SHORT COMMUNICATIONS

Photochemical Decomposition of 2,4,6-Trinitrophenyl Amino Acids and Peptides

By Kazuo SATAKE and Tsuneo OKUYAMA

(Received February 9, 1959)

The unstability of dinitrophenyl amino acids to sunlight was already described by Sanger[1], Mills[2], and Blackburn[3]. Akabori et al.[4] also studied the rate of the photochemical decomposition of various dinitrophenyl amino acids by exposure to a tungsten lamp.

Trinitrophenyl (TNP) amino acids and peptides, synthesis of which is going to be published elsewhere, have similar properties to dinitrophenyl derivatives and are also labile photochemically.

A saturated solution of TNP-amino acid in 1 N hydrochloric acid was prepared 20 ml. of the solution was placed in a petri dish (8 cm. in diameter) and exposed to ultraviolet light (Mazda UV-sterilizing lamp)or sunlight. The absorption spectrum of the solution changed gradually to that of picramide. It suggests the formation of picramide by the photochemical decomposition of TNP-amino acids. When the solution showed the absorption spectrum of picramide, it was concentrated to dryness under reduced pressure. The residue was dissolved in a small amount of ethanol and applied to filter paper. The presence of picramide and the absence of free amino acids were verified by means of one-dimensional paper chromatography on Toyo No. 51 filter paper. The solvent systems used were phenol-water (4 : 1, v/v), 1.5 M phosphate buffer (pH 5.6) or butanol saturated with 2 N ammonia.

By further exposure to light (after 24 ~44 hr.), no ninhydrin positive substance was detected by two-dimensional paper chromatography of the concentrated samples, although the decoloration of the solution was observed, indicating the complete decomposition of the TNP-amino acid. A yellow substance, probably resulting from picramide, was then precipitated. Conditions of the two-dimensional paper chromatography were as follows: ascending method, on Toyo No. 51 filter

TABLE I. PHOTODECOMPOSITION OF TNP-AMINO ACIDS AND -PEPTIDES

TNP-compound	Solvent	Photodecomposition products: Ninhydrin positive substance	Photodecomposition products: Recovery of amino acid (%)
TNP-glycine	1 N HCl	none	0
TNP-DL-alanine	1 N HCl	none	0
TNP-L-leucine	1 N HCl	none	0
TNP-L-valine	1 N HCl	none	0
〃	1 N HCl-Dioxane*	valine (?), unknown	—
〃	1 N HCl-Ethanol*	none	0
TNP-glycyl-glycine	1 N HCl	glycine	93
〃	1 N HCl-Dioxane*	glycine, glycyl-glycine, unknown	—
TNP-glycyl-leucine	1 N HCl	leucine	—
TNP-glycyl-DL-valine	1 N HCl	valine	87
TNP-glycyl-DL-serine	1 N HCl	serine	91
TNP-alanyl-DL-asparagine	1 N HCl	asparagine	83
TNP-L-leucyl-L-tyrosine	1 N HCl	tyrosine	92
TNP-glycyl-glycyl-glycine	1 N HCl	glycyl-glycine	—

* 1:1, v./v.

1) F. Sanger, *Biochem. J.*, **45**, 563 (1949).
2) G. L. Mills, ibid., **50**, 707 (1952).
3) S. Blackburn, ibid., **45**, 579 (1949).
4) S. Akabori, T. Ikenaka, Y. Okada and K. Kohno, *Proc. Japan Acad.*, **29**, 509 (1953).

paper, using butanol-acetic acid-water (4 : 1 : 1, v/v) as the first developer and phenol-water (4 : 1, v/v) as the second.

In the cases of TNP-peptides, the similar photochemical decomposition was observed. Only the trinitrophenylated amino-terminal amino acid residue was decomposed photochemically, while the second residue was liberated in a free state.

The liberated amino acid was estimated by the TNP-method, which was composed of the trinitrophenylation of amino acids with picryl sulfonate, followed by the colorimetry at 340 mμ[5]. The recoveries of amino acids were listed on Table I. The amino acid liberation was caused exclusively by the photochemical decomposition of the TNP-amino-terminal amino acid residue, since no amino acid was released by the similar treatment of TNP-peptide without exposure to light, or of free dipeptide with exposure to light.

In the case of TNP-triglycine, glycylglycine was liberated, but no release of glycine could be observed.

These results suggest a probable application for a new method of the stepwise degradation of peptide from the amino terminal. Detail of these studies will be the subject of the future communication.

Department of Chemistry
Faculty of Science
Tokyo Metropolitan University
Fukazawa, Setagaya-ku, Tokyo

5) K. Satake, T. Okuyama and M. Ohashi, unpublished paper.