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A New Mechanism Involving Cyclic Tautomers for the Reaction with Nucleophiles of the Water-Soluble Peptide Coupling Reagent 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC)[1a]

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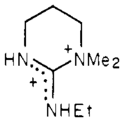
Abstract: Rate constants for the hydrolysis of the water-soluble peptide coupling reagent 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide (EDC) exhibit a pH dependence including an acid plateau region with $\mathrm{p}K_{\mathrm{apparent}} = 3.2$, a basic plateau from pH 6 to pH 10, and a dependence on hydroxide ion concentration from pH 11 to pH 14. Potentiometric titration shows two ionizations of $\mathrm{p}K = 3.1$ and 11.1. The pH dependence of the rate constant for acetate attack on (EDC) is "bell" shaped with pK's equal to 4.5 and 3.1. Reaction of EDC with strong amines reveals a nonlinear dependence of the rate constant on the fraction of amine present as its base form. The hydrolysis of model carbodiimides including 1-ethyl-3-(3-(trimethylammonio)propyl)carbodiimide perchlorate (ETC) was measured over a pH range in order to elucidate the mechanism for the EDC reactions. The major part of the reaction flux in the acid and neutral hydrolysis of EDC passes through the cyclic ammonioamidine dication I to product. At alkaline pH the neutral carbodiimide tautomer takes the reaction flux for hydrolysis.



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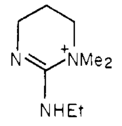
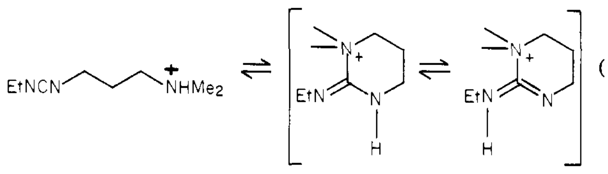


II

Attack of strong amines in the alkaline pH range involves both cyclic II and carbodiimide species; the favored mechanism at the lower pH involves the cyclic species II and at the higher pH the carbodiimide. Acetate reacts with EDC through attack on the dication I. Studies with the model carbodiimides demonstrate that the minor reactions of water and acetate ion with EDC through the carbodiimide tautomer suffer a change in rate-limiting step from attack of nucleophile on carbodiimide to the formation of the latter as the pH is lowered. The cause of the predominant reaction through the cyclic dication is the blocking of the carbodiimide mechanism; the rate-limiting formation of the carbodiimide is inhibited by acid.

Water-soluble carbodiimides are important reagents used extensively in condensation reactions[1b] and for the modification of naturally occurring macromolecules.[2] One of the originally prepared carbodiimides (EDC) was proposed to exist as a tautomeric mixture;[3] this has been shown to be the case in NMR studies on aqueous and dimethyl sulfoxide solutions of the reagent (eq 1).[4,5]



(1)

A significant problem is the question of which tautomeric species is involved in addition reactions of nucleophiles to EDC and similar water-soluble carbodiimides capable of tautomerism. Both tautomers are potentially capable of addition, and the mode of action is clearly relevant to the action of these reagents as peptide couplers and protein modifiers. There have been no studies of this problem, and moreover the proposed mechanism for the action of these tautomeric carbodiimides have all assumed reaction with the carbodiimide tautomer.

In the present study we investigate the pH dependence of the hydrolysis of model carbodiimides including a model of the carbodiimide tautomer in eq 1, namely, 1-ethyl-3-(3-(trimethylammonio)propyl)carbodiimide (ETC). Combining these data with the equilibrium data of Tenforde, Fawwaz, and Freeman[4] we are able to show that both tautomeric types from EDC are involved in hydrolysis. The reaction of EDC with strong amines proceeds via both cyclic and noncyclic tautomers, but reaction of acetate is with the cyclic tautomer alone.

Experimental Section

Materials. Triethylenediamine (DABCO), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride salt, and *N,N'*-diisopropylcarbodiimide were purchased from the Aldrich Chemical Co. Trimethylamine hydrochloride salt was prepared by bubbling HCl through the amine solution in water; evaporation of the solvent gave the salt which was recrystallized from ethanol. DABCO (1,4-diazabicyclo[2.2.2]octane) was purified by sublimation.

N,N'-Di-*n*-propylthiourea was prepared by adding *n*-propylamine (1 mol) in benzene (100 mL) to an ice-cooled solution of carbon disulfide (47 g, 0.62 mol) in benzene (250 mL). The dithiocarbamate product soon started to precipitate, and after complete addition of the amine the reaction mixture was kept for 12 h at room temperature. The mixture was then refluxed for 50 h, the solution cooled to room temperature, and the thiourea precipitate filtered, washed with petroleum ether, and dried. The thiourea (10 g) was refluxed in benzene (60 mL) with dry freshly prepared yellow mercuric oxide (40 g). Water formed in the reaction was absorbed with BaO in a Soxhlet extractor. After being refluxed for 6 h, the mixture was filtered and evaporated. Distillation gave the *N,N'*-di-*n*-propylcarbodiimide as an oil in 32% yield; bp 40-42 °C (5 torr) (lit.[6] bp 53 °C (11 torr)).

1-Ethyl-3-(3-(dimethylamino)propyl)urea was prepared by adding a solution of ethyl isocyanate (41 g, 0.57 mol) in anhydrous ether (400 mL)

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(1) (a) ITI is grateful to the Iraqi Ministry of Education for support; a preliminary account of this work has been reported (I. T. Ibrahim and A. Williams, *J. Am. Chem. Soc.*, **100**, 7420 (1978). (b) F. Kurzer and K. Douraghi-Zadeh, *Chem. Rev.*, **67**, 107 (1967).

(2) K. L. Carraway and D. E. Koshland, *Methods Enzymol.*, **25** (part B), 616 (1972).

