(13) Pentadeuteriocyclopentadiene (97.3% isotopic purity) was prepared by base catalyzed H–D exchange of cyclopentadiene with D_2O.
(14) Dry deuterium gas was slowly bubbled through a purple toluene solution containing $RuHCl(PPh_3)_3 \cdot CH_3Ph$ (1 g) and PPh_3 (7 g) at 90 °C for 2 days. After removal of the solvent under vacuum, tris(2,6-dideuteriophenyl)-phosphine was obtained by sublimation from the white, purple residue (90–100 °C, 0.01 mmHg), 6.1 g (87% yield, 97.8% isotopic purity): G. W. Parshall, W. H. Knoth, and R. A. Schunn, *J. Am. Chem. Soc.*, **91**, 4990 (1969).
(15) T. J. Katz, *Adv. Organomet. Chem.*, **16**, Chapter 9 (1977).
(16) Isolated by GLC. The isotopic purity was determined by mass spectrometry and the position of the label was determined by NMR spectroscopy.
(17) W. J. Kelly and N. Calderon, *J. Macromol. Sci., Chem.*, **9**, 911 (1975).
(18) The titanocene analogue **4** did not catalyze the formation of metathesis products. Whether this was due to rapid destruction of the methylene by H abstraction or to electronic effects will require further investigation.
(19) As further evidence, the intermediate produced from the reaction of 1,3-dibromobutane with $Ni(COD)_2$ yields ethylene (21%), methylcyclopropane (20%), and butenes (59%).[10,22]
(20) S. J. McClain, C. O. Wood, and R. R. Schrock, *J. Am. Chem. Soc.*, **99**, 3519 (1977).
(21) C. P. Casey and H. E. Tuinstra, *J. Am. Chem. Soc.*, **100**, 2290 (1978).
(22) P. Diversi, G. Ingrosso, and A. Lucherini, *J. Chem. Soc., Chem. Commun.*, 52 (1977).
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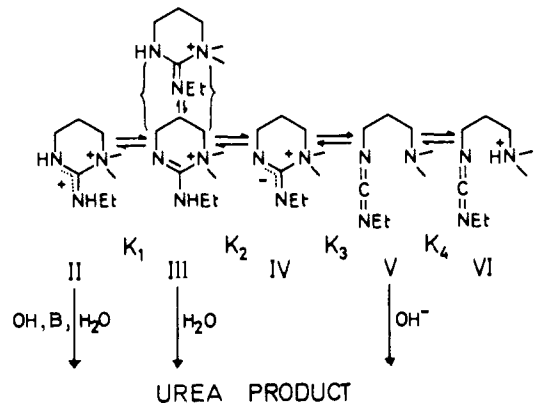
Reaction of the Water-Soluble Reagent *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide with Nucleophiles: Participation of the Tautomeric Cyclic Ammonioamidine as a Kinetically Important Intermediate

Sir:

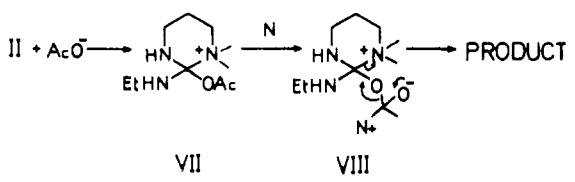
Sheehan, Cruickshank, and Boshart[1] reported that *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (I), a water-soluble peptide coupling reagent, could undergo ring-chain tautomerism. This hypothesis was confirmed experimentally by Tenforde, Fawwaz, Freeman, and Castagnoli[2a] who used spectroscopic evidence to show that only some 7% of the total reagent exists as carbodiimide in neutral aqueous solution.

This communication reports data on the reactions of acetic acid and water with I consistent with the mechanisms of Schemes I and II where the only participation of free carbodiimide is in the alkaline region of pH.

