Elimination–Addition Mechanisms of Acyl Group Transfer: Transcarbamoylation in Aminoalkylimidazoles carbamoylated on the Heterocyclic Nitrogen

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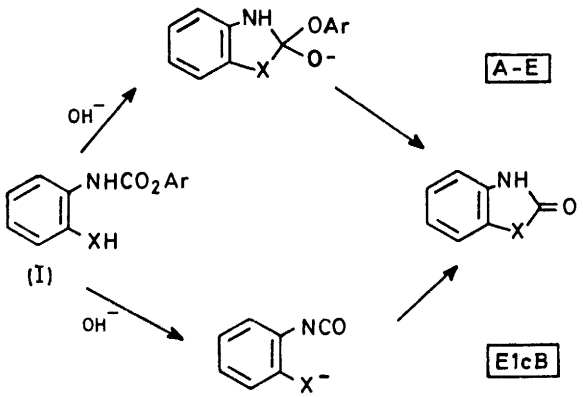
Histamine and histidine are carbamoylated on the ring nitrogen (probably N^{π}) in aqueous solutions of cyanic acid from pH 3 to 11. A further reaction occurs where the carbamoyl group is transferred from the ring to the amino nitrogen to form a urea; the major part of the reaction flux for this reaction passes through an intermolecular E–A process involving cyanate ion as an intermediate and not through intramolecular nucleophilic attack by amine on the N^{π}-carbamoylimidazolyl function.

WORK of Fife [1] and of Hegarty [2] has shown that intramolecular transcarbamoylations *via* a nucleophilic substitution mechanism (A–E) † appear to be favoured over the corresponding *E*1cB process (Scheme 1); at



SCHEME 1 X = CO_2, NH_2, O

alkaline pH values the *E*1cB mechanism predominates because the intramolecular nucleophilic path is pH-independent in these media whereas the *E*1cB pathway is proportional to hydroxide ion concentration. There are only three [1,2] well-studied examples where an intramolecular A–E path could compete with an E–A (elimination–addition) path and of these only the phenylenediamine derivative (X = NH)[2] possesses an efficient *E*1cB mechanism at neutral pH. We present here a study of a further reaction where there is a choice between E–A or A–E transcarbamoylation; in this case (see Scheme 2) the E–A mechanism is intermolecular as against an intramolecular A–E path.

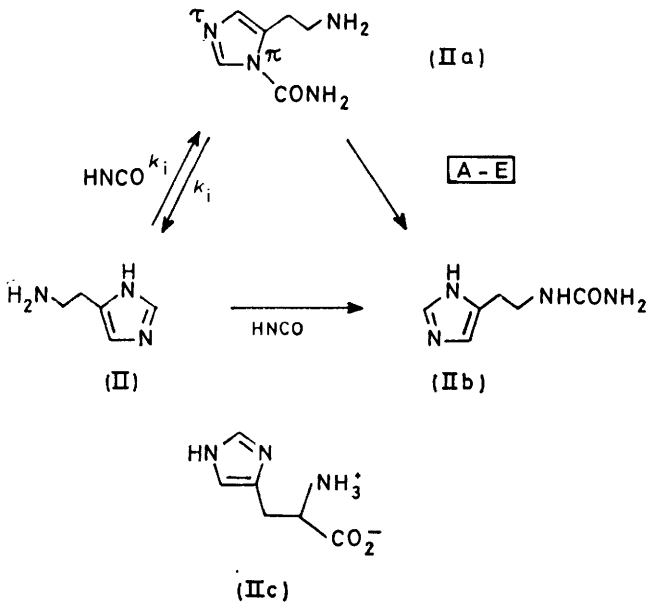
We noted during a study of the reaction of some substituted imidazoles with cyanic acid that the initial carbamoylation on the heterocyclic nitrogen as indicated by an increase in absorption at around 240 nm was followed by a decrease in absorption for those species possessing an amino-function. We trace this decrease to a transcarbamoylation to give a urea (Scheme 2); it is the purpose of this report to describe experiments indicating that this reaction involves an *intermolecular* transcarbamoylation *via* an *E*1cB process rather than the intramolecular A–E mechanism.

† The terms A–E, E–A, and *E*1cB are defined in ref. 3.
‡ 1 λ = 1 microlitre

EXPERIMENTAL

Materials.—Histamine (II) and histidine (IIc) were obtained from Sigma Chemical Co. Potassium cyanate was recrystallised from bench grade material using aqueous ethanol and stored over calcium chloride. Ethanolamine hydrochloride was prepared from the free base by evaporating a solution in hydrochloric acid and recrystallising the product from ethanol. Imidazole was recrystallised from benzene and dried *in vacuo*. Water used throughout the investigation was doubly distilled from glass. Other materials such as buffer components were of analytical reagent grade or were recrystallised or redistilled from bench grade products.