(3) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961).

(4) T. Tenforde, R. A. Fawwaz, and N. K. Freeman, *J. Org. Chem.*, **37**, 3372 (1972).

(5) I. Yavari and J. D. Roberts, *J. Org. Chem.*, **43**, 4689 (1978).

(6) H. Z. Lecher and K. Gubernator, *J. Am. Chem. Soc.*, **75**, 1087 (1953).

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to *N*,*N*-dimethyl-1,3-diaminopropane (58 g, 0.57 mol) in anhydrous ether (200 mL). The temperature was moderated by external cooling, and after the mixture was stirred for 2 h at room temperature, the ether was evaporated to give a quantitative yield of the urea as a yellow oil. The crude urea (84 g, 0.48 mol) was dissolved in dichloromethane (150 mL) together with triethylamine (20 mL); toluenesulfonyl chloride (94 g, 0.96 mol) in dichloromethane (1 L) was added to the urea solution and the mixture kept at 5 °C. The mixture was allowed to warm to room temperature and then refluxed for 3–4 h; the product was stirred with 40% aqueous K_2CO_3 (3 × 600 mL). The organic layer was collected, concentrated, and extracted with several portions of anhydrous ether. Evaporation of the ether gave an oil which was distilled in vacuo to yield the carbodiimide, bp 43–45 °C ((0.3 torr) (lit.[3] bp 53–54 °C (0.6 torr)) in 60% yield. The carbodiimide (30 g, 0.2 mol) was shaken with methyl iodide (57 g, 0.4 mol) in ether (500 mL) at room temperature for 2 days to give the methiodide salt in 85% yield. The material was recrystallized from chloroform/ethyl acetate to give plates, mp 99–100 °C (lit.[3] mp 106.5–107.5 °C).[3] Anal. Calcd for $C_9H_{20}N_3I$: C, 36.4; H, 6.8; N, 14.1. Found: C, 36.2; H, 6.8; N, 14.1.

1-Phenyl-3-(3-(dimethylamino)propyl)carbodiimide was prepared from phenyl isocyanate and *N*,*N*-dimethyl-1,3-diaminopropane by a method similar to that above. The free carbodiimide distilled to give an oil, bp 101–102 °C (0.1 torr) in 33% yield. Anal. Calcd for $C_{12}H_{17}N_3$: C, 70.9; H, 8.4; N, 20.7. Found: C, 70.7; H, 8.9; N, 20.7. IR: λ_{max} 2130 cm^{-1} (NCN). NMR ($CDCl_3$): δ 2.12 (6 H, s), 2.3 (2 H, t), 1.72 (2 H, m), 3.38 (2 H, t), 7.03 (5 H, m). The 1-phenyl-3-(3-(dimethylamino)propyl)carbodiimide methiodide (PTC) was prepared from the carbodiimide as above in 95% yield and was recrystallized from acetone/ether to give plates, mp 163–164 °C. $C_{13}H_{20}N_3I$: C, 45.2; H, 5.8; N, 12.2. Found: C, 45.2; H, 5.7; N, 12.3. IR: λ_{max} 2130 cm^{-1} (NCN). NMR (Me_2SO-d_6) δ 3.1 (9 H, s), 3.2 (2 H, t), 2.06 (2 H, m), 3.6 (2 H, t), 7.06 (5 H, m). ^{13}C NMR (Me_2SO-d_6): δ 24.06 ($CH_2{}^{13}CH_2CH_2$), 43.31 ($CH_2{}^{13}CH_2N^+$), 52.29 ($-N^+({}^{13}CH_3)_3$), 63.09 ($=N^{13}CH_2CH_2$), 123.4, 124.86, 128.5, 129.47 (aromatic carbon), 139.66 ($N{=}{}^{13}C{=}N$).

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

Methods. A stock solution of ETC perchlorate in water at approximately 0.4 M was prepared by adding silver perchlorate (0.166 g) in water to the ETC iodide (0.276 g) in water. The silver iodide precipitate was filtered and a drop of KCl solution added to the filtrate to ensure complete precipitation of any excess silver ions. The solution was centrifuged, the volume made up to 2 mL, and the stock used within 1 day. The phenyl analogue was prepared as a stock in water in the same way.

The kinetics of the hydrolysis of carbodiimide were measured spectrophotometrically. A typical experiment involved adding a solution of carbodiimide (50λ) on the flattened tip of a glass rod to a buffer solution (2.5 mL) in a silica cell in the thermostated cell holder of a Unicam SP 800 spectrophotometer. Two to three vertical strokes of the glass rod in the solution effected complete stirring, and the absorbance at a fixed wavelength was recorded with time on an external servoscribe recorder. The pH of the solution in the cell was measured before and after the reaction by using a Radiometer pH meter PHM 26 calibrated with E.I.L. buffer powders to ±0.01 unit. Pseudo-first-order rate constants were computed from plots of $A_t - A_\infty$ vs. time on two-cycle semilogarithmic graph paper. The more rapid reactions were followed to completion and measured values of absorbance obtained for infinite time (A_∞). Values of A_∞ for the slower reactions were calculated by assuming the first-order kinetics were obeyed from the eq 2 where A_1, A_2, and A_3 are absorbancies and $t_2 - t_1 = t_3 - t_2$; the reaction was always followed to 90% completion where the rate of reaction had decreased by tenfold.

$$A_\infty - A_1 = (A_2 - A_1)^2/(2A_2 - A_3 - A_1) \qquad (2)$$

Other reactions, including very slow ones, were also measured by assaying the residual carbodiimide. The carbodiimide stock solution (0.2 mL) was added to 2.5 mL of buffer to yield a reaction mixture. The decay of the carbodiimide concentration was measured by adding aliquots of the reaction mixture (100λ) at regular intervals to anilinium hydrochloride (100λ, 1 M) and incubated for approximately 5 min; the solution was then diluted by adding 50λ to dilute HCl (3 mL, 1 M) and the ultraviolet absorption read at 230 nm. The reading at infinite time for these reactions was measured by the assay procedure using the buffer *without* added carbodiimide; in this case water was substituted for the carbodiimide.

