A Stepwise Mechanism in the Reaction of Amines with Carbodi-imides to form Guanidines

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Reaction of amines with the water-soluble *N*-ethyl-*N'*-(3-trimethylammoniopropyl)carbodi-imide (ETC) perchlorate in aqueous solution is not catalysed by general acids or bases. Both acid and base forms of the amine may be involved in the kinetic expression; with weakly basic amines the former predominate whereas with strongly basic amines the latter do. Amines of intermediate basicity involve both acid and base forms. Guanidine formation is shown not to interfere with the synthesis of amides from relatively basic amines except at pH values above 8 where *O*-acylisourea formation is not efficient. On the contrary, guanidine formation will strongly compete with anilide synthesis in aqueous solution at acid pH.

Guanidine formation is a possible complicating feature in the carbodi-imide method of peptide synthesis from carboxylic acids and amines. Although the carbodi-imide procedure continues to be a popular coupling method [1] guanidine by-products [equation (1)] have never been observed in peptide synthesis. It is not surprising that nobody has advanced an explanation of the absence of guanidine by-products but the possibility is intriguing and this paper is addressed to this problem.

No one has reported satisfactory kinetic data on guanidine formation from carbodi-imide and amine although there are many reports of synthetic studies.[1] The development of sensitive assays for carbodi-imides [2] and the observation of u.v. spectral changes with reactions of these species [3] prompted us to investigate the mechanism of this reaction. We use as the standard substrate *N*-ethyl-*N'*-(3-trimethylammoniopropyl)-carbodi-imide (ETC) perchlorate as this is water-soluble; the reaction is studied using aqueous solution because this has the advantage of no ion-pair formation, the ability to buffer the solution accurately, and to have high concentrations of ionic species without markedly altering the physical chemistry of the solution. Preliminary studies indicated that simple carbodi-imides such as dipropyl- or di-isopropyl-carbodi-imide were not sufficiently water-soluble for easy kinetic studies to be carried out.

Experimental

Materials.—Amines were obtained as free bases or hydrochlorides and were purified by recrystallisation or redistillation. *N*-Ethyl-*N'*-(3-trimethylammoniopropyl)carbodi-imide (ETC) iodide was synthesised as described in an earlier paper and converted into the perchlorate salt with silver perchlorate.[3]

Buffer components were of analytical reagent grade or were purified from bench-grade materials by recrystallisation or distillation. Water used throughout the kinetic investigation was doubly distilled from glass.

Methods.—Rates of reaction of amines with ETC were measured by reacting the carbodi-imide with the amine present as a buffer component at an appropriate pH; portions of the solution were withdrawn at intervals and assayed for carbodi-imide by reacting with aniline as described previously.[2a,3] When the reaction with amine gave a useful spectral change the decay of carbodi-imide was followed continuously at the appropriate wavelength. In the case of some anilines the carbodi-imide was present in excess and the decay of aniline was followed continuously. Pseudo-first-order rate constants were calculated from linear logarithmic plots *versus* time using semi-logarithmic graph paper.

$$R'NH_2 + RNCNR + H^+ \longrightarrow R'NH\!-\!C(=NHR)^+ \qquad (1)$$

Ionisation constants for anilines were measured spectrophotometrically using a Unicam SP 500 instrument coupled with a pH-stat apparatus as described in the accompanying paper.[3,4a]

Results

There have been many synthetic studies on the reaction of amines with carbodi-imine [1] and it is unlikely that the reaction of ETC yields anything other than guanidines. Owing to the presence of the polar side-chain in ETC we were not able to isolate guanidines easily from the reaction product. Spectroscopic analysis of the product of reaction of di-isopropylcarbodi-imide with aniline in aqueous solution under the conditions of the kinetic analysis (Table 1) indicated that *NN'*-di-isopropyl-*N''*-phenylguanidine was formed in theoretical yield. The latter compound was synthesised and had m.p. 135—136 °C (lit.,[4b] 131—132 °C); it had an absorption maximum at 238 nm with an extinction coefficient 7 020. The reaction of aniline with ETC under the conditions of the kinetic analysis gave a product with spectrum ($\lambda_{max.}$ 238 nm) identical with that for the di-isopropyl case and use of the molar extinction coefficient of the latter indicated a theoretical yield. A further piece of evidence in favour of guanidine formation is that the rate constants for ETC and di-isopropylcarbodi-imide with aniline are similar (Table 2). Glycine, ethyl glycinate, β-alanine, and ethanolamine might be expected under prolonged heating to gives cyclic products.[1,4c] Rate constants measured for these amines are close to those expected for guanidine formation by comparison with amines of similar pK (Table 1).