Methods.—Reactions were followed spectrophotometrically by adding an aliquot of reagent (*e.g.* potassium cyanate *ca.* 20 λ‡) to a solution of the appropriate buffer (2.5 ml) in a silica cell in the thermostatted cell compartment of a recording spectrophotometer (Beckman DBG or Unicam SP 800). Repetitive scanning of the spectrum during reaction gave an indication of stoicheiometry and the best wavelength to employ to determine rate constants.



SCHEME 2

Pseudo-first-order rate constants were obtained from plots of $A_t - A_\infty$ *versus* time on semilogarithmic graph paper; infinity readings were taken after approximately 10 half-lives. In the case of reactions exhibiting intermediates the kinetics were treated as for a consecutive reaction [equation (1)].

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (1)$$

The value of k_1 was determined as follows: a plot of log $A_t - A_\infty$ *versus* time was non-linear but became linear as time (t) increased. The slope of the linear portion gave k_2 and extrapolation to time (t) gives an absorption denoted by $A_\infty{}^t$. A plot of $A_t - A_\infty{}^t$ *versus* time on semilogarithmic graph paper gives a straight line the slope of which may be used to determine $k_1.\alpha$

N^α-*Ureidohistamine Picrate.*—This material was prepared by allowing a solution of histamine·2HCl (3 g), KNCO (1.3 g), and $KHCO_3$ (1.6 g) in water (50 ml) to react overnight. The solution was evaporated *in vacuo* and the alcoholic suspension warmed with picric acid (3.5 g). The filtrate was allowed to cool to yield crystals of the picrate which were recrystallised from ethanol: m.p. 151—152 °C (lit. m.p.,[4] 150 °C) (Found: C, 37.2; H, 3.1; N, 24.7. Calc. for $C_6H_{11}N_4O{\cdot}C_6H_2N_3O_7$: C, 37.6; H, 3.4; N, 25.6%); $\nu_{max.}$ (Nujol mull) 3 500, 3 400, 1 610, and 1 630 cm^{-1}; $\delta[(CD_3)_2SO]$ 8.95 (s, 1 H), 8.55 (s, 2 H), 7.4 (s, 1 H), 6.8 (t, 1 H), 5.5br (2 H), 3.24 (q, 2 H), and 2.7 (t, 2 H); the NH^+ signals appeared to be completely decoupled. The yield was 81% theoretical based on recovery of the picrate.

N^α-*Ureidohistidine Picrate.*—This material was prepared *via* the same procedure as for the histamine species: it was recrystallised as the ethanolate from ethanol and had m.p. 120—122 °C (Found: C, 37.3; H, 3.4; N, 21.6. $C_7H_{11}N_4O_3{\cdot}C_6H_2N_3O_7{\cdot}C_2H_5OH$ requires C, 38.1; H, 4.0; N, 20.7%) * $\nu_{max.}$ (Nujol mull) 3 450, 3 500, 1 710, 1 620, and 1 650 cm^{-1}; $\delta[(CD_3)_2SO]$ 8.96 (s, 1 H), 8.56 (s, 2 H), 7.35 (s, 1 H), 6.36 (d, 1 H), 4.50 (q, 1 H), 3.45 (q, 2 H), 3.1 (m, 2 H), and 1.05 (t, 3 H); the NH^+ and CO_2H and OH signals were decoupled but a very broad peak was observed at δ 14 possessing approximately four protons. The yield was 75% of the theoretical based on the recovery of the picrate.

Product Analysis for Transcarbamoylation Experiments.—Identical conditions for the product analyses as for the kinetics were not possible because the concentration of KNCO in the latter was very low. The only difference in conditions was that the KNCO concentration was raised to that of the histamine or histidine nucleophile. Histamine or histidine solution (5 ml; 2M) was treated with KNCO (5 ml; 2M) and the pH maintained for 20 h at 6.55 in a pH-stat (Radiometer of Copenhagen). The solution was then evaporated *in vacuo* and the product isolated with picric acid as described in the preparative method. The yield of picrate salts was 2.8 and 3.9 g of the crude product (dry) which represents a yield of 73 and 82% for the histamine and histidine respectively. I.r. spectra and m.p.s were identical with those from the preparative experiments.

In-situ *Preparation of* N^π-*Carbamoylhistamine and Its Decomposition.*—Histamine·2HCl (0.92 g, 5 mmol) and KNCO (0.4 g, 5 mmol) were dissolved in water (5 ml) and the solution kept for 20 min. The stock solution containing the N^π-carbamoylhistamine was then used in decomposition experiments in selected buffers at 25 °C and 1M-ionic strength at different pH values. The reactions were followed spectrophotometrically at 240 nm using 1-cm path-length cells.

The determination of pK_a values under the conditions of the kinetic experiments was carried out titrimetrically using a Radiometer REC 61/REA 160 recording titration system coupled with a pH-meter PHM 62 and an autoburette ABU 11.