Measurement of ionization constants of species was carried out potentiometrically by using a Radiometer pH titration set comprising REC 61 Servograph, REA titratigraph, pH meter PHM 26, titrator TTT60, and Autoburette ABU 11.

Fitting of rate parameters to theoretical kinetic laws was carried out by using "Grid-Search" programs of the type previously described by us,[7] we are grateful to Mr. S. V. Hill for help with the computer programs.

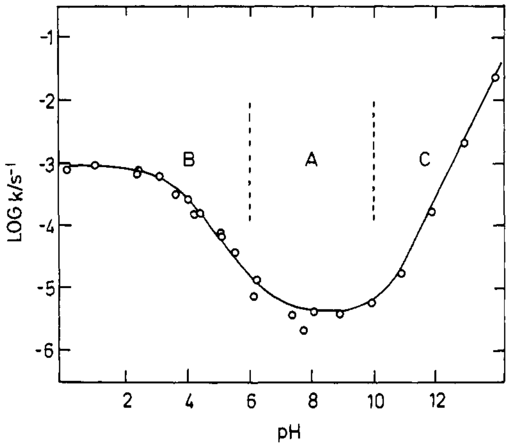

Figure 1. Dependence on pH of the hydrolysis of EDC at 25 °C, 1 M ionic strength and extrapolated to zero buffer concentration. Buffers used were as follows: pH 0–3, HCl/KCl; pH 3–8, 1,4-diazabicyclo[2.2.2]-octane (DABCO); pH 8–11, trimethylamine; pH 11–14, KOH/KCl. The regions A, B, and C are described in the text; the line is theoretical from eq 4.

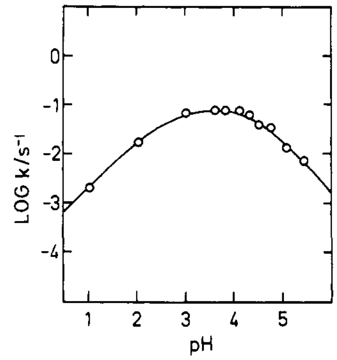

Figure 2. Dependence on pH of the reaction of EDC with acetate (1 M) at 1 M ionic strength and 25 °C. No buffering species other than acetate is present (see text); the line is theoretical from eq 5 by using parameters given in the text and in Table II.

Results

Model Carbodiimides. The hydrolysis of the model carbodiimides obeys good pseudo-first-order kinetics over at least 90% of the reaction. The pseudo-first-order rate constants depend on hydroxide and hydronium ion concentration according to eq 3.

$$k = k_H[H^+] + k_{OH}[OH^-] \qquad (3)$$

The results are collected in Table I. Buffer catalysis of the hydrolysis of the bona fide carbodiimides was found to be absent; the hydrolysis rate constants of diisopropyl carbodiimide and ETC are invariant in DABCO concentration from 0.1 to 1 M in buffers at pHs of 3.86 and 5.51. No explicit pH-independent term was observed corresponding to the hydrolysis in water alone; an upper limit for such a term for the hydrolysis of ETC is 10^{-6} s^{-1} corresponding to the changeover rate constant between hydronium ion and hydroxide ion at pH 8.5.

Reaction of EDC with Water. Potentiometric titration of EDC (HCl salt) was effected by using HCl and KOH as titrants. Two apparent p*K*'s were determined from the sigmoid titration curves obtained by subtracting the solvent titration curve; values of p*K* at 3.1 and 11.1 at 1 M ionic strength and 25 °C were obtained. The hydrolysis of EDC at zero buffer concentration follows a complex pH profile (Figure 2) which fitted a kinetic expression (eq 4). Hydrolysis of EDC is buffer dependent, and the data

$$k = 10^{-3}/(1 + [H^+]/10^{-3.2}) + 4 \times 10^{-6} + 2.5 \times 10^{-2}[OH^-] \qquad (4)$$

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[7] A. Williams, "Introduction to the chemistry of enzyme action", McGraw-Hill, London, 1969, p 124.

Table I. Collection of Rate Constants for the Hydrolysis of Some Heterocumulenes

heterocumulene	k_H/ $M^{-1}\,s^{-1}$	$k_{OH}/M^{-1}\,s^{-1}$
diisopropylcarbodiimide[a-c]	63	1.1×10^{-3}
di-*n*-propylcarbodiimide[a-c]	630	1.4×10^{-2}
ETC[a-c]	320	0.16
PTC[a,b]	10	13
isocyanic acid[a,e,8b]	0.16	980
carbon dioxide[i]		4000[h]
carbon oxysulfide		4.2[j]
carbon disulfide		$1.1\ 10^{-3}$[j]
phenyl isothiocyanate[d]		0.125
phenyl isocyanate[f,8b]		7.5×10^{3}
diphenylcarbodiimide[g,10]		3.2×10^{-2}
1-cyclohexyl-3-(2-morpholinoethyl)-cabodiimide methotoluenesulfonate[9]		0.65

[a] Ionic strength 1 M, 25 °C. [b] This work; error limits on the data ±5%. [c] An upper limit on the water term for these substrates is $10^{-6}\,s^{-1}$. [d] Value for 30 °C. A. M. Kardos, J. Volke, and P. Kristian, *Collect. Czech. Chem. Commun.*, **30**, 931 (1965). [e] Water term $7.9 \times 10^{-2}\,s^{-1}$. [f] Water term $5 \times 10^{-2}\,s^{-1}$ data for 0.1 M ionic strength and 30 °C. [g] Data for 75% tetrahydrofuran/water at 20 °C. [h] M. B. Jensen, *Acta Chem. Scand.*, 13, 289 (1959). [i] Water term $1.45 \times 10^{-2}\,s^{-1}$; B. R. W. Pinsent, L. Pearson, and F. J. W. Roughton, *Trans. Faraday Soc.*, 52, 1512 (1956). [j] B. Philipp and H. Dautzenberg, *Z. Phys. Chem. (Leipzig)*, 231, 270 (1966).