Reactions were accurately first order over at least 90% of the progress; the derived pseudo-first-order rate constants for the reaction of carbodi-imide with amines are linearly related to the total amine concentration. Where carbodi-imide is in excess the rate constants are a linear function of the concentration of the latter. Figure 1 illustrates a typical plot for the reaction of piperidine with ETC; the intercept at zero buffer concentration agrees in all cases with the background hydrolysis rate constant from a previous study.[3] The rate constants for anilines were dependent only on the concentration of the conjugate acid. The value of k_A [equation (2)] was determined from the pH profile and the pK of the aniline (measured separately). The strongly basic primary amines only reacted as their conjugate bases and a typical plot to determine k_B [equation (2)] is illustrated in Figure 2. The general kinetic

Table 1. Reaction of amines with *N*-ethyl-*N'*-(3-trimethylammoniopropyl)carbodi-imide (ETC) perchlorate [a]

Amine	p*K*	No.[d]	$10^4 k_A$/ l mol^{-1} s^{-1}	$10^3 k_B$/ l mol^{-1} s^{-1}	pH range	λ/nm
1 Ethanolamine	9.48	16	<2	5.4	8.5—10.5	248
2 Ethyl glycinate	7.8	10	<10	1.6	7.2—8.5	255
3 Hydrazine	8.39	10	<20	22	7.8—9.1	250
4 Ethylamine	10.96	8	<10	15	10.6—11.3	240
5 Diaminoethane	10.03	8	<2	12	9.6—10	240
6 Diaminoethane monocation	7.55	4	<2	12	7.5	250
7 Glycine	9.77	8		7.5	8.9—10.1	250
8 β-Alanine	10.35	8	<1.5	9.9	9.9—10.7	245
9 Piperazine monocation	5.72	8	34	1.5	5.3—6.3	250
10 Piperazine	10.04	8		12.8	9.4—10.5	255
11 Morpholine	8.87	8		3.18	8.4—9.2	240
12 Piperidine	11.42	8		19.2	11—11.7	250
13 2,2,2-Trifluoroethylamine	5.92	12	129		5.1—6.3	225
14 Benzylamine	9.46	10	<5	7.1	8.8—9.8	*c*
15 Aniline	4.92	11	6 800		4.6—5.9	230 [b]
16 4-Toluidine	5.16	11	5 500		4.9—5.8	230 [b]
17 4-Sulphanilic acid	3.11	6	120 000		5.0—6.7	290
18 4-Chloroaniline	4.26	5	7 700		5.1—6.8	290
19 4-Anisidine	5.46	6	3 500		5.1—7.0	265

[a] 25 °C, 1M ionic strength. [b] Quench method. [c] Measured by the assay of carbodi-imide *via* the aniline quench method. [d] Number of data points.

Table 2. Reaction of amines with di-isopropylcarbodi-imide [a]

Amine	p*K*	k_A/l mol^{-1} s^{-1}
Aniline	4.92	0.35
Aminoacetonitrile	5.56	0.3

[a] 25 °C, 1M ionic strength.

