotting $-\,d \log (I_0/I)/dt$ against $\log (I_0/I)$ a

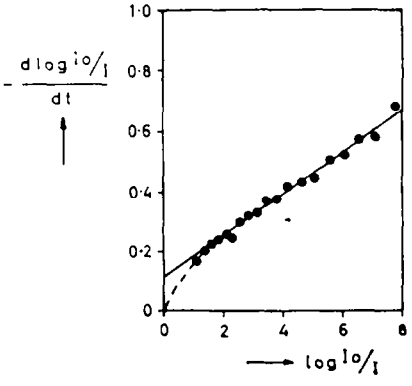


FIG. 2. The determination of $(k_E)_{obs}$ and $(k_0)_{obs}$.

* Details of the kinetic measurements at 25° and at an ionic strength of 0·5 of the respective imidazole derivatives are to be found in the thesis of one of us.[1]

TABLE 3. THE IODINATION OF HISTIDINE AT 25° AND IONIC STRENGTH 0·5. THE CONCENTRATIONS ARE EXPRESSED IN mol/l. $(k_E)_{obs}$, k_0 AND k_{calc} ARE EXPRESSED IN SECONDS^{-1}, $(k_0)_{obs}$ IN mol . l^{-1}. sec^{-1}. B ARE THE SOLUTIONS BUFFERED BY BORATE. THE KINETIC RUN MARKED WITH* IS REPRESENTED IN FIG. 2

$H^+ . 10^7$	$His^- . 10^4$	I^-	$HPO_4^{2-} . 10^3$	$(k_E)_{obs} . 10^3$	$(k_0)_{obs} . 10^5$	$k_0 . 10^5$	$k_{calc} . 10^3$
0·316	2·81	0·050	B	1·27	1·61	5·7	1·40
0·398	2·20	0·050	B	0·90	1·37	6·3	0·87
0·502	1·78	0·050	B	0·58	0·97	5·5	0·56*
0·630	1·37	0·050	B	0·36	0·81	5·9	0·34
0·331	2·68	0·050	B	1·29	2·42	9·0	1·27
0·331	2·68	0·075	B	0·64	1·92	7·2	0·65
0·331	2·68	0·100	B	0·52	1·41	5·3	0·37
0·331	2·68	0·125	B	0·33	1·16	4·3	0·24
0·512	1·76	0·100	B	0·15	0·90	5·1	0·16
0·407	2·16	0·100	B	0·25	1·18	5·5	0·24
0·331	2·68	0·100	B	0·44	1·15	4·3	0·37
0·126	6·94	0·100	B	2·61	3·52	5·1	2·51
0·126	10·40	0·100	B	3·25	4·73	4·5	3·77
0·126	6·94	0·100	B	2·40	3·87	5·6	2·51
0·126	3·47	0·100	B	1·07	3·02	8·7	1·26
0·513	1·74	0·050	6·09	2·34			2·47
0·513	1·74	0·050	7·18	2·57			2·80
0·513	1·74	0·050	8·26	2·92			3·19
0·513	1·74	0·050	9·35	3·12			3·47
0·260	3·47	0·050	6·76	6·76			6·50
0·260	3·47	0·050	7·97	7·81			7·23
0·260	3·47	0·050	9·19	8·31			7·98
0·260	3·47	0·050	10·40	9·85			8·72
0·913	0·91	0·050	5·24	1·05			1·02
0·913	0·91	0·050	6·17	1·19			1·17
0·913	0·91	0·050	7·11	1·30			1·32
0·913	0·91	0·050	8·04	1·33			1·47
0·550	1·59	0·050	2·78	1·42			1·29
0·550	1·59	0·050	4·93	1·86			1·89
0·550	1·59	0·050	7·07	2·42			2·49
0·550	1·59	0·050	9·21	3·00			3·09
3·31	0·19	0·050	3·90	0·148			0·144
1·55	0·50	0·050	5·83	0·531			0·571
0·960	0·87	0·050	7·03	1·085			1·245
0·560	1·55	0·050	8·10	2·45			2·72
0·520	1·28	0·050	9·30	2·56			2·52
0·520	1·70	0·050	9·30	3·14			3·35
0·520	2·02	0·050	9·30	4·04			4·00
0·520	2·55	0·050	9·30	5·36			5·04
1·097	0·75	0·050	2·29	0·41			0·42
1·258	0·64	0·050	2·16	0·36			0·33
1·430	0·56	0·050	2·07	0·33			0·27
1·396	0·57	0·050	2·09	0·32			0·28
0·398	2·21	0·083	8·64	1·78			1·58
0·389	2·27	0·067	8·70	2·75			2·52
0·389	2·27	0·050	8·70	4·65			4·44
0·912	0·91	0·100	7·10	0·362			0·334
0·912	0·91	0·075	7·10	0·631			0·588
0·912	0·91	0·050	7·10	1·27			1·32
0·912	0·91	0·025	7·10	4·95			5·24

