Rapid Mass-spectrometric Identification of the *N*-Terminal Amino-acid Residue in Terminal *N*-Thiobenzoyl Polypeptides

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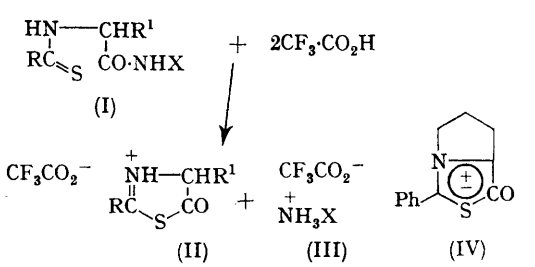
ONE of us has recently reported[1] the ready cleavage of terminal *N*-thiobenzoyl peptides (I; R = Ph, X = peptide residue) by trifluoroacetic acid, with the suggestion that the use of this reaction in a stepwise degradation of polypeptides should offer advantages over the Edman method [using derivatives (I; R = PhNH)], and other related methods (I; R = SH, *O*-alkyl).[2] In particular, pathways by which the 5-thiazolone intermediates (II) can undergo either rearrangement (to phenylthiohydantoins, when R = PhNH), or fragmentation, are not available to the corresponding 2-phenyl-5-thiazolone salts. In consequence, accumulation of artifacts during repetitive cycles of the method is minimised; and near-quantitative conversion of (II; R = Ph), after its separation from the shortened peptide (III), into the corresponding



N-thiobenzoylamino-acid anilide (I; R = X = Ph) can be achieved.[1]

Identification of *N*-terminal residues released in each cycle of a sequential analysis usually involves chromatographic methods.[2] Recently, however, the application of mass spectrometric methods has been proposed[3] for the identification of thiohydantoins obtained through Edman degradations. We now find that the mass spectra† of *N*-thiobenzoyl-amino-acid anilides, and of the *N*-thiobenzoyl derivatives of the "natural" amino-acids, can be readily interpreted (*e.g.* leucine and isoleucine are easily distinguishable). An *N*-terminal analysis of a polypeptide is achieved by placing one drop of its *N*-thiobenzoyl derivative in trifluoroacetic acid on the direct-insertion probe of the mass spectrometer, warming off most of the trifluoroacetic acid, and then introducing the probe directly into the mass spectrometer source. Particularly clean spectra (*cf.* Figure) are thus obtained, clearly derived from the 2-phenyl-5-thiazolone (II; R = Ph), and containing no fragments from the shortened peptide (III); a few additional fragment ions at low mass numbers (*e.g.* *m/e* 69) are derived from trifluoroacetic acid. Dipeptides, and a tripeptide,‡ with *N*-terminal glycine, alanine, leucine, valine, serine, and proline have been subjected successfully to this simple *N*-terminal analysis technique.

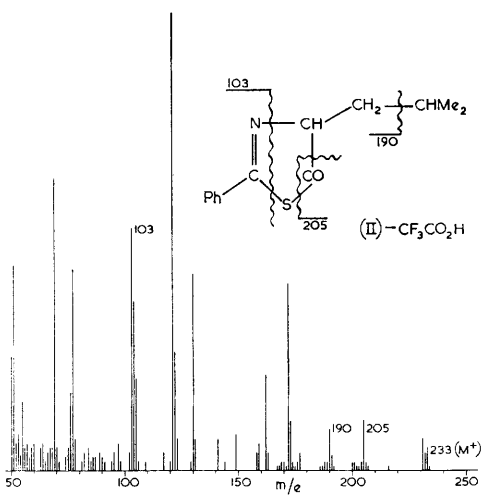


FIGURE. *Mass spectrum of* N-*thiobenzoyl-leucylglycine after dissolution in trifluoroacetic acid.*

In spite of this clear evidence for the course of the cleavage reaction, u.v. spectra of solutions of the cleavage products from terminal *N*-thiobenzoyl peptides differ from those reported[4] for compounds claimed to be 2-phenyl-5-thiazolones. 2-Phenyl-4-isobutyl-5-thiazolone, m.p. 122°, with satisfactory elemental analysis and spectroscopic properties, was prepared both by cyclisation of *N*-thiobenzoyl leucine with phosphorus tribromide[4] and by dissolution of *N*-thiobenzoyl leucylglycine in trifluoroacetic acid, followed by treatment with excess aqueous sodium hydrogen carbonate, and extraction into ether. The thiazolone was readily soluble in dilute sodium hydroxide solution, and differed in this and in other respects from the compound, m.p. 41°, obtained[4] by acetic anhydride cyclisation of *N*-thiobenzoyl-leucine. In fact, we find that *N*-thiobenzoyl amino-acids are invariably converted into 2-phenyl-5-acetoxythiazoles when treated with warm acetic anhydride; again, support for this statement derives from mass spectrometric data and full elemental analyses. As with analogous *N*-thiobenzoyl imino-acids,[5] however, acetic anhydride cyclisation of *N*-thiobenzoylproline gives[6] the meso-ionic thiazolone (IV), and *N*-terminal proline cleaved from a polypeptide in this form is readily identifiable with the present technique.

We feel that, as with solid-state Edman degradation procedures,[7] the full potential of a sequence analysis based on the present method will be realised by prior attachment of the polypeptide, *via* its carboxyl terminus, to a resin; studies of this modification are in progress.

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† Mass spectra were run on an A.E.I. MS9 mass spectrometer.
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