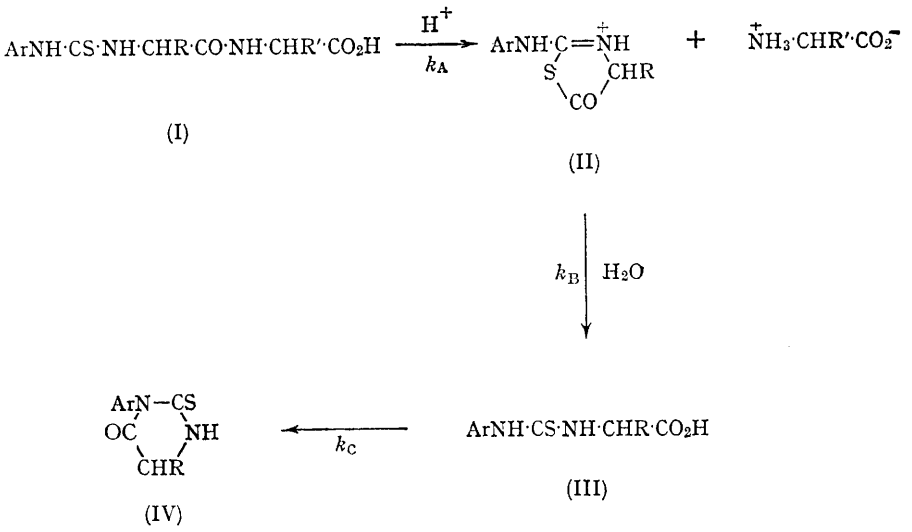
Kinetics and Mechanism of the Edman Degradation

By D. BETHELL, G. E. METCALFE, and R. C. SHEPPARD
(*Robert Robinson Laboratories, University of Liverpool*)

THE Edman procedure[1] for *N*-terminal stepwise degradation of polypeptides finds widespread use in structural studies. Although Edman himself has discussed the mechanism of the reaction,[2] few quantitative data are available. With a view to providing a basis for improving the procedure, we have undertaken a kinetic re-examination of the mechanism of the degradation in aqueous solution using leucylglycine as the model peptide.



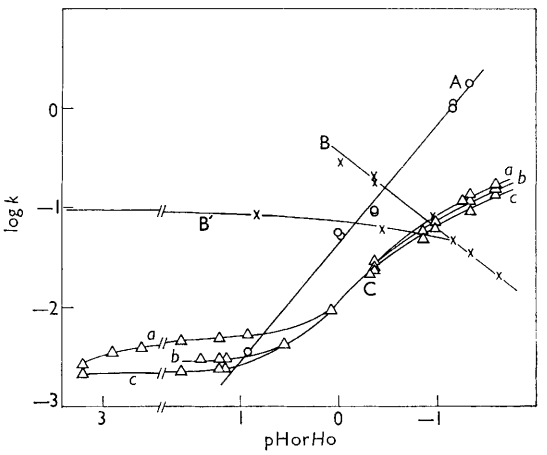
R=$Me_2CH·CH_2$; R′=H; a, Ar=*p*-Cl·C_6H_4; b, Ar=Ph; c, Ar=*p*-MeO·C_6H_4

On treatment with aqueous perchloric (or hydrochloric) acid the *para*-substituted phenylthiocarbamoyl-leucylglycines (Ia,b,c; λ_{max} 245 mμ, $\epsilon \approx 13{,}000$) were quantitatively converted into the corresponding 3-arylthiohydantoins (IV a,b,c; λ_{max} 265mμ, $\epsilon \approx 16{,}000$). Observation of the ultraviolet spectrum of reaction mixtures as a function of time showed rapid disappearance of the peak due to (I) with concurrent, transient increase in absorption around 230 mμ. As this increased absorption decayed, a second transient increase occurred at around 245 mμ, absorption at 265 mμ increasing continuously thereafter until the reaction was complete. The wavelengths of the transient absorptions correspond to those of protonated 2-anilino-4-isobutylthiazolinone (II; shoulder at 228 mμ, $\epsilon \approx 11{,}000$) and phenylthiocarbamoyl-leucine (III; λ_{max} 245mμ, $\epsilon_{max} \approx 13{,}000$), supporting Edman's suggestions[2] concerning the course of the reaction (see Chart).