Table II. Collection of Rate Constants for Attack of Nucleophiles on the Tautomers of EDC[a]

nucleophile	tautomer	rate constant
acetate ion	I	$0.12\ M^{-1}\,s^{-1}\ (k_{OAc})$
ethanolamine	II	$7.5 \times 10^{-5}\ M^{-1}\,s^{-1}\ (k_5)$
ethanolamine	I	$<3 \times 10^{2}\ M^{-1}\,s^{-1}\ (k_7)$
ethanolamine	IV (V)	$3.3 \times 10^{-3}\ M^{-1}\,s^{-1}\ (k_6)$
ethylamine	II	$1.8 \times 10^{-3}\ M^{-1}\,s^{-1}\ (k_5)$
ethylamine	I	$<4 \times 10^{4}\ M^{-1}\,s^{-1}\ (k_7)$
ethylamine	IV (V)	$8.3 \times 10^{-3}\ M^{-1}\,s^{-1}\ (k_6)$
water	I	$10^{-3}\,s^{-1}$
hydroxide	I	$3.2 \times 10^{5}\ M^{-1}\,s^{-1}$[b]

[a] Conditions 25 °C, 1 M ionic strength. [b] The rate constant for attack of hydroxide ion on I is kinetically indistinguishable from water reacting with II; the latter has the unimolecular rate constant $4 \times 10^{-6}\,s^{-1}$. The ionic product of water at 1 M ionic strength and 125 °C is $10^{-13.97}$ (S. Thea, N. Kashefi-Naini, and A. Williams, *J. Chem. Soc., Perkin Trans. 2* 65 (1981).

for the pH-profile are from extrapolation to zero buffer concentration. The buffers employed in the construction of the pH profile are quoted in the legend to Figure 1 and parameters in Table II.

Reaction of Acetate Buffers with EDC. EDC decomposes in aqueous buffers containing acetate with a pseudo-first-order rate constant which is proportional to the total acetate concentration up to 1 M. The reaction was followed by utilizing the large decrease in absorbance at 250 nm. No evidence of a square power term in acetate concentration was found in the concentration range studied. The pH dependences of the rate constant at 1 M total acetate is illustrated in Figure 2, and the data fit a kinetic equation (eq 5) involving the pK values 3.1 (K_1), 4.55 (acetic acid, K_a),

$$k_{obsd} = \frac{k_{OAc}[\text{total acetate}]}{(1 + [H^+]/K_1 + K_a/[H^+])} \qquad (5)$$

and k_{OAc} (0.12 $M^{-1}\,s^{-1}$). No evidence of poor buffering capacity was observed over the whole of the pH range illustrated in Figure 2.

Reaction of Strong Amines with EDC. The reaction of EDC with ethanolamine and ethylamine buffers followed at 250 and 245 nm, respectively, exhibited good pseudo-first-order kinetics up to 90% of the total reaction. There is an overall *decrease* in absorbance. The rate constants were accurately first order in the concentration of amine from 0.1 to 1.0 M. The slopes of the rate constants vs. total amine buffer concentration depend on the fraction of amine present as base in a nonlinear law (Figures 3 and 4). The kinetic rate law governing these results is discussed later.

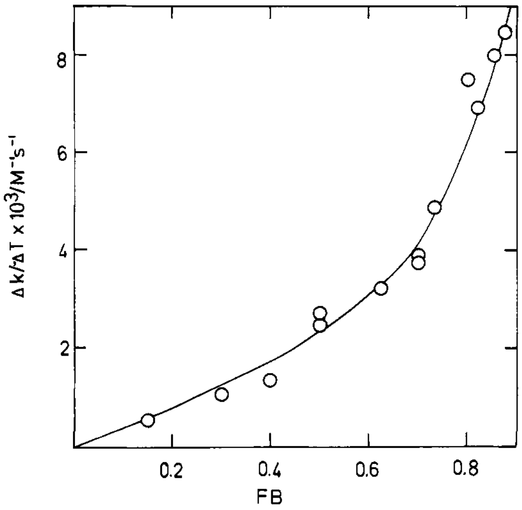

Figure 3. Variation of the slope of the pseudo-first-order rate constants for reaction with EDC against the concentration of total ethanolamine ($\Delta k/\Delta T$) as a function of the fraction of amine present as base (FB). The line is theoretical from eq 13 by using parameters in Table II and equilibrium constants given in the text (conditions: ionic strength 1 M, 25 °C).

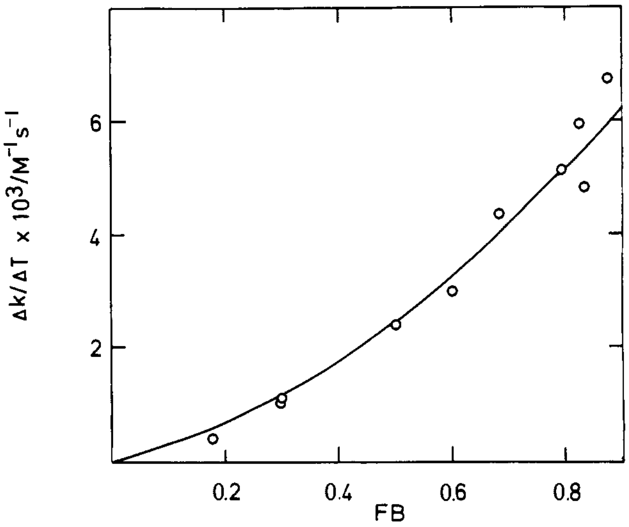

Figure 4. Reaction of ethylamine with EDC (details and conditions as in Figure 3).