The pH of the buffer in the spectrophotometric cell was measured after reaction using a Pye–Dynacap or a Radiometer PHM 26 instrument calibrated with E.I.L. buffers to ±0.02 units. Buffers at constant total histamine and histidine and imidazole concentration were prepared by adjusting a solution of very nearly the correct volume (containing the appropriate imidazolyl species and salt concentrations) to give on dilution the buffer at correct ionic strength and concentration.

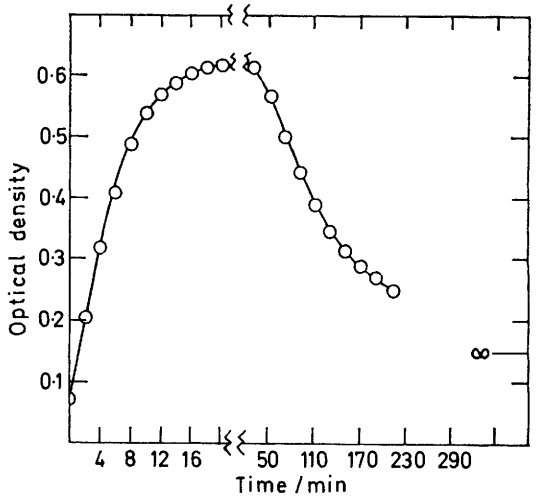


FIGURE 1 The time course of the absorbance at 240 nm for reaction of KNCO (2.4×10^{-3}M) with histamine (0.2M) at pH 6.52, 25 °C, and ionic strength made up to 1M with KCl

Some reactions, especially those of histamine, were followed at concentrations where the u.v. absorption was in excess of 2 optical density units and for these a slightly different technique was employed for the kinetic measurement. An aliquot of KNCO (20 λ) was added to histamine or imidazolyl buffer and the chart recorder initiated. The solution was then introduced into a 1-mm path-length silica cell with a pasteur pipette and the progress of the reaction followed at the appropriate wavelength.

RESULTS

The reaction of histamine and histidine buffers with potassium cyanate exhibit an intermediate (Figure 1) and the kinetics were analysed in terms of a consecutive process [equation (1)]. The intermediate could be a ' blind-alley ' species but this would not affect the kinetics. Since the rate constants for formation of the intermediate are more than 10-fold greater than those of the decay the kinetic analysis is a simple case. The products of the reactions of KNCO with histamine and histidine carried out under preparative-scale conditions similar to those of the kinetics gave negative ninhydrin reactions consistent with the mask-

* No explanation is offered for the poor analysis; the error seems to be instrumental since individual analyses varied for a series of measurements on the homogeneous samples. Spectroscopic data, however, yield the identity and, within the limits of the n.m.r. data, the purity of the salts.

TABLE 1

Reaction of histamine and histidine with KNCO [a,c,f]

pH	$k_1 \times 10^3$/ s^{-1}	k_{-1} [d] $\times 10^3$/ s^{-1}	$k_2 \times 10^5$/ s^{-1} [e]
	pK_a values		
Histamine (0.2M)	6.55, 10.20		
2.56	1.1 [b]		
3.05	2.4 [b]		
3.49	3.0 [b]		
3.82	3.3 [b]		7.1, 16
4.05		1.88	
4.70	3.4		5.6, 12
5.10		1.85	
5.34	3.3		9
5.50		1.75	
6.00	2.9	1.9	1.3, 7.7
6.42		1.80	
6.50	3.3, 2.0		1.8, 1.1,
	3.0		6
6.51	3.1		
6.52	2.6		
7.00		1.83	
7.20	2.6		1.8, 5.7
8.03	1.5		
8.25		1.78	
6.55 (0.1M)		1.80	3.6
6.55 (0.2M)			7.3
Histidine (0.1M)	pK_a values 6.38, 9.40		
5.44	3.3	2.03	10
6.43	2.8	2.13	6
7.50	2.7	2.14	15

[a] Ionic strength maintained at 1M with KCl; 25 °C; KNCO at 1 to 5 $\times 10^{-3}$M; wavelength for kinetics 240 nm. [b] At this pH a correction for spontaneous hydrolysis of the cyanate was necessary (derived from data reported in Supplementary Publication no. SUP 21136 *); values quoted are the corrected ones. [c] Values of k_1, k_{-1}, and K for histamine best fitting the data are 12 l mol^{-1} s^{-1}, 1.8 $\times 10^{-3}$ s^{-1}, and 1.5 $\times 10^{-4}$ mol l^{-1} respectively. The value of k_1 [equation (7b)] for the interaction of the β-amino-group of histamine with isocyanic acid which best fits the data is 4 000 l mol^{-1} s^{-1}. This is somewhat high for an amine of pK_a 10.2 (ethylamine has k_1 = 840 l mol^{-1} s^{-1});[7] we have no explanation for this except to note that the pK_a values 10.2 and 6.55 may not be the correct ones to use in equation (7b). [d] These values are of the decomposition of the N^{π}-carbamoylated species prepared *in situ*. [e] Scatter in k_2 is referred to in the text [f] *N*-Carbamoylimidazole has an absorption maximum at 206 nm and a shoulder at 230 nm (pH 6.00); the corresponding intermediate from the reaction of histamine with KNCO had an absorption maximum at 216 nm under the same conditions.