straight line was obtained from which $(k_E)_{obs}$ and $(k_0)_{obs}$ could be computed (Fig. 2). (When the I_3^- concentration becomes small, this line bends towards the origin.)

This procedure could only be performed with some accuracy in the borate buffered solutions as the catalysis by phosphate ions predominates over the zero order reaction, thus making it negligible.

The kinetic data are summarized in Table 3. The zero order reaction in iodine appears to be first order in His^-.

$$(k_0)_{obs} = 5{\cdot}9 \,.\, 10^{-5}[His^-],$$

or as

$$[His^-] = 10^{-9{\cdot}02}[His_0]/[H^+],$$

$$(k_0)_{obs} = 5{\cdot}6 \,.\, 10^{-14}[His_0]/[H^+].$$

In the case of $[I^-] = 0{\cdot}050$ mol . l^{-1} the first order reaction in iodine obeys the equation:

$$(k_E)_{obs} = 1{\cdot}57 \,.\, 10^{-7}\frac{[His]}{[H^+]} + 1{\cdot}77 \,.\, 10^{3}[His^-][HPO_4^{2-}], \text{ (Fig. 4).}$$

If the iodide concentration is taken into account, the rate of iodination of His at 25° and at an ionic strength of 0·5 can be represented by the expression:

$$-\frac{d[I_3^-]}{dt} = 5{\cdot}6 \,.\, 10^{-14}\frac{[His_0]}{[H^+]} + 5{\cdot}0 \,.\, 10^{-10}\frac{[His^-][I_3^-]}{[H^+]([I^-]^2 + 0{\cdot}015[I^-])} + 4{\cdot}2 \,.\, 10^{-10}\frac{[His_0][I_3^-][HPO_4^{2-}]}{[H^+][I^-]^2}$$

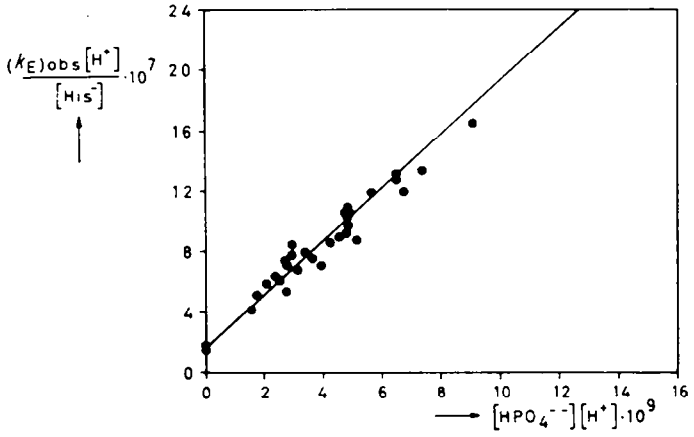


FIG. 3. Histidine: the determination of k_1' and k_2 at $[I^-] = 0{\cdot}050$ mol . l^{-1}.