Discussion

Model Carbodiimides. The reactivities of the model compounds are readily understandable; steric factors in both acid and base catalysis limit the attack of oxygen at the carbon center causing the isopropyl to be less reactive than the normal isomer. The alkaline hydrolysis of ETC is tenfold faster than the di-*n*-propyl case probably due to the electrostatic effect between the reacting ions. The hydrolysis of the phenyl derivative (PTC) is more rapid in alkali than the alkyl analogue (ETC), and this is analogous to the greater reactivity of phenyl isocyanates[8a] compared with that of the alkyl species.[8b] An explanation of this difference in reactivity has not so far been discussed for the isocyanate case, and we believe that the enhancement in the carbodiimide arises from a similar cause, namely, that the phenyl group is conjugated more effectively with the amidino-like transition state than with

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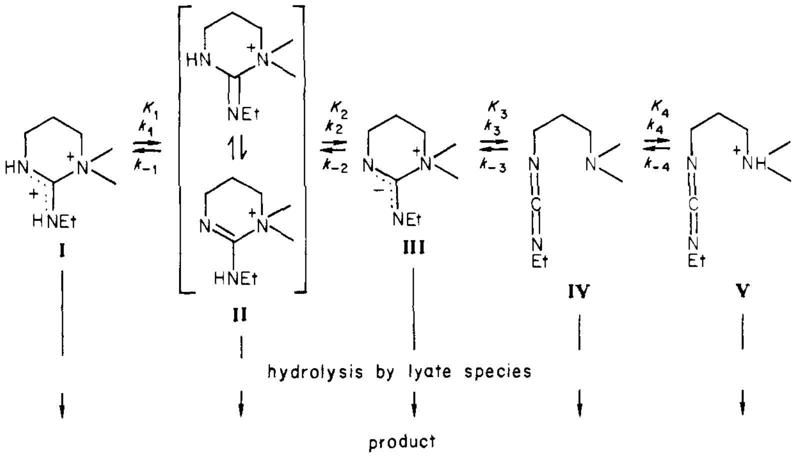
[8] (a) J. Longridge, unpublished observations. (b) A. Williams and W. P. Jencks, *J. Chem. Soc., Perkin Trans. 2*, 1753 (1974).



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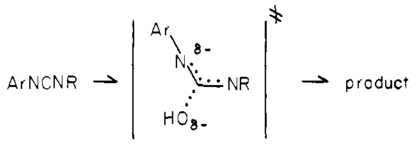
the carbodiimide ground state because of the larger π system in the former state.



(6)

Table I reveals that aliphatic carbodiimides have a reactivity towards hydroxide ion which is close to that of carbon disulphide and isothiocyanates; the oxygen containing heterocumulenes are generally much more reactive.

Our results for the carbodiimides are in agreement with the reactivity to acid and base measured by other workers using different media.[9,10]

Titration of EDC. The ionization and tautomeric equilibria of the water-soluble species (EDC) may be summarized in (eq 7); the tautomeric species react with the various lyate forms of the solvent to yield the urea product. With use of the data of Tenforde and his co-workers for aqueous solutions, the proportion of free carbodiimide in the pH region 7–10 is 0.07.[4] With the assumption that both III and IV are present in only negligable proportions, then the ratio [V]/[II] = 0.07/(1 - 0.07) = 0.075 = K_2K_3/K_4. The equilibrium constants are defined as follows: $[\mathrm{II}][\mathrm{H}^+]/[\mathrm{I}] = K_1$; $[\mathrm{III}][\mathrm{H}^+]/[\mathrm{II}] = K_2$; $[\mathrm{IV}]/[\mathrm{II}] = K_3$; $[\mathrm{IV}][\mathrm{H}^+]/[\mathrm{V}] = K_4$. We treat the tautomers in parentheses in eq 7 as a single entity for ease of working. Assuming K_4 is a normal ionization constant for a dimethyl tertiary amine (the p*K* of *N*,*N*-dimethylpropylamine is 10.1),[11] we may estimate the product K_2K_3 to be 6×10^{-12} ($10^{-11.2}$).

With the assumption of negligible hydrolysis during the titration and a mobile equilibrium between the species of eq 7, the pH dependence of I is given by eq 8. The p*K* of the ammonioamidinium ion I may be calculated approximately from the data of Charton;[12] this leads to an estimate of 3.83 taking the ρ_I of the ammonium substituent to be 0.73.[13] Charton's correlation is based on data bunched together over a small range of p*K* values[12] and a conservative estimate of the error would be ±2 p*K* units; this encompasses the value of 3.1 and 3.2 determined titrimetrically and kinetically. The good correlation between kinetic and thermodynamic p*K* values indicates that the former is not the result of a change in rate-limiting step but results from an ionization. The agreement between the calculated and observed p*K* values also indicates that the latter value is not a composite one. We are therefore justified in making the assumption that the species is not a major one in the pH range 7–10. Substituting values of K_2K_3 ($10^{-11.2}$) and K_4 ($10^{-10.1}$) in eq 8 confirms that the experimental titration involves a simple ionization (K_1).

$$\mathrm{I} = ([\mathrm{EDC}]K_4[\mathrm{H}^+])/\{K_1K_2K_3(1 + K_4/[\mathrm{H}^+] + K_4/[\mathrm{H}^+]K_3 + K_4/K_2K_3 + [\mathrm{H}^+]K_4/K_1K_2K_3)\} \quad (8)$$