* For details of the Supplementary Publications scheme see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1978, Index issue.

TABLE 2

Reaction of KNCO with different concentrations of histamine at constant pH [a,c]

[histamine]/M	$k_1 \times 10^3$/s^{-1} [d]
0	1.8 [b]
0.1	1.8.4, 2.16, 2.21
0.2	2.52, 2.12, 2.44
0.3	2.60, 3.00, 2.76
0.4	2.80, 3.30, 3.19

[a] pH 6.55, fraction of histamine as free base (imidazolyl) = 0.5, ionic strength maintained at 1M with NaCl. [b] Value obtained from the decomposition of N^{π}-carbamoylhistamine prepared *in situ* (k_{-1}, see Table 1). [c] The equilibrium constant $K = 1.5 \times 10^{-4}$ [see equation (4)] is derived using pK_a (HNCO) = 3.29 and the values $k_{-1} = 1.8 \times 10^{-3}$ s and $k_1 = 12$ l mol^{-1} s^{-1}; the latter rate constant is derived from the slope of the graph in Figure 2 (see Results section). [d] See the results section for a discussion of the poor reproducibility of the rate constants (k_1).

ing of the α-amino-function by a carbamoyl group. The products were shown to be substantially the corresponding urea by isolation as the picric acid salts and comparison with authentic samples (see Experimental section).

The k_2 values at pH 6.55 were proportional to the concentration of the histamine nucleophile (Table 1). Values of k_1 are linearly dependent on the concentration of the histamine. Table 2 collects the data for k_1 at varying concentrations of histamine at a fraction of base 0.5; the rate constant k_1 is a linear function of the histamine concentration and the intercept (Figure 2) is taken from the decomposition rate constant for N^{π}-carbamoylhistamine (prepared *in situ*) at the same pH. Values of k_1 and k_2 for different pH values are set out in Table 1.

Figure 3 illustrates the pH-dependence of k_1 and Figure 4 that for k_2 for the reaction of histamine at 0.2M with KNCO.

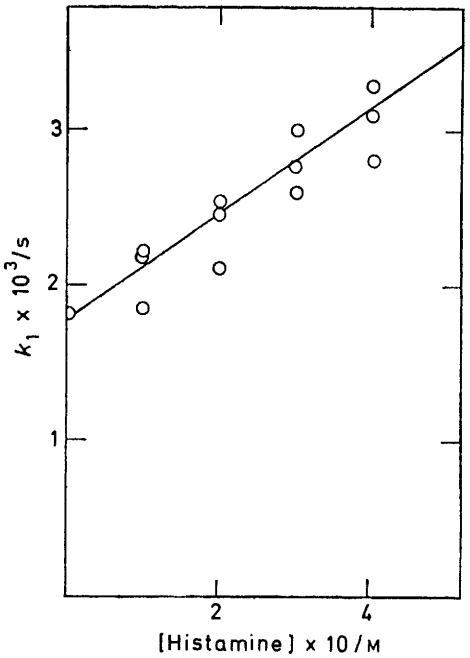


FIGURE 2 The dependence of k_1 for the reaction of histamine with KNCO on the concentration of histamine. Data are taken from Table 2; line is theoretical from parameters given in Table 2

Owing to the complication of the carboxylate group a full pH-profile was not attempted for the histidine species. The rate constant k_1 is corrected for the spontaneous hydrolysis of the cyanate.

Even with histamine a full pH-profile was not easily obtained due to the low equilibrium constant for formation of the N^{π}-carbamoyl species at high pH and the consequent low changes in the u.v. absorption. The histamine experiments were complicated by the large background absorbance in the wavelength region where the reaction was being followed. These considerations give rise to considerable scatter in the repeat rate constants. The apparent pH-independence of k_1 within the limits of the experimental error is probably due to the predominating influence of the decomposition rate constant for the N^{π}-carbamoylhistamine owing to the low formation constant [equation (2)].

In order to check this possibility in an experimentally

$$k_1 = k_{\text{formation}} + k_{\text{decomposition}} \quad (2)$$

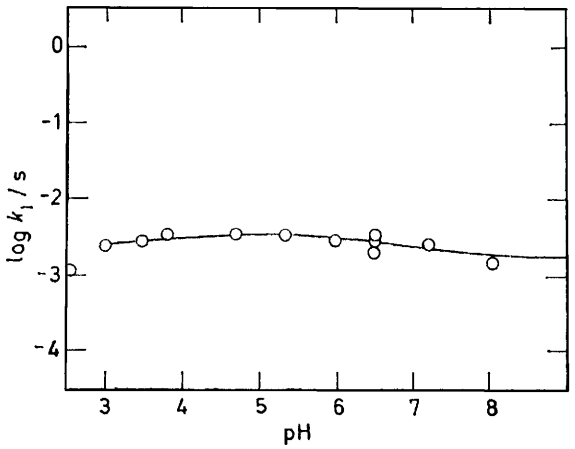


FIGURE 3 Dependence of k_1 for histamine reacting with KNCO on pH; concentration of histamine is 0.2M, 25 °C, ionic strength maintained at 1M with KCl. Line is theoretical from equation (3) using parameters from Table 2; data are from Table 1.