Scheme I



Scheme II



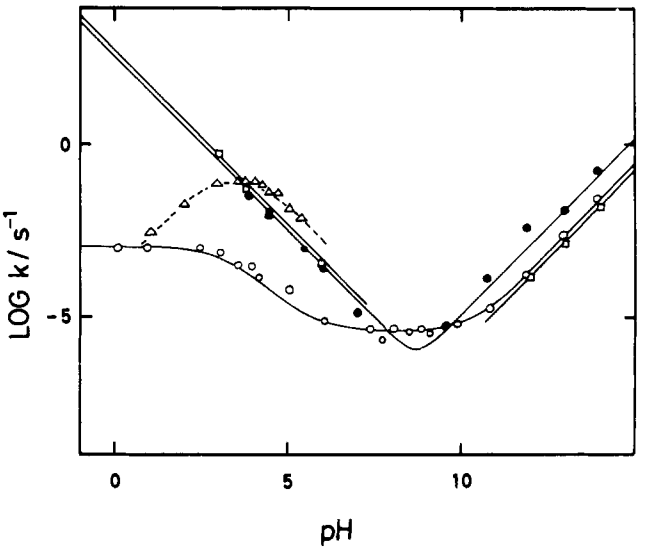


Figure 1. Dependence of rate constant on pH for hydrolysis of I (○); *N*-ethyl-*N'*-(3-trimethylammoniopropyl)carbodiimide (●); *N,N'*-di-*n*-propylcarbodiimide (□). The pH dependence for the reaction of I in 1 M acetate buffer (△). Lines for the hydrolyses of carbodiimides are theoretical and are from the following equations: $k = 4 \times 10^{-6} + 10^{-3}/(1 + 10^{-3.2}/a_H) + 2.5 \times 10^{-2}[OH^-]$ (○); $k = 320a_H + 0.14[OH^-]$ (●); $k = 400a_H + 1.4 \times 10^{-2}[OH^-]$ (□). Conditions are 25 °C, aqueous solution, ionic strength made up to 1 M with KCl.

We report here the pH profiles for hydrolysis of *N*-ethyl-*N'*-(3-trimethylammoniopropyl)carbodiimide perchlorate, *N,N'*-di-*n*-propylcarbodiimide, and the coupling reagent I at zero buffer concentration; good pseudo-first-order kinetics were observed which obey equations given in the legend to Figure 1.

Water hydrolysis of the trimethylammonio carbodiimide model for VI has an upper limit for the rate constant (10^{-6} s^{-1}) close to that estimated from the Brønsted plot for nucleophilic attack.[2b] The proportion of free carbodiimide in the region of pH 7–10 present as the form VI is ~0.1 using data from Tenforde and co-workers.[2a] Thus $[VI]/[III]$[2c] $= K_2K_3/K_4 \approx 0.1$ and, assuming K_4 represents a normal pK_a for a dimethyl tertiary amine (9.99 for dimethylpropylamine),[3] then $K_2K_3 \approx 10^{-11}$. The rate constant for the mechanism through water attack on the carbodiimide VI therefore has the upper limit $0.1 \times 10^{-6} = 10^{-7}$ s^{-1}; the observed value of 4×10^{-6} s^{-1} indicates that the carbodiimide mechanism for this region of pH can only take at most 1/40 of the total reaction flux. At this stage we are not able to say whether the plateau is due to water attack on III or hydroxide attack on II.

Titration of reagent I with acid and base reveals two acidic groups of $pK_a = 3.1$ and 11.1, respectively. We may calculate a pK_a for an ammonioamidinium dication such as II, using the data of Charton,[4] to be 3.83; we take the σ_I of the ammonio substituent to be 0.73.[5a] Perusal of Charton's correlation indicates that a conservative estimate of error would be ±2 pK units. The apparent titration pK_a in the acid region may be derived from Scheme I (eq 1); substituting for K_2K_3 and K_4 leads to an apparent pK_a corresponding to K_1 which therefore has a value (3.1) consistent with that for ammonioamidinium ionization within the limits of the prediction.

$$[II] = \frac{[\text{total I}]K_4a_H}{K_1K_2K_3(1 + K_4/a_H + K_4/a_HK_3 + K_4/K_2K_3 + a_HK_4/K_1K_2K_3)} \quad (1)$$

The value of 11.1 is high for the pK_a of a dimethyl tertiary amine.[3] The apparent pK_a may be derived assuming equilib-

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rium from Scheme I with an equation similar to eq 1;[5b] using the known values for K_2K_3 and K_4 we may estimate K_2 and K_3 to be $<10^{-11}$ and >1, respectively. The ionization pK_a for the amidine (K_2) is relatively low presumably owing to the powerfully electron-withdrawing ammonio group.