Confirmation of the correctness of the scheme comes from the kinetic results. The intermediates (II) and (III) have been synthesised as their bisulphate or chloride and dicyclohexylammonium salts respectively, thus enabling the kinetics of this hydantoin formation from (I), (II), and (III) to be studied separately. Values of k_C were determined spectrophotometrically by direct observation of the reaction (III) → (IV). Treating the conversion (II) → (III) → (IV) as a sequence of irreversible, first-order reactions and observing the

[1] P. Edman, *Acta Chem. Scand.*, 1950, **4**, 283.
[2] (*a*) P. Edman, *Nature*, 1956, **177**, 667; *Acta Chem. Scand.*, 1956, **10**, 761; (*b*) D. Ilse and P. Edman, *Austral. J. Chem.*, 1963, **16**, 411.

time (t) at which the concentration of (III) reached its maximum value, k_B was calculated from the equation[3] $t = (k_C - k_B)^{-1} \ln k_C/k_B$. Values of k_A were determined in an analogous fashion: within the experimental error, values so obtained agreed with those determined by direct observation of the rate of appearance of glycine. This agreement supports our assumptions concerning the irreversibility of the three steps and indicates that there are no competing routes for transforming (I) into (IV). The variation of k_A, k_B, and k_C with the acidity of the reaction medium as measured by pH or H_0* is shown in the Figure.



Dependence of velocity constants, k (*min.*$^{-1}$), *on acidity:*
A ⊙ *Peptide cleavage*
B × *Thiazolinone hydrolysis* (*B', constant ionic strength*)
C △ *Thiohydantoin formation from* p-*chloro-* (a), *unsubstituted-* (b), *and* p-*methoxy-* (c) *phenylthiocarbamoylleucine.*

We draw attention to the following points:

(i) At acid concentrations greater than ~0·2M, the conversion (III) → (IV) is much slower than the peptide cleavage reaction, (I) → (II), the rate difference becoming progressively greater as the acid concentration increases. In the past, users of the Edman procedure have often adopted acidic conditions which optimise production of thiohydantoin.[5] In so doing they may use more severe conditions than are necessary for cleavage, and this could lead to further reaction of the residual peptide by normal hydrolysis with consequent low recovery (*cf.* ref. 2b).

(ii) The peptide cleavage shows all the characteristics of a normal A-1 reaction, *viz.*, $k_A \propto (h_0)^{1,2}$, $\Delta S^{\ddagger} = -4$ cal. mole.$^{-1}$ deg.$^{-1}$, $k_A^{H_2O}/k_A^{D_2O} = 0{\cdot}6$. Somewhat surprisingly *para*-substituents in the phenyl group have a negligible effect on the reaction rate.

(iii) The hydrolysis of (II) is inhibited in strongly acidic media. By maintaining a constant ionic strength (by addition of sodium perchlorate) this variation is much reduced (curve B'). Similar observations have been made on the hydrolysis of succinic anhydride.[6] Again there is no effect due to substituents.

(iv) Cyclization of (III) shows a rate plateau between pH 3 and 1, and marked curvature below $H_0 = -1$. Analysis of the curves Cabc indicates that, down to pH 1, the phenylthiocarbamoylamino-acid, but not its anion, cyclises (pK_a of IIIa estimated to be 3·38). In more acidic media a further route becomes available involving protonation, presumably on the carboxyl group. Analysis of the kinetic results in this region suggests that the curvature† may be due to appreciable protonation of (III). The estimated pK_a of the protonated form is $\sim -1{\cdot}6$, too high to be a protonated carboxyl group and suggesting that the deviation from linearity and order of substituent effects may be due to protonation of the thioureido-group (reported pK_a values: $C_6H_5.NH.CS.NH_2$,[7] −2·3; $CH_3.NH.CS.NH_2$,[8] −1·12).

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* Protonation of amides is not well correlated by H_0,[4] but should only be shown by linear log k against H_0 plots of non-unit slope.

† Plots of log k against log $[HClO]_4$ in this region are approximately linear, but with slopes in the range 1·4—1·6 for IIIabc. We prefer, however, to discuss the results in terms of H_0 here.

[3] A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," Wiley, New York, 1953, p. 153.
[4] J. T. Edward and I. C. Wang, *Canad. J. Chem.*, 1962, **40**, 966; A. R. Katritzky, A. J. Waring, and K. Yates, *Tetrahedron*, 1963, **19**, 465; K. Yates, J. B. Stevens, and A. R. Katritzky, *Canad. J. Chem.*, 1964, **42**, 1957.
[5] See, for example: H. Fraenkel-Conrat, J. I. Harris, and A. Levy in "Methods of Biochemical Analysis," Vol. II, Ed. D. Glick, Interscience, New York, 1955, p. 359; J. I. Harris and V. I. Ingram in "A Laboratory Manual of Analytical Methods of Protein Chemistry," Ed. P. Alexander and R. J. Block, Pergamon, Oxford, 1960, p. 482.
[6] C. A. Bunton, J. H. Fendler, N. A. Fuller, S. Perry, and J. Rocek, *J. Chem. Soc.*, 1963, 5361.
[7] R. Zahradnik, *Coll. Czech. Chem. Comm.*, 1959, **24**, 3678.
[8] M. J. Janssen, *Rec. Trav. chim.*, 1962, **81**, 650; see also *idem, ibid.*, 1963, **82**, 1197.