more amenable system we looked at the formation of *N*-carbamoylimidazole at different pH values at a constant imidazole concentration choosing the best wavelength for study at each pH. Reaction of imidazole with KNCO followed good pseudo-first-order kinetics as expected from previous work; the pH-dependence for the reaction at ionic strength 1M and imidazole at 1M is illustrated in Figure 5 and the data are collected in Table 4. The reaction became progressively more difficult to follow as the pH increased and different wavelengths were employed at each pH value. Moreover 1-mm path-length cells were used at high pH values owing to increased background absorbance. As the pH increased the total absorbance change at 240 nm decreased to small values and a plot of this change *versus* pH is illustrated in Figure 5: the plot agrees well with a theoretical line calculated from literature data.[5]

The rate constant for approach to the equilibrium con-

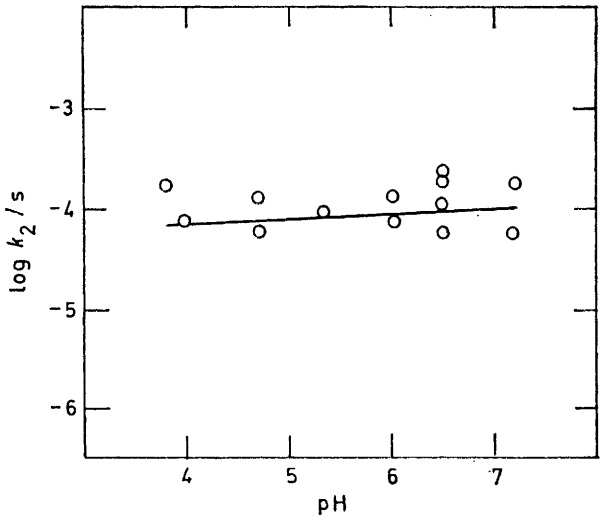


FIGURE 4 Dependence of k_2 on pH for the decomposition of N^{π}-carbamoylhistamine in the presence of histamine buffers (0.2M); 25 °C, ionic strength maintained at 1M with KCl. Data are taken from Table 1; line is theoretical from equation (7b) using parameters from Table 1

TABLE 3

Reaction of imidazole with KNCO in the presence of varying concentrations of ethanolamine [a,b]

[Ethanolamine]/M	$k_1 \times 10^2$/s^{-1}	$A_{250\ nm}$ [c]
0	1.39	0.2
0.05	1.24	0.2
0.1	1.31	0.19
0.15	1.30	0.19
0.20	1.39	0.19

[a] Imidazole concentration kept at 0.2M, pH 7.26, 25 °C, ionic strength maintained at 1M with KCl; KCNO concentration was 2.4×10^{-3}M. [b] Rate constant for the decay of the intermediate at 0.2M-ethanol amine concentration = 3.31×10^{-5} s^{-1}. [c] Absorbance change at pH 6.5 for histamine (0.2M) with KNCO at 2.4×10^{-3}M at 240 nm is 0.62; absorbance change for the same amount of cyanate in a mixture of ethanolamine (0.2M) and imidazole (0.2M) at pH 6.5 is 0.78.

centration of *N*-carbamoylimidazole follows the expression [equation (3)]:

$$k_1 = k_i\,[\mathrm{ImH}][\mathrm{frHNCO}] + k_{-i} \qquad (3)$$

The symbol ImH refers to neutral imidazole and frHNCO to free isocyanic acid. The data agree well with the values

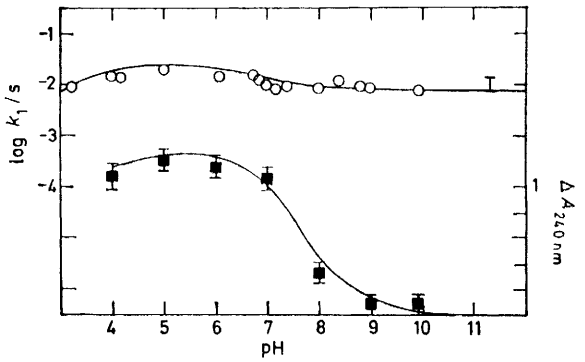


FIGURE 5 Dependence on pH of the reaction between imidazole and KNCO at concentrations 1M and 3.05×10^{-3}M respectively; ○ = rate constant for approach to equilibrium of the system; ■ = total change in absorbance at 240 nm for the reaction. Data are taken from Table 4 and the lines are theoretical from parameters given in previous work [5] as described in the text [equation (3)].

calculated from previously determined parameters for the system.[5] At this stage we should state that we discovered an arithmetical error in the previous work (A. Williams and W. P. Jencks, *J.C.S. Perkin II*, 1974, 1760) where k_1 should be double that quoted for imidazole (142 l mol^{-1} s^{-1}) and the equilibrium constant K should be 6.7×10^{-5} mol l^{-1}; it is also noted that in Table 4 of ref. 5 the units for the equilibrium constants should be in molarity.

