Cleavage of *N*-Thiobenzoyl-dipeptides with Trifluoroacetic Acid: the Basis of a New Stepwise Degradation of Polypeptides

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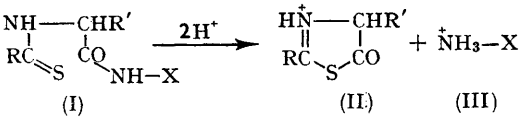
THE Edman procedure[1] for the stepwise degradation of a polypeptide depends upon the rapid cleavage which follows protonation of a terminal *N*-phenylthiocarbamoylated peptide (I; R = Ph·NH, X = peptide residue):



(I) (II) (III)

The unstable 2-anilinothiazolin-5-one (conjugate base of II; R = Ph·NH) thus split from the *N*-substituted polypeptide is converted during isolation procedures into the isomeric *N*-phenylthiohydantoin,[2] identification of which defines the *N*-terminal amino-acid residue of the polypeptide; alternatively, the same information is provided by the "subtractive method", amino-acid analysis of the shortened peptide (III). The procedure is used with some confidence in protein chemistry, since its limitations[3] are well-understood; and it has virtually eclipsed two closely-related methods, the acid-catalysed cleavage of peptide dithiocarbamic acids (I; R = SH)[4] and of peptide thioncarbamates (I; R = *O*-alkyl).[5]

We now find† that terminal *N*-thiobenzoyl peptides (I; R = Ph, X = peptide residue) readily undergo an analogous cleavage reaction; in a typical procedure, a solution of an *N*-thiobenzoyl-peptide in anhydrous trifluoroacetic acid was prepared and evaporated without delay. Model experiments involved a number of dipeptide derivatives, for one of which (*N*-thiobenzoyl-glycyl-leucine) the time required for fission was up to 20 min. The fission products were cleanly separated by trituration of the resulting oil with ether, or by its partition between ether and water. The *N*-terminal amino-acid residue was isolated in near-quantitative yield as the anilide of its *N*-thiobenzoyl derivative (I; R = X = Ph), by treatment of the ether extracts with excess of aniline in warm toluene,[6] and identified as such (physical methods used[1] in micro-scale Edman *N*-terminal analyses seem readily applicable to these yellow derivatives).

A stepwise degradation of polypeptides based on the present method should lack many of the inherent difficulties[3] of the Edman method; thiobenzoylation of amines is achieved in a number of ways,[6,7] reaction of peptides in neutral aqueous solution with sodium thiobenzoylthioglycollate[7] overcoming the solubility problems encountered[3] in the reaction of a peptide with an essentially non-polar reagent such as phenyl isothiocyanate, and presenting fewer difficulties in the separation of excess of reagent. Also, the quantitative nature of the fission and separation steps, and most important of all, the stability of the fission products of *N*-thiobenzoyl peptides during separation procedures, should prevent the accumulation of artifacts during repetitive cycles of a sequence analysis based on the method (the cleavage step in the Edman degradation may have a competitive side-reaction which results in loss of yield[3]). The shorter exposure to anhydrous acid apparently‡ required for fission of *N*-thiobenzoyl-peptides also minimises the possibility of side-reactions within the peptide residue. Finally, whereas electron-releasing substituents in the phenyl group of *N*-phenylthiocarbamoyl dipeptides (I; R = Ph-NH) have no effect on the rate of the fission step,[2b] their thiobenzoyl analogues (I; R = Ph) are likely to be susceptible to substituent effects, investigation of which may lead to a stepwise degradation employing milder conditions.

Impressive recent developments[8,9] in the technique of the Edman method can be incorporated also in procedures based on alternative chemical methods, evaluation of which may accelerate progress towards improved chemical methods of sequence analysis in protein chemistry.

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† The possibility of using *N*-thioacyl-peptides in sequence analysis was recognised by Kenner and Khorana (*cf.* ref. 5) but not explored by them.

‡ The fission of R·NH·CS-peptides requires exposure to anhydrous trifluoroacetic acid during 1 hr. (ref. 3) or 0·5 hr. (ref. 8), though some model *N*-phenylthiocarbamoyl dipeptides are cleaved within 2 min. by this reagent (W. Konigsberg and R. J. Hill, *J. Biol. Chem.*, 1962, **237**, 2547).

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