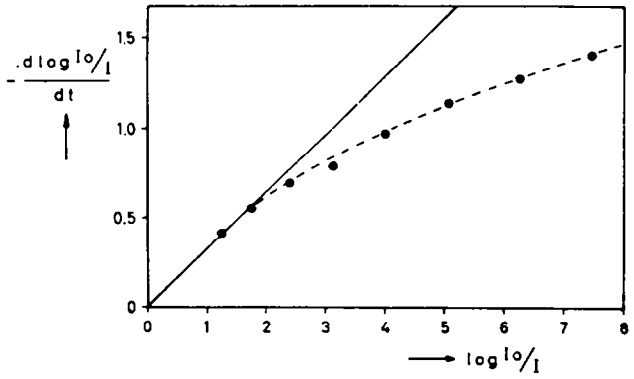


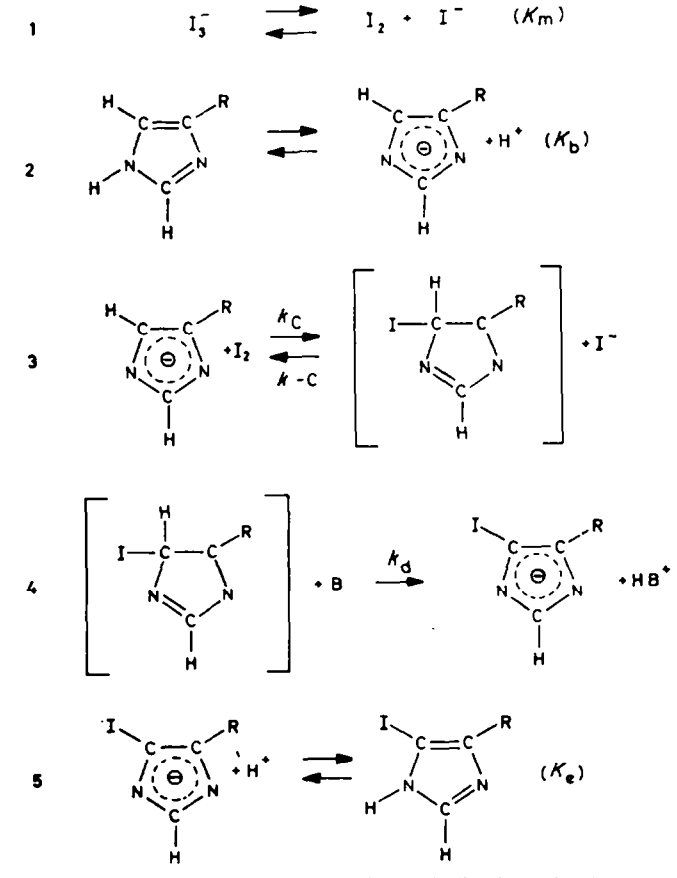
FIG. 4. Monoiodohistidine: the determination of k_{obs}.

During the iodination of MIH the first order and zero order reactions could not be distinguished, as the zero order reaction probably is followed by a first order reaction, that is partly rate determining in this case (Fig. 4). The first order constant of each kinetic run, k_{obs}, was obtained from the tangent from the origin to the curve in figure 4; k_{obs} comprises all first order reactions including the reaction following the zero order reaction (cf. scheme 3).

During the iodination of DIH a brown color slowly appeared in the solution, probably due to degradation of the direct product of iodination. Up to 50% conversion the reaction followed first order kinetics; at longer periods the brown color interfered by raising the extinctions.

DISCUSSION

The kinetic equations derived for the iodination of various imidazole derivatives are consistent with a mechanism as presented in scheme 2. The representation includes the electrophilic attack by molecular iodine on an anionic imidazole ring system, followed by proton abstraction from the sigma complex as the rate determining step (4). Other authors[11,13,15,28,29] suggest a similar mechanism for the iodination of aromatic compounds in aqueous solutions. Of course, other steps and sequences leading to the same transition state as supposed in step (4) may be envisaged as well.