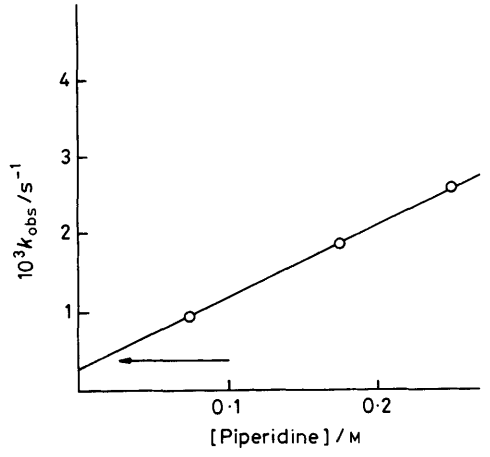


Figure 1. Reaction of *N*-ethyl-*N'*-(3-trimethylammoniopropyl)-carbodi-imide (ETC) perchlorate with piperidine; pH 11.42, 25 °C, ionic strength 1M. The line is theoretical from data in Table 1 and the arrow indicates the intercept expected from previous data for the hydrolysis of ETC [3]

equation for reaction of all the amines in this study is given in equation (2) where k_W is the background hydrolysis rate constant of ETC in water. The parameters k_A and k_B are

$$k_{obs} = k_W + k_A\,[\text{Ammonium ion}] + k_B\,[\text{Amine}] \quad (2)$$

given in Table 1; the strongly basic amines have an upper limit quoted for their value of k_A which is obtained from the error on the intercept at zero fraction of base (FB) of the plot of $(k_{obs} - k_W)$/[total amine] *versus* FB (see Figure 2).

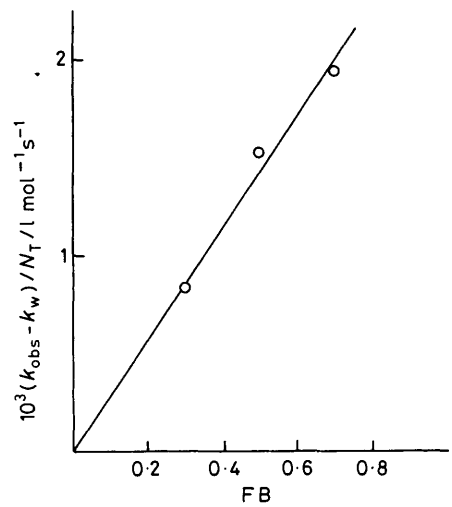
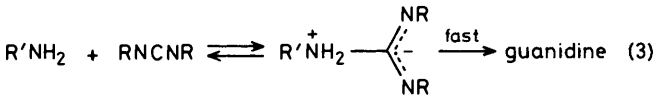


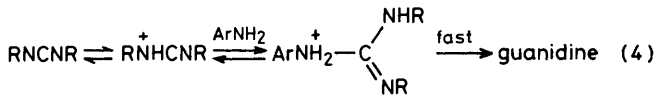
Figure 2. Reaction of ETC with ethanolamine over a range of base fractions (FB); ionic strength 1M, 25 °C. Line is theoretical from Table 1

Buffer catalysis of the reaction of 4-anisidine and aniline with ETC was shown to be absent for DABCO (1,4-diazabicyclo[2.2.2]octane) at pH 5 and 7 and for *N*-propargylmorpholine and *N*-methylmorpholine at pH 4.69 and 7, respectively.

Discussion

The base term (k_B) can only arise from reaction of neutral amine with carbodi-imide. It is unlikely that the reaction is catalysed by water acting as an acid as no general acid catalysis is observed. Thus the mechanism probably involves the formation of a zwitterion which decomposes in a fast step [equation (3)] to guanidine. This might be expected because the acid p*K* of the guanidine [$R'\overset{+}{N}H_2C(NHR)NR \rightleftharpoons R'\overset{+}{N}H_2\bar{C}(NR)_2 + H^+$] will be much larger than that for the zwitterion adduct of amine with isocyanic acid [5] which has a