Assuming equilibrium of the species II-VI and rate-limiting proton-catalyzed hydrolysis of the free carbodiimide VI, we may estimate the acid plateau rate constant (pH 0-4) from proton attack on the trimethylammonio model ($k_H = 3 \times 10^2$ M^{-1} s^{-1}). The overall rate constant for reagent I becomes $k_H K_1 K_2 K_3 / K_4$[5c] in the acid plateau region which gives 3×10^{-2} s^{-1} on substituting values for the equilibrium constants and k_H. The estimated value is much larger than that observed (10^{-3} s^{-1}) indicating that one of the steps K_1-K_4 is rate limiting for the carbodiimide mechanism. The decomposition rate constant for VI increases with decrease in pH and it is likely that K_3 becomes the rate-limiting step because this is pH independent and the other steps involve proton transfer to and from electronegative atoms. The overall rate constant for production of V is hydroxide ion dependent and the observation of the low, pH independent rate constant in the acid region is therefore consistent with a different mechanism, namely water attack on the ammonioamidinium dication (II).

Hydrolysis of I in the alkaline pH region follows the hydrolysis of the neutral model *N,N'*-di-*n*-propylcarbodiimide. Assuming that species II-VI are in equilibrium and using values of the equilibrium constants deduced above, we estimate that the fraction of reagent I present as V is 50% in the alkaline region; the other 50% is IV. Thus we should expect an observed rate constant some 50% less than that of the model at the corresponding pH. The close proximity of the data (Figure 1) confirms that the alkaline hydrolysis proceeds via the neutral carbodiimide V.

Attack of acetate (1 M) buffer on the trimethylammoniocarbodiimide at pH 5.4 has the rate constant 1.6×10^{-3} s^{-1}; at pH 5.4 the proportion of protonated carbodiimide VI is calculated to be 0.091 using the equilibrium constants determined above. The predicted rate constant for I with 1 M acetate is thus $0.091 \times 1.6 \times 10^{-3} = 1.46 \times 10^{-4}$ s^{-1}. This is considerably less than the observed value (see Figure 1) and the reaction is therefore considered to proceed via attack of acetate ion on the ammonioamidinium dication (II) as in Scheme II. The adduct from this reaction (VII) is probably decomposed by reaction with further acetate to yield acetic anhydride or by other nucleophiles.

The observation of buffer catalysis in decomposition of I in the region pH 3.5-7 is consistent with other observations of catalysis of amidinium ion hydrolysis.[6,7] We have shown that regular carbodiimides hydrolyse without buffer effects.[2b]

The data of DeWolfe[6] and Robinson and Jencks[7] suggest that the reactivity of II with water is consistent with amidinium ion hydrolysis.[8]

The water-soluble carbodiimide I and its analogues have been used in peptide synthesis[10] and in protein modification.[11] While the fate of the initial complex (analogous to VII) via direct attack by nucleophile or via anhydride is yet to be determined, this work indicates that initial reaction occurs at neutral and acid pH's via the ammonioamidinium dication and not via carbodiimide.

We point out here the possible synthetic utility in dehydration reactions of amidinium cations activated by powerful electron-withdrawing substituents.

References and Notes

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

(2) (a) T. Tenforde, R. A. Fawwaz, N. K. Freeman, and N. Castagnoli, *J. Org. Chem.*, **37**, 3373 (1972). (b) I. T. Ibrahim and A. Williams, unpublished work. (c) At neutral pH the major species will be III and VI; strictly, the ratio of [VI]/[III] = 0.07/(1-0.07) and this is rounded to 0.1 since the arguments do not involve small differences.

(3) H. K. Hall, *J. Am. Chem. Soc.*, **79**, 5441 (1957).