The value of 11.1 for the higher titration p*K* of EDC is high for the p*K* of a normal *N*,*N*-dimethyl tertiary amine (see earlier discussion); the apparent p*K* may be derived from the expression for the concentration of IV as a function of hydrogen ion concentration (eq 9). With use of values already estimated, the term

$$\mathrm{IV} = \mathrm{EDC}K_4/([\mathrm{H}^+] + K_4 + K_4/K_3 + K_4[\mathrm{H}^+]/K_2K_3 + [\mathrm{H}^+]^2K_4/K_1K_2K_3) \quad (9)$$

$[\mathrm{H}^+]K_4/K_1K_2K_3$ is negligible between pH 8 and pH 14. The value of $[\mathrm{H}^+]$ corresponding to the apparent titration p*K* is given by expression 10. Substituting values for K_2K_3 ($10^{-11.2}$) and K_4

$$[\mathrm{H}^+] + [\mathrm{H}^+]K_4/K_2K_3 = K_4 + K_4/K_3 \quad (10)$$

($10^{-10.1}$) and $[\mathrm{H}^+]$ at half neutralization ($10^{-11.1}$) leads to a value of $K_3 = 26$; since $K_2K_3 = 10^{-11.1}$, K_2 is then $10^{-12.5}$; these values will of course possess considerable error and are probably correct to within 1 order of magnitude.

The Hydrolysis of EDC. The reagent (EDC) hydrolyzes over a pH range via three different mechanisms as evidenced by three regions in the pH profile at zero buffer concentration (Figure 2): the region between pH 6 and 10 (A); the acid plateau region (B), and the alkaline region (C).

Let us consider first the mechanism for *region A*: the hydrolysis of ETC the analogue of V, has an upper limit for water attack of 10^{-6} s^{-1}. It is doubtful whether we could obtain this rate constant accurately because there is no evidence that the observed rate constants are any greater than twice that at the crossover point between oxonium and hydroxide ion catalysis (Figure 1). The upper limit, however, fits a Brønsted-type relationship for the reactivity of several nucleophiles against ETC.[14] The rate constant arising from the mechanism through water attack on V therefore has the upper limit, $10^{-6}[\mathrm{V}]/[\text{total EDC}] = 10^{-7}$ s^{-1}. The observed value of 4×10^{-6} s^{-1} for the region A indicates that the carbodiimide mechanism for this region of pH can only at most take one-fortieth of the total reaction flux. We are not able to prove that the reaction flux is taken by water attack on II or the kinetically equivalent hydroxide attack on I for this pH region. We shall see later that acetate ion reacts with I but not with II; water, which is much less nucleophilic than acetate, is therefore unlikely to react with II, and we believe that region A involves attack of hydroxide ion on I (Table II).

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(9) D. G. Knorre, V. A. Kurbatov, G. S. Mushinskaya, and E. G. Saikovich, *Izv. Sib. Otd. Akad. Nauk SSSR., Ser. Khim. Nauk*, 137 (1967).

(10) S. Hünig, H. Lehmann, and G. Grimmer, *Justus Liebigs Ann. Chem.* **579**, 77, 87 (1953).

(11) J. Hansson, *Sven. Kem. Tidskr.*, **67**, 256 (1955); we synthesized *N*,*N*-dimethyl-*N′*-(3-(dimethylamino)propyl)urea from dimethylcarbamoyl chloride and the appropriate amine (bp 113–115 °C (0.4 torr); $n^{25}{}_\mathrm{D} = 1.4735$; anal. Calcd for $C_8H_{19}N_3O$ C, 55.5; H, 11.0; N, 24.3; found C, 55.5; H, 10.7; N, 24.3) and determined the p*K* at 25 °C by using 1 M ionic strength (p*K* = 10.1).

(12) M. Charton, *J. Org. Chem.*, **30**, 969 (1965).

(13) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).

(14) I. T. Ibrahim and A. Williams, unpublished observations.

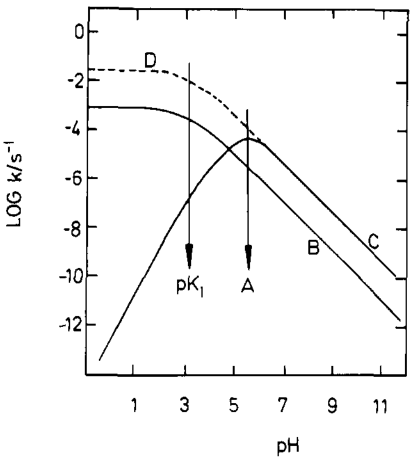

Figure 5. Hypothetical pH dependence of the rate constant for hydrolysis of EDC through oxonium ion catalyzed hydrolysis of V (line C). The dotted line D represents the rate constant without "K_3" being a rate-limiting step. Line B represents the observed decomposition of I. Point A marks the pH where the change in rate-limiting step occurs; the slope of the line between A and pK_1 is unity and +2 below pK_1. At pHs less than A the line C also measures the attack of acetate ion on V. The position of A will be different for acetolysis and hydrolysis but is hypothetical in this diagram.