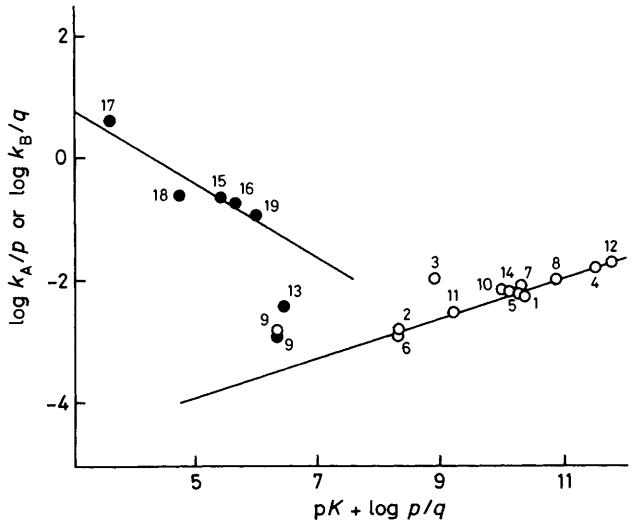


Figure 3. Brønsted-type plots for k_A (●) and k_B (○) *versus* the pK of the attacking nucleophile. The points are corrected statistically and the numbering is as in Table 1; the slopes are −0.59 (k_A) and 0.3 (k_B) and are derived by omitting points 3, 9, and 13 from the correlations

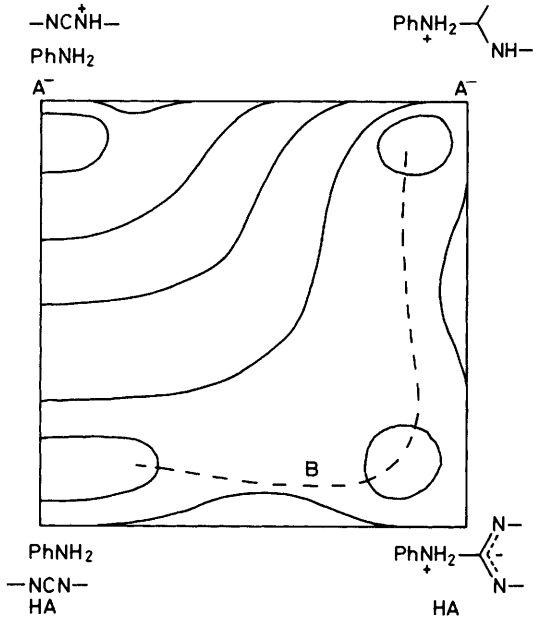


Figure 4. Diagrammatic potential energy surface for the attack of aniline on a carbodi-imide in the presence of an acid of pK 7

value of *ca.* 10; it is quite possible that the acid pK is higher than 14 because other studies show that *O*-phenyl-*NN'*-diisopropylisourea has a pK greater than 14.[6] Transfer of a proton from water is therefore thermodynamically favourable and the decay of the zwitterion [equation (3)] will be diffusion controlled.

The Brønsted-type exponent (β_N) for attack of amine on carbodi-imide is 0.3 (see Figure 3) and similar values are observed for the attack of amines on ethyl isothiocyanate (0.28),[7] carbon dioxide (0.26),[8] carbon disulphide (0.26),[9] carbonyl sulphide (0.26),[9] and isocyanic acid (0.3).[7] In the case of all these electrophiles the rate-limiting step is the addition to give a zwitterion; addition to isocyanic acid refers to strongly basic amines (anilines are known to involve rate-limiting proton transfer).[6,7] The ratio of reactivities for reaction with an amine of pK 11 is carbodi-imide : ethyl isothiocyanate : carbon dioxide : carbon disulphide : carbonyl sulphide : isocyanic acid = 0.015 : 0.042 : 4300 : 0.013 : 13 : 1 300. Thus carbodi-imide is quite close in reactivity to isothiocyanate and carbon disulphide for amine attack; replacement of nitrogen by an oxygen naturally increases the reactivity.

The slight positive anomaly of piperazine monocation may be due to an internal solvation effect caused by the neighbouring ammonium ion. The same effect is not seen with the diaminoethane monocation but this may be due to the weaker acidity of the ammonium and the less favourable entropy required to allow solvation to occur with this ion. We are not suggesting the participation of *general acid* catalysis here. The base term for attack of anilines is not observed because of the preponderant acid (k_A) component for these species.