Scheme 2. Possible mechanism of the substitution of iodine in an imidazole ring system.

[28] W. E. Mayberry, J. E. Rall and D. Bertoli, *J. Amer. Chem. Soc.* **86**, 5302 (1964).
[29] W. E. Mayberry and D. Bertoli, *J. Org. Chem.* **30**, 2029 (1965).

On the basis of the scheme represented the kinetics would be

$$-\frac{d[I_3^-]}{dt} = K_m K_b \frac{k_c k_d}{k_{-c}} \frac{[Im_0][I_3^-][B]}{[H^+]([I^-]^2 + k_d/k_{-c}[I^-][B])},$$

where $k_d/k_{-c}[B] = a^*$ and B is water or HPO_4^{2-}.

Thinking in terms of scheme 2 it has to be assumed that the reactivity of the dissociated imidazole system ($pK = 10^{-14}$)[30] exceeds that of the neutral imidazole ring by at least a factor 10^8. In His_0, Ham^+, Hes^+ and MIH_0 the ammonium group in the side chain forms a hydrogen bond with the imidazole nucleus (cf. Table 1). In spite of the fact that this hydrogen bond may facilitate the dissociation of the imidazole nucleus (K_b), it will decrease the overall reactivity because of its desactivating influence on the imidazole anion. The existence of the intramolecular hydrogen bond probably may account for the fact that species with a protonated ammonium group do not occur in the "noncatalysed" terms of the kinetic equations for these compounds. For Nor_0 a similar reasoning may hold on the basis of an inductive effect of the ammonium group at the carbon atom directly attached to the imidazole ring.

It is somewhat surprising that the "phosphate" terms of the kinetic equations do contain the species with a protonated ammonium group. This may be due to either a rupture and compensation of the intramolecular hydrogen bond by phosphate ions or to a more effective path of iodination opened by the phosphate ions, which also may be followed by the less reactive hydrogen-bonded imidazole nuclei. These findings and those reported by Vaughan *et al.*[13] would point to a specific function of phosphate ions in aromatic iodination. Some speculations as to the mechanism are to be found in loc. cit.[1] Further experiments with other basic agents are being carried out (cf. also[28,29]). The exceptional position of Hes may be connected with the low pK value of its α-ammonium group.

The iodination of Homo proceeds with high velocity (k_1 in Table 3). This can be attributed to the conformational ease for the α-amino group of this compound to function as an intramolecular proton acceptor, thus accelerating the deprotonation of the sigma complex. The numerical values of k_1 (Table 3), exceeding those of ILA and BH and following the sequence of the basicities of the amino groups in the side chain, suggest that in the case of His, Ham and Hes this intramolecular catalysis also takes place, though to a much smaller degree. (The higher k_1 values of ILA and BH as compared with that of Im may be brought about by the inductive effect of the side chain. The ratio of k_1 for the iodination of 2-methylimidazole and imidazole is reported to be about ten[12].)

The zero order term (k_0) appearing in the kinetic Eqs 5, 6, 7 and 9 may be accounted for by the rate determining formation of a reactive species (Fig. 5). In this species the imidazole anion can be regarded as a tautomeric form. (As the negative charge density in neutral imidazole is reported to be less at C-2 than at C-4,[31] C—H-dissociation seems more likely to occur at C-2. This would mean that the position of substitution may be different for the "first order" and for the "zero order" reaction.) As

* As pointed out by Mayberry *et al.*[28,29] the broken order in I^- in the denominator may arise from the equilibria $I_3^- \rightleftharpoons I_2 + I^-$ and $I_2Cl^- \rightleftharpoons I_2 + Cl^-$. The numerical value of a, however, if only accounting for these equilibria would not exceed 0·0025 under our conditions.