The approximately pH-independent profile for k_1 is due to the k_1 term being of the same order as k_{-i} even in the pH region where the former predominates. At high pH values we are able to follow k_1 even though this then refers to k_{-i} because the background u.v. absorption is not large enough to hamper the observation of the very small changes.

Analysis of the pH-Profile for the Rate Constant (k_1) *for the Formation of* N^{π}*-Carbamoylhistamine.*—Figure 2 indicates that histamine is reacting with KNCO to form an equilibrium mixture. We assume that this involves formation of the N^{π}-carbamoylhistamine because the major tautomer of the imidazolyl group of the basic histamine possesses an NH^{τ} group.[6] The rate law for the approach to equilibrium

TABLE 4

Reaction of KNCO with imidazole at constant concentration (1M) over a pH-range.[a,b]

pH	$k_1 \times 10^3/s^{-1}$	$\Delta A_{240\ nm}$ [c]
3.2	8.57	
4.00	13.0	1.06
4.15	12.8	
5.00	19.0	1.20
6.05	13.8	1.14
6.70	15.4	
6.84	11.8	
7.00	10.0	1.06
7.14	8.2	
7.36	8.8	
8.00	9.0	0.32
8.35	11.5	
8.78	9.4	
8.98	9.0	0.1
9.93	7.5	0.1
11.27	12.0, 9.0	

[a] 1M imidazole, 25 °C, 1M ionic strength maintained with KCl. [b] Wavelength employed for following the reaction varied from 230 to 245 nm depending on the pH. In some cases 1-mm path-length cells were employed. [c] The absorption was measured in 1-mm path-length cells and corrected for 1-cm path-length.

is similar to equation (3). The equilibrium constant and forward and reverse rate constants may be obtained from the slope and intercept of the line of Figure 2: the forward rate constant k_i and the equilibrium constant obtained from the data have large errors because the overall change in rate constant over the concentration range is less than 100%. The difficulty in measuring the rate constants in the presence of histamine buffers also gives rise to uncertainty in the values for the parameters in question. The equilibrium constant for formation of the N^{π}-carbamoylhistamine is given by equation (4). It is a reasonable assumption that the ionisation of the β-ammonium species has little effect on the value of

$$K = k_{-i}/k_i = [HNCO][ImCH_2CH_2NH_3^+]/[N^{\pi}\text{-carbamoylhistamine}] \quad (4)$$

the equilibrium constant; we assume that K is constant, thus the fraction of KNCO converted into N^{π}-carbamoylhistamine at equilibrium varies from 0.27 at pH 6.55 to 0.002 6 at pH 9 for buffers containing 0.2M-histamine.*

The directly observed values of k_{-i} lie on a line almost indistinguishable from that for the rate constant for the approach to equilibrium. The low equilibrium proportions of N^{π}-carbamoylhistamine at high pH bear out the observation that the rate constants are difficult to measure in the forward direction.

Carbamoylation of Ethanolamine in the Presence of N-Carbamoylimidazole.—A stock solution of KNCO was added to buffer adjusted to pH 7.2 (the pK_a of imidazolium ion) containing imidazole (0.2M) and ethanolamine (0.2M). Sufficient KCl was present to maintain the ionic strength at 1M. The same experiment was carried out using buffers containing no ethanolamine. The absorbance, monitored at 240 nm, was observed to increase obeying a first-order rate law and then decrease slowly in the presence of ethanolamine; in the absence of ethanolamine no decrease in absorbance was observed after the initial increase. Details of the kinetics are recorded in Table 3. The rate constants for the initial increase and the absorption changes were independent of ethanolamine concentration up to 0.2M.

Perusal of Tables 1—3 indicates that the rate constants for formation and decay in the N-carbamoylimidazole–ethanolamine system are similar to those for histamine and histidine attack on cyanate as are also the absorbance changes. Using data from previous studies [6] for the reaction of ethanolamine with HNCO and the equilibrium constant between isocyanic acid and N-carbamoylimidazole we estimate using equation (5) the rate constant for decay of the intermediate to be 2.03×10^{-5} s^{-1}. This agrees remarkably well with the directly observed value (Table 3) taking

$$\text{Decay rate} = [\text{frHNCO}][RNH_2]\ 310 \quad (5)$$

into account the indirect nature of the calculations. A control study with cyanate in imidazole buffer in the absence of ethanolamine showed no decay over the period where ethanolamine induced complete decay of the intermediate. The free HNCO concentration (frHNCO) is calculated according to equation (6) where $K_a^{HNCO} = 10^{-3.29}$ (ref. 7) and

$$[\text{frHNCO}] = [\text{total KNCO}]/(1 + K_a^{HNCO}/a_H + [\text{frImH}]/K) \quad (6)$$