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

(5) (a) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964). (b) $[V] = [\text{total I}]K_4/(a_H + K_4 + K_4/K_3 + K_4a_H/K_2K_3 + a_H^2K_4/K_1K_2K_3)$. At pH 8-14 the term $a_H^2K_4/K_1K_2K_3$ is negligible and the value of a_H corresponding to the apparent titration pK_a is given by $a_H + a_HK_4/K_2K_3 = K_4 + K_4/K_3$; substituting values for K_2K_3 and K_4 yields K_3 and then K_2. (c) The rate expression for the hydrolysis of I via acid attack on VI is $\text{rate} = [\text{VI}]k_Ha_H = [\text{total I}]k_Ha_H/(1 + K_4/a_H + K_4/a_HK_3 + K_4/K_2K_3 + a_HK_4/K_1K_2K_3)$. Substituting values for the equilibrium constants reduces this expression to that in the text when $a_H > K_1$.

(6) (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).

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

(8) Robinson and Jencks[7] find a rate constant of 10^{-6} s^{-1} for ater attack on 1,3-diphenyl-2-imidazolium chloride. Allowing ρ_I values[9] of 10 and 19.3 for substituent change on C-2 and nitrogen, respectively, we may estimate a rate constant for attack of water on a C-2 ammonio-substituted tetraalkylamidinium dication of type II. The estimated value (10^{-2} s^{-1}), in view of the large assumption made, is remarkably close to the rate constant observed in the acid plateau region.

(9) Values of ρ_I are estimated from the data of DeWolfe[6] using the Charton relationship $\rho_I = 6.23\rho_{\text{Hammett}}$.[5a]

(10) H. D. Law, "The Organic Chemistry of Peptides", Wiley-Interscience, London, 1970.

(11) (a) D. G. Hoare and D. E. Koshland, *J. Biol. Chem.*, **242**, 2447 (1967); (b) T. E. Banks, B. K. Blossey, and J. A. Shafer, *ibid.*, **244**, 6323 (1969); (c) D. G. Hoare, A. Olson, and D. E. Koshland, *J. Am. Chem. Soc.*, **90**, 1638 (1968).

(12) I.T.I. thanks the Iraqi government for a postgraduate scholarship.

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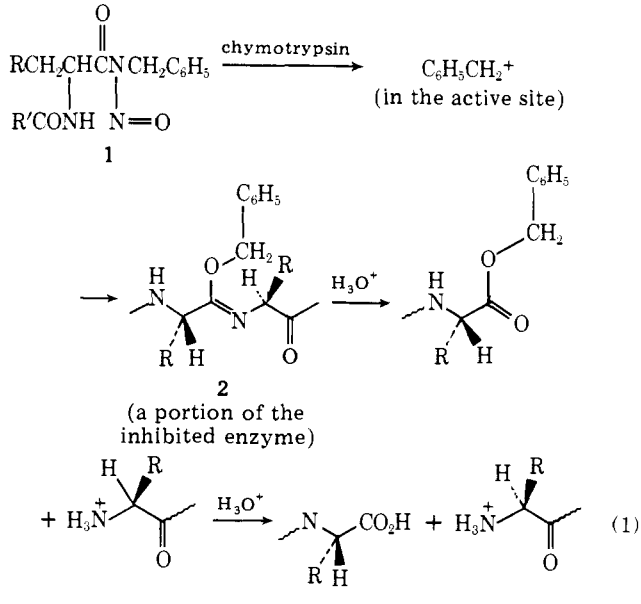
Labeling of Amide Linkages in Active Site Mapping: Carbonium Ion and Extended Photoaffinity Labeling Approaches

Sir:

Nitrosoamides of amino acids (**1**) are active site directed inhibitors[1] of the suicide type[2] for the enzyme α-chymotrypsin. Work with model systems now show that the carbonium ions generated by these reagents alkylate amide linkages along the backbone of the protein to give preferentially O-alkylation. The resulting imidate ester groups (**2**) are readily hydrolyzed at pH ~5 to give the amine and carboxylic acid fragments[3] (eq 1). Thus, each "hit" leads to a break in the chain at that point to give two unlabeled peptides.

An important aspect of these observations is that the normal peptides resulting from imidate hydrolysis can be analyzed by standard techniques. Thus, end-group analysis would lead to



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