For region B the species (I to V) are assumed to be in mobile equilibrium, and the hydrolysis is due to proton-catalyzed reaction with V; we may then estimate the rate constant for the "acid" plateau from the model reaction of ETC and oxonium ion. The rate expression for the hydrolysis through V is given in eq 11.

$$\text{rate} = k_H[V][H^+] = ([\text{total EDC}][H^+]k_H)/(1 + K_4/[H^+] + K_4/[H^+]K_3 + K_4/K_2K_3 + [H^+]K_4/K_1K_2K_3) \quad (11)$$

When $[H^+] > K_4$, expression 11 leads to a value for the rate constant for the acid plateau region of $k_HK_1K_2K_3/K_4$; substituting the value of k_H for (ETC) and the values for the equilibrium constants gives a rate constant 2×10^{-2} s^{-1} which is 20-fold *larger* than the observed value. This result is very interesting because a mechanism different from the model proposed in eq 11 should give a rate constant larger than the calculated one. We believe that the pathway taken by the major part of the reaction flux is through I, and the reason for the higher rate constant predicted from eq 11 is the neglect of a change in rate-limiting step in that mechanism. The step most likely to limit the reaction through V is that between II and IV since the others in the scheme (eq 7) are proton-transfer steps to and from electronegative atoms; the "K_3" step is pH independent. A change in rate-determining step can occur because the decomposition of V through reaction with oxonium ions will increase with a decrease in pH, and the rate constant for production of III from V will decrease with decrease in pH. We are not able to estimate the pH at which the change in rate-limiting step will occur as this requires a knowledge of k_3 (see eq 7), but it must be greater than neutrality and probably less than about pH 10 (see later discussion). Figure 5 illustrates a hypothetical pH dependence for the reaction through V.

Since the observed rate constant for the "acid" plateau region is lower than that predicted for the reaction through V under equilibrium conditions, it is possible that the mechanism could still hold and the lower rate constant could be due to the change in rate-limiting step. However, the pH dependence at pH less than 3.1 should involve a logarithmic plot of slope +2 (Figure 5). We are therefore confident that the plateau is caused by reaction through species I.

In the alkaline region of pH (region C) the hydrolytic reactivity of EDC follows that of the neutral model *N*,*N'*-di-*n*-propylcarbodiimide. Assuming that the species (I to V) are in equilibrium and using the values of the equilibrium constants already deduced, we estimate that the ratio of reagent present as IV and III is greater than 10:1. We should therefore expect a rate constant approximately the same as that of the neutral model; the closeness of the model rate constants (Figures 1 and 2) leads us to suppose that alkaline hydrolysis passes through the carbodiimide IV.

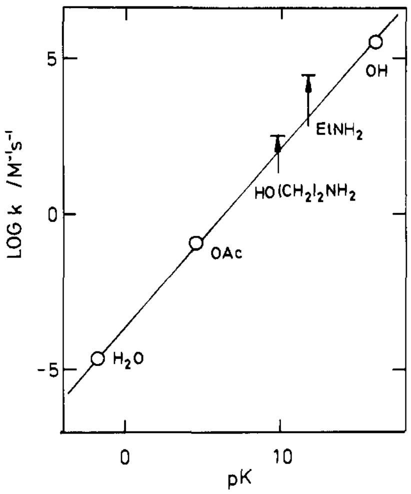

Figure 6. Brønsted-type dependence for the reaction of nucleophiles with I. The line is arbitrary and has a slope of 0.56 (data and conditions in Table II).

Reaction of Acetate Buffers with EDC. Attack of acetate ion on the dication I is believed to be the mechanism for the reaction of acetate buffers with EDC. This mechanism is consistent with the linear dependence of rate constant on total acetate concentration; reaction with a bona fide carbodiimide, namely ETC involves a squared term in acetate concentration.[15a] Acetate attack on ETC involves an increase in absorbance at 250 nm,[15a] whereas the attack on EDC involves a decrease.

The rate constant for attack on ETC buffer at one molar concentration and pH 5.4 is 0.63 s^{-1}.[15b] The proportion of V, calculated for pH 5.4 by using the equilibrium constants deduced above, is 0.071 and leads to a predicted rate constant for the reaction of EDC with acetate through V of $0.071 \times 0.63 = 4.5 \times 10^{-2}$ s^{-1}; this is larger than the observed value at pH 5.4.[15c] The difference in magnitude between observed and calculated quantities is only small, but we believe it to be significant. Consideration of the rate constant for reaction of acetate with V obtained from the model (0.63 s^{-1})[15b] indicates that it is considerably larger than that estimated for the attack of proton at that pH ($3.2 \times 10^2 \times 10^{-5.4} = 1.3 \times 10^{-3}$ s^{-1}). In the region of pH in question proton attack is not the rate-limiting step, and therefore we can conclude that acetate attack at the concentration in question is also not rate limiting. These considerations lead to two predictions for the carbodiimide pathway for acetate reaction which are not fulfilled: (a) there should be no dependence of rate constant on acetate concentration; (b) the pH dependence of the rate constant (at 1 M total concentration) in the pH region in question should be linear with a slope of 1; this corresponds to the ionization (K_2) required to yield III prior to the pH-independent rate-limiting step k_3. At pHs below 3.1 the slope of the pH profile will be 2 corresponding to the effect of the two ionizations prior to III (K_1 and K_2) (see Figure 6).

We cannot exclude the kinetically equivalent mechanism, namely, attack of acetic acid on the monocation II. It is difficult to see how this reaction could occur except by concerted proton transfer (VI); under these circumstances there would be no necessity to invoke a concerted mechanism[16-18] as at the pHs concerned the amidine will undergo a fast protonation to yield a

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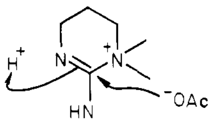
(15) (a) I. T. Ibrahim and A. Williams, *J. Chem. Soc., Chem. Commun.*, 25 (1980). (b) This value is calculated from data for attack of acetate ion on ETC.[15a] (c) The observed value (see Figure 2) is 7.1×10^{-3} s^{-1}.

(16) W. P. Jencks, *Acc. Chem. Res.*, **9**, 425 (1976).

(17) W. P. Jencks, *Acc. Chem. Res.*, **13**, 161 (1980).