$K = 6.7 \times 10^{-5}$ mol l^{-1} (see earlier); frImH is free neutral imidazole and the concentration of ethanolamine is calculated using the pK_a 9.71.[7]

DISCUSSION

Formation of N$^{\pi}$*-Carbamoylimidazolyl Species.*—There is little doubt from previous work [5] that the initial reaction of histamine or histidine with potassium cyanate involves carbamate formation on the heterocyclic nitrogen. The final product of the reaction namely the N^{α}-carbamoyl species is indicated by the isolation of substantial amounts from reaction of both histamine and histidine with cyanate. The nature of the intermediate comes from secondary data; *in situ* preparation with histamine yields a species which has u.v. absorption and kinetic and equilibrium parameters similar to those for the characterised N-carbamoylimidazole (see Tables 1 and 2).

The site of carbamoylation is difficult to determine for histamine and histidine species but it is almost certainly at the N^{π} nitrogen as the N^{τ} nitrogen in the basic imidazolyl function in the free species is protonated.[6] The pH-dependence for the attainment of equilibrium is largely a measure of that for the decomposition step k_{-i} of the carbamate.

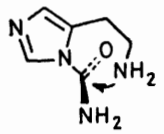
Decay of N$^{\pi}$*-Carbamoylimidazolyl Species containing Free Amino-groups.*—The decay rate constant for the intermediate in histamine solutions is pH-independent (Figure 4) and is proportional to the concentration of the histamine buffer. This behaviour is not to be expected if the transcarbamoylation involves intramolecular amine attack (III) which should be concentration independent. A further feature of this mechanism is that the rate constant should be dependent on the ionisation of the β-ammonium group and therefore have a pH-profile

* The fraction of N^{π}-carbamoylated histamine at different pH values is estimated from the equation:

$$[N^{\pi}\text{-carbamoylhistamine}]/[\text{total cyanate}] = \{1 + K(1 + K_a^{HNCO}/a_H)(1 + a_H/K_a^{ImH_2^+})/[ImH_{total}]\}$$

The parameters are given in the text and Tables.

(log k_2/pH) with unit slope in the region of pH covered by our observations (Figure 4). The observed pH-independence of k_2 is consistent with the reaction of



(III)

amine base with the isocyanic acid in equilibrium with the carbamate [equation (7a)]; the kinetic expression

$$\text{(IIa)} \longrightarrow \text{(II)} + \text{HNCO} \xrightarrow{\text{(II)}} \text{urea (IIb)} \qquad (7a)$$

$$\text{(II)} \rightleftharpoons \text{NCO}^- + \text{H}^+$$

$$k_2 = \frac{K_a^{RNH_3^+}\,[\text{total amine}]\,k_i}{\left[1 + K_a^{HNCO}/a_H + \dfrac{[\text{total imidazole}]}{K(1 + a_H/K_a^{ImH_2})}\right]} \qquad (7b)$$

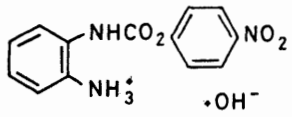
for this reaction (7b) is pH-independent in the observed region of pH.

Addition of potassium cyanate to a mixture of imidazole and ethanolamine (Table 3) results in a reaction which exhibits the formation and decay of an intermediate. The rate constant for the decay reaction is similar to that for the histamine system at similar pH and concentration and, moreover, may be predicted from previously determined parameters.[5,7] A control experiment with cyanate and imidazole in the absence of ethanolamine exhibited no decay.

Changeover in Mechanism.—The pH-profiles for the decomposition of phenyl carbamates (X = NH, O, and CO_2 in Scheme 1) possess plateau regions corresponding to the ionisation of the *ortho*-acid (I).[1,2] In the case of the 2-hydroxy- and 2-carboxy-derivatives these plateaux arise from intramolecular nucleophilic attack on the carbamate moiety (Scheme 1); the hydrolysis of these derivatives also possess a hydroxide term as evidenced by the upward swing of the pH-profile in the alkaline pH-region. The second-order rate constant for reaction of hydroxide ion and the conjugate base of substrate is close to that for the *E*1cB hydrolysis of the carbamate unsubstituted on the *N*-phenyl ring;[8] an approximate allowance for the presence of CO_2^-, NH_2, and O^- in the *ortho*-position to the carbamate ester may be calculated from data from Hegarty's laboratory[2] but does not alter the expected rate constant significantly. The hydroxide term is also absent for compounds where the NH group on the carbamate ester is methylated.[1,2] This is good evidence that the alkaline limb of the pH-profile for these compounds is due to an *E*1cB process involving an isocyanate intermediate; the formation of isocyanate must, of necessity, be followed by intramolecular attack by the internal nucleophile.