[30] H. Walba and R. W. Isensee, *J. Org. Chem.* **21**, 702 (1956).

[31] H. Hamano and H. F. Hameka, *Tetrahedron* **18**, 985 (1962).

its negative charge is localized on a carbon atom, a great reactivity towards iodine can be expected. Since this zero order reaction is found only in the case of His, Ham, Hes and MIH the formation of this "tautomer" must be favoured by the ammonium group in the side chain. Independent indications for this "tautomerization" may be found by a kinetic investigation into the iodination of 2-methylhistidine and of the study of H—D exchange in alkaline D_2O.

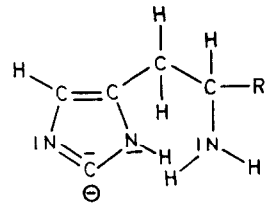
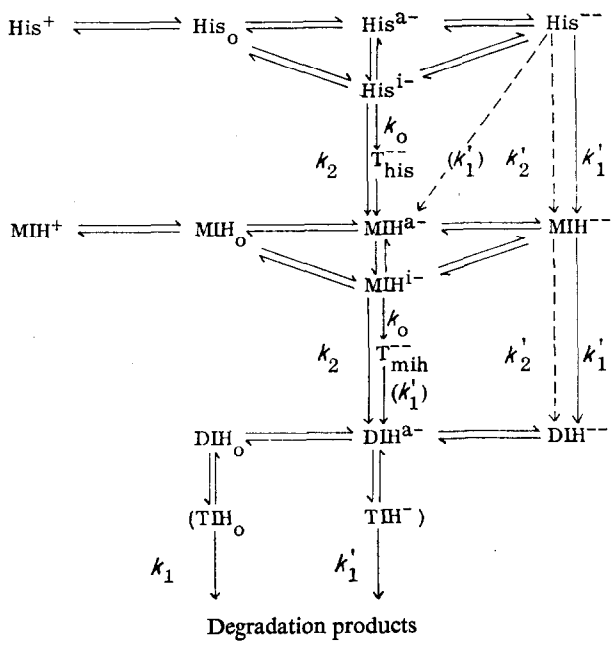


FIG. 5. A "tautomeric form" of the anionic imidazole ring, capable of rapid reaction with iodine.

The reaction of DIH with iodine proceeds partly through the single negative species DIH^- and partly through a doubly negative species DIH^{2-}. It seems implausible that triiodohistidine with an iodine atom on the imino nitrogen is the product



SCHEME 3. A schematic representation of the reaction with iodine of histidine (His) via monoiodohistidine (MIH), diiodohistidine (DIH) and possibly triiodohistidine (TIH) to degradation products. His^{a-} represents the histidine-molecule with a neutral imidazole ring and an unprotonated α-ammonium group (alanine radical is negative); His^{i-} is the histidine-molecule with a negative imidazole ring and a protonated ammonium group in the side chain. In His^{2-} both the ring and the side chain are negatively charged. T^{2-}_{his} represents the high energy "tautomer" of histidine (cf. Fig. 5). The rate constants k_0, k_1, k_1', k_2 and k_2' correspond with those of Table 2.

of reaction. The occurrence of a triiodo-compound has not been demonstrated for any of the imidazole derivatives, except for imidazole itself.[32] Also the kinetic Eq. 10 probably does not describe a substitution reaction. Since rate determining proton abstraction is not to be expected in the case of the iodination of an imino nitrogen atom, the reaction with molecular iodine should require a different iodide dependence ($1/[I^-]$ instead of $1/[I^-]^2$). The assumption seems fair that the kinetic equation refers to the formation of an oxidation product which decomposes to the brown degradation products observed in the course of reaction. This oxidation reaction will be dealt with in a subsequent paper.[16]

Scheme 3 summarizes schematically the reaction of histidine with iodine as it emerges from the experimental data and the discussion.

[32] H. Pauly, *Chem. Ber.* **43**, 2243 (1910).