ACID HYDROLYSIS OF 3-PHENYL-2-THIOHYDANTOINS*

by

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The observations by the FRAENKEL-CONRATS[1] following the suggestion of OTTESEN AND LINDERSTRØM-LANG, to the effect that the EDMAN procedure of phenylisothiocyanate degradation[2] can be extended to proteins if the acidification stage is carried out in aqueous solution, has quickened interest in this approach to the elucidation of amino acid sequences. The above investigators have accomplished the identification of the cleaved 3-phenyl-2-thiohydantoin(s) by hydrolysis with baryta at 140° C for 48 hours, followed by the removal of barium as the carbonate and the identification of the resulting amino acid(s) on paper chromatograms. We wish to report that phenylthiohydantoins are hydrolysed more satisfactorily with acid[3], a procedure which possesses the advantage of experimental simplicity.

Kinetic studies with a representative selection of phenylthiohydantoins showed that the slowest (phenylalanine) was completely hydrolysed by 16 hours of digestion with 20% hydrochloric acid at 150°. Under these conditions, which were then adopted as standard, the phenylthiohydantoins of glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, ε-phenythiocarbamyl (PTC)- lysine, and histidine were hydrolysed quantitatively to the corresponding amino-acids. Arginine gave an additional 10% of ornithine. Tryptophan was broken down completely, giving glycine and a little alanine as ninhydrin positive degradation products. Serine phenylthiohydantoin yielded a weak alanine spot on the chromatogram. Both threonine phenylthiohydantoin and its corresponding 5-unsaturated derivative gave spots due to glycine, alanine and α-amino-butyric acid, although they were very faint, appearing just at the threshold of visibility. Cystine, cysteine, and S-PTC-cysteine phenylthiohydantoins all yielded cystine plus alanine. The above group of phenylthiohydantoins which are decomposed by acid, also suffer destruction during alkaline hydrolysis.

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A SIMPLE METHOD OF APPLYING THE BECKMAN SPECTROPHOTOMETER TO THE MEASUREMENT OF PAPER CHROMATOGRAMS

by

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Following the widespread acceptance of paper chromatography by biochemists during the past few years, a number of devices have been developed for the quantitative measurement of chromatograms by photometric methods. Such devices may be purchased from commercial sources, and others have been described in the literature. However, all of these require a certain financial investment.