The changeover in mechanism from intramolecular attack (A–E) in the plateau region of pH to *E*1cB at alkaline pH values for X = O^- and CO_2^- occurs because the reactivity of the former mechanism is pH-independent above the pK_a of XH whereas the latter is proportional to hydroxide ion concentration; of course, the reactivity of the *E*1cB mechanism is only proportional to hydroxide ion concentration below the pK_a of the carbamate ester NH but this is relatively high (*ca.* 15).[9]

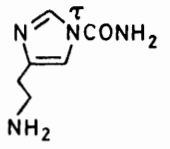
Hegarty shows,[2b,c] by comparison with the behaviour of the 4-aminophenyl isomer of (IV) that the *E*1cB mechanism holds for the hydrolysis of 4-nitrophenyl-*N*-(2-aminophenyl)carbamate (IV) over the whole pH-



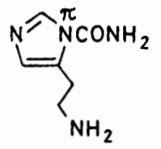
(IV)

range. The hydroxide term from the alkaline region of the pH-dependence is close to that for the *E*1cB hydrolysis of a 4-nitrophenylcarbamate with an unsubstituted *N*-phenyl group. Moreover the ' plateau ' region of pH corresponds to a rate constant which is similar to that calculated for the alkaline hydrolysis rate constant for the *ortho*-ammonium species from the 4-nitrophenyl *N*-phenylcarbamate using the Hammett equation and data from reference 2*b* assuming a σ value of +1.7 for the 2-NH_3^+ group.

The transcarbamoylation of N^{π}-carbamoylhistamine or histidine involves an intermolecular process rather than the direct intramolecular attack of the amino-function at the carbamate centre. The greater efficiency of the intermolecular *E*1cB process may reside in the planar stereochemistry of the *N*-carbamoylimidazole moiety; thus relatively large amounts of energy are required to force the carbonyl group perpendicular to the plane of the imidazolyl function and into alignment for nucleophilic attack by the amine. The *N*-carbamoyl-histamine or -histidine intermediates consist of two isomers [(Va) and (Vb)] one of which (the N^{τ}-isomer) is precluded from a nucleophilic transcarbamoylation process on steric grounds. At least half the bulk of the



(Va)

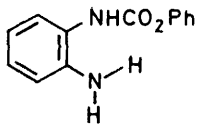


(Vb)

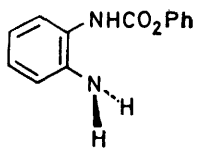
intermediate and probably the whole of it (see earlier) will be that isomer (the N^{π}-carbamoyl species) where intramolecular attack is possible; since the isomers are in rapid equilibrium (*via* the isocyanic acid path) there is not likely to be any blockage in a possible intramolecular pathway when the reactive isomer is used up.

A possible explanation for the *E*1cB mechanism in the spontaneous reaction of phenyl *N*-(2-aminophenyl)-

carbamate (VI) is that the intramolecular process is also inhibited by a process similar to that proposed for the histamine case; for intramolecular attack to occur a perpendicular configuration is required for the amine



(VI)

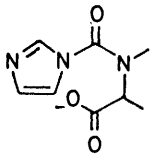


(VII)

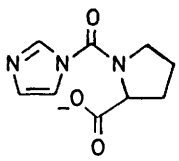
relative to the plane of the aromatic nucleus (VII). The *ortho*-carboxylate and phenolate derivatives do not suffer from this requirement.

It could be argued that in the case of histamine the proportion of the N^{π}-carbamoyl isomer (favourable for the intramolecular path is small and therefore there is insufficient intermediate to support a reaction flux through the intramolecular route. Although the fraction of total intermediate is small at high pH (see earlier) the proportion rises to 0.27 for 0.2M-histamine at the pH corresponding to the pK_a of the imidazolyl species.

An example where the intramolecular addition–elimination mechanism predominates in the transfer of a carbamoyl group from an imidazolyl acceptor is the decomposition of *N*-(2-carboxylalkyl)carbamoylimidazoles (VIII).[10] This involves formation of an *N*-carboxyanhydride; the elimination–addition mechanism is impossible for the corresponding proline derivative (IX) and since there is considerable ring strain in the



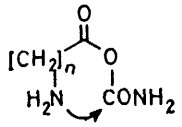
(VIII)



(IX)

carboxyanhydride from this species a reduced rate is observed. Alkylation of the α-amino-group has no effect on the rate constant.

Amino-acids, even at low pH, carbamoylate directly to nitrogen in the presence of cyanate and no observable reaction flux is taken by the intramolecular transcarbamoylation mechanism through the anhydride (X).[11] The reason for this is probably the poor equili-



(X)

brium constant for formation of the anhydride; this is borne out by previous work from this laboratory[9] which shows that formation constants for attack by oxyanions on isocyanic acid decrease with decreasing pK_a of the conjugate acid of the nucleophile. A further complication is that water hydrolysis of the anhydride could compete efficiently with the intramolecular reaction.

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