(18) W. P. Jencks, *Chem. Rev.*, **72**, 705 (1972).

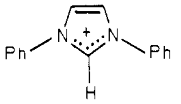


VI

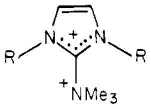
thermodynamically stable conjugate acid.

The adduct from the reaction of acetate ion with I is probably decomposed in the present case by fast reaction with excess acetate to yield the urea product and acetic anhydride which further decomposes to acetic acid; the absence of a squared term in total acetate concentration is consistent with this conclusion. When stoichiometric amounts of EDC and acetate are employed the pathway will involve either hydrolysis of the adduct or reaction with an amine (if present) by attack at the acetyl function.

There are no direct procedents in the literature to enable us to compare the rate constants for the reaction with nucleophiles on I. Attack at amidine centres has been investigated, however,[19,20] and water attack on 1,3-diphenyl-2-imidazolium chloride (VII)



VII



VIII

has the rate constant 10^{-6} s^{-1}.[20] Values of ρ_I for substituent change at C-2 (10) and N (19.3) may be estimated from data of DeWolfe[19] by using the Charton relationship ($\rho_I = 6.23\ \rho$ Hammett).[13] We may therefore estimate by using the rate constant for water attack on VII as the standard the hydrolysis rate constant for VIII a possible model for I. The estimated value (10^{-2} s^{-1}) is tolerably close to that observed in the acid plateau region. Of course there is considerable uncertainty in the comparison because VIII is a five-membered ring and is aromatic.

The data for the reaction of oxygen nucleophiles with I may be correlated with their p*K* by a Brønsted-type relationship (Figure 6).

Reaction with Strong Amines. The reaction of strong amines with bona fide carbodiimides exhibits simple second-order kinetics involving bimolecular reaction of the basic amine species with neutral substrate.[14] The kinetic rate law for the reaction of strong amines with EDC is more complicated than that of the carbodiimides due to the tautomerism. The results for both ethanolamine and ethylamine fit the theoretical equation for the mechanism where both ammonioamidine II and the carbodiimide tautomers IV and V react with the basic amine species (eq 12).

$$\begin{tikzcd}
\mathbf{I} \arrow[r, shift left, "\overset{K_1}{k_1}"] \arrow[drr, "k_7[\mathrm{RNH_2}]"'] & \mathbf{II} \arrow[l, shift left, "k_{-1}"] \arrow[r, shift left, "\overset{K_2}{k_2}"] \arrow[dr, "k_5[\mathrm{RNH_2}]"] & \mathbf{III} \arrow[l, shift left, "k_{-2}"] \arrow[r, shift left, "\overset{K_3}{k_3}"] & \mathbf{IV} \arrow[l, shift left, "k_{-3}"] \arrow[r, shift left, "\overset{K_4}{k_4}"] \arrow[dl, "k_6[\mathrm{RNH_2}]"'] & \mathbf{V} \arrow[l, shift left, "k_{-4}"] \arrow[dll, "k_6^{+}[\mathrm{RNH_2}]"] \\
 & & \text{products} & &
\end{tikzcd} \tag{12}$$

In order to estimate the various parameters, we make the reasonable assumption that the rate constants for reaction of neutral amine with charged (V) and neutral (IV) carbodiimides are identical ($k_6 = k_6{}^+$).

The kinetic equation consistent with the above mechanism, assuming a mobile equlibrium among the tautomeric species at the pHs in question, is given in eq 13, where [amine] and [EDC]

$$\text{rate} = [\text{amine}][\text{EDC}]\frac{(k_5(1 + [\mathrm{H}^+]/K_4) + k_6[\mathrm{H}^+]/K_2K_3)}{([\mathrm{H}^+]/K_2K_3 + 1/K_3 + 1 + [\mathrm{H}^+]/K_4)} \tag{13}$$

are the concentrations of free basic amine and *total* reagent, respectively. Using the parameters already determined, we may analyze the data to give the best fit for k_5 and k_6 and this is illustrated in Figures 3 and 4 and rate constants collected in Table II.

Attack of amines on amidinium ions is known,[21] but no reactivity studies using water medium have been made. The value of k_6 should approximate to rate constants for amine attack on regular carbodiimides.[14] Reaction of ETC with ethanolamine and ethylamine has been shown to possess the bimolecular rate constants 5.7×10^{-3} and 1.5×10^{-2} M^{-1} s^{-1}, respectively;[14] these values compare well with the values of k_6 for reaction of the same amines with IV or V (3.3×10^{-3} and 8.3×10^{-3} M^{-1} s^{-1}, respectively). The mechanism carrying the majority of the reaction flux changes with the fraction of basic amine (FB). At low FB the path through the ammonioamidine is predominant, but at high FB the carbodiimide pathway is the favored one.

In the above analysis we neglect the possibility of reaction of neutral amine with the dicationic species I; the parameter $\Delta k/\Delta T$ (see Figures 3 and 4) for this mechanism should be independent of pH up to the p*K* of the attacking amine. The absence of such an effect indicates that the cation does not contribute a significant proportion to the total reaction flux. The dicationic species I reacts with nucleophiles, and we may estimate an upper limit to the reaction of ethanolamine and ethylamine from the rate constant at the lowest pH measured by using eq 8. These upper limits are consistent with the rate constants for other nucleophiles (Figure 6).

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[19] (a) R. H. DeWolfe and M. W.-L. Cheng, *J. Org. Chem.*, **34**, 2595 (1969). (b) R. H. DeWolfe, *J. Am. Chem. Soc.*, **86**, 864 (1964).

[20] D. R. Robinson and W. P. Jencks, *J. Am. Chem. Soc.*, **89**, 7088 (1967).

[21] W. Kantlehnev, *Adv. Org. Chem.*, **9** (part 2), 321 (1976).