The acid term (k_A) is observed only for weakly basic amines such as anilines. Stronger amines have a dominating base term and it may be calculated that the k_A term for these species give an intercept on the plot at FB = O (Figure 2) less than the error if the slope of the Brønsted-type plot is about unity (Figure 3). Attack of protonated amine on the carbodi-imide is an unlikely mechanism and we suggest that the rate-limiting step is attack of free amine on protonated carbodi-imide [equation (4)]. The cation formed is most likely a strong acid and its decomposition to guanidine diffusion controlled. Concerted proton transfer is unlikely in view of the absence of buffer catalysis in the presence of relatively strongly acidic components. If the mechanism is as in equation (4) then the Brønsted-type exponent (β_N) for attack of amine on the protonated carbodi-imide is +0.2.

General acid–base catalysis is observed in the corresponding reactions of anilines with isocyanic acid and in the reaction of carboxylate ion with carbodi-imide in water. The latter mechanism must be close to the changeover to stepwise because the stepwise proton-catalysed mechanism for attack of carboxylate is acting concurrently.[10] The absence of general acid catalysis in aniline attack on carbodi-imides can be understood from the diagrammatic potential energy surface (Figure 4). In contrast to the attack of carboxylate ion (see following paper) [10] the system in the bottom right corner is considerably more stable due to the extra electrostatic effect. The overall effect of this stabilisation will be to skew the surface to favour path B; the stability gained in the zwitterion is probably not sufficient to lower its pK below that of water.

Merrifield and his co-workers excluded guanidine formation in peptide syntheses using carbodi-imines.[11] Muramatsu [4c] and DeTar [12] gave an indication of the rate of guanidine formation in non-aqueous solvents. DeTar [12] showed that the ammonium form of the amine nucleophile was the kinetically important species. The results of this work now enable us to explain why guanidine formation is not important in peptide synthesis in water; they do not allow a direct explanation for non-aqueous solvents and a rigorous mechanistic treatment

for these is difficult. Let us consider a typical amide synthesis in water from ethyl glycinate (pK 7.8) and acetic acid (pK 4.5) mediated by carbodi-imide at equimolar concentrations at 1M. Inspection of the data in Table 1 shows that guanidine formation has the second-order rate constant at pH 6 of 1.6 $10^{-3} \times 1.58 \times 10^{-2} = 2.54 \times 10^{-5}$ l mol^{-1} s^{-1}. Isourea formation at the same pH has 0.1 l mol^{-1} s^{-1} under the same conditions.[10] Thus the amine at pH 6 does not compete efficiently with the carboxylic acid for the carbodi-imide. At pH 7.8 (pK of the amine) the rate constant for isourea formation is *ca.* 10^{-3} l mol^{-1} s^{-1} and for guanidine $0.5 \times 1.6 \times 10^{-3} = 80 \times 10^{-5}$ l mol^{-1} s^{-1}; coupling must therefore be carried out at pH values <6 if guanidine by-products are not to be obtained. In a typical coupling procedure with water-soluble carbodi-imides the pH values will be in the region 5—6 using typical pK values for amine and carboxy-protected amino-acids. In non-polar solvents with water-insoluble carbodi-imides the *O*-acylisourea formation is probably even more efficient than the guanidine route as the concerted mechanism for the former (involving little charge separation) will be favoured over stepwise routes. The second reaction in peptide synthesis, namely acylation of the amine, is most efficient at high pH where the amine is predominantly in its basic form; thus the pH should be kept as high as possible consistent with the competition between acid and amine for the carbodi-imide. It should be noted here that Hegarty and his co-workers have indicated that O ⟶ N rearrangement of the *O*-acylisourea is suppressed in acid media.[13]

Perusal of Table 1 indicates that reaction of aniline at pH 4.5 with carbodi-imide in the presence of 1M-acetic acid total buffer has a rate constant of 0.49 l mol^{-1} s^{-1}. The rate constant for isourea formation under the same conditions is 2.8 l mol^{-1} s^{-1} indicating that guanidine by-products are a serious problem in amide formation from weakly basic amines. The problem is even more serious for amines with lower basicity than aniline where the k_A term is larger.

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