On the Photochemical Decomposition of 2,4-Dinitrophenyl Peptides

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In recent years, Blackburn[1], Mills[2] and others have pointed out the fact that

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1) S. Blackburn, *Biochem. J.*, **45**, 579 (1949).
2) G. L. Mills, ibid., **50**, 707 (1952).

DNP-amino acids are unstable toward light, and Akabori et al.[3] have previously reported a semiquantitative investigation on the photochemical decompsition of the DNP-amino acids.

In the present investigation, photochemical decomposition of DNP-dipeptides, DNP-L-leucyl-glycine and DNP-glycyl-L-leucine, was examined. It was thus confirmed that the *N*-terminal amino acid residues of these peptides, together with the attached DNP-group, were completely decomposed under suitable conditions, resulting in the liberation of the *C*-terminal residues, glycine and leucine, in the form of free amino acids. The starting materials, DNP-L-leucyl-glycine and DNP-glycyl-L-leucine, were prepared from L-leucyl-glycine[4] and glycyl-L-leucine[5] according to Sanger's method[6] and their purity was confirmed by nitrogen analysis and two-dimensional paper chromatography under improved Levy's condition[7].

Anal. Found: N, 15.68**; N, 15.78***. Calcd. for $C_{14}H_{18}O_7N_4$: N, 15.81%. Found: N, 15.40****. Calcd. for $C_{14}H_{18}O_7N_4 \cdot 1/2\,H_2O$: N, 15.42%. Two milligrams of DNP-L-leucyl-glycine (m.p. 154~157°) on a glass dish (9 cm. in diameter) were dissolved in a small amount of ethanol or ether and then diluted with 20 ml. of suitable solvent such as distilled water, 1 N-hydrochloric acid, ether, benzene, chloroform and carbon tetrachloride. The solution was covered with cellophane sheet (0.02 mm. thick) and exposed to a 15 W. Mazda UV-sterilizing lamp for about 20 hr. at a distance of 20 cm., the principal line of the lamp being 2537 Å. The exposed solution was evaporated to dryness in a vacuum desiccator at room temperature and the residue was extracted as completely as possible with 1 ml. of water. One tenth milliliter of the extract was subjected to paper chromatography using a mixture of butanol, acetic acid and water (4:1:1) as solvent in order to detect the ninhydrin-positive products. The water-insoluble part of the photodecomposed residue was extracted with ethyl acetate and the extract was similarly analyzed by two-dimensional paper chromatography under improved Levy's condition (Fig. 1, Table I). When water or 1 N-hydrochloric acid was used as the solvent during irradiation, rather complicated results were obtained; dinitroaniline, dinitrophenol, glycine and other ninhydrin-positive materials, though small in amount, being detected as the products. In the case of benzene as the solvent,

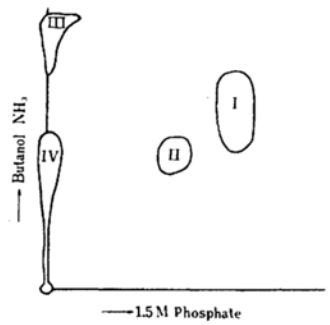

Fig. 1. An example of two-dimensional paper chromatograms of ethyl-acetate soluble parts of the photodecomposed products, the details of which are listed in Table I.

Spot I (yellow) corresponds to the DNP-leucyl-glycine and to the DNP-glycyl-leucine, spot II (yellow) to dinitrophenol, spot III (deep yellow) to dinitroaniline and sport IV (brown) probably to basic materials derived from dinitroaniline.

TABLE I

Starting material	DNP-leucyl-glycine						DNP-glycyl-leucine		
Reaction solvent	—[a]	Ether[b]	Benzene	Benzene[b]	Chloroform[b]	Carbon tetrachloride[b]	—[a]	Chloroform[b]	Carbon tetrachloride[b]
Spot I	###	###	###	###	+	−	###	+	±
Spot II	±	±	±	±	±	±	±	±	±
Spot III	−	+	+	##	##	−	−	##	##
Spot IV	−	−	−	−	−	###	−	−	+

a Starting material before irradiation.
b Solvent which was saturated with 1N-hydrochloric acid.

3) S. Akabori, T. Ikenaka, Y. Okada and K. Kohno, *Proc. Japan Acad.*, **29**, 509 (1953).
4) M. Bergmann, L. Zervas and J. S. Fruton, *J. Biol. Chem.*, **111**, 238 (1935).
5) E. Fischer and J. Steingroever, *Ann.*, **365**, 167 (1909).
6) F. Sanger, *Biochem. J.*, **39**. 507 (1945); ibid., **45**, 563 (1949).
7) A. L. Levy, *Nature*, **174**, 126 (1964); G. Koch and W. Weidel, *Z. physiol. Chem.*, **303**, 213 (1956).

** DNP-L-leucyl-glycine (dried in vacuo at room temperature)
*** DNP-glycyl-L-leucine (dried in vacuo at 80° for 3hr.)
**** DNP-glycyl-L-leucine (dried in vacuo at room temperature)

especially when saturated with 1N-hydrochloric acid, only one faint but clear spot corresponding to glycine was obtained as ninhydrin-positive substance, but greater part of the starting material remained unchanged. More promising results were obtained in the case of chloroform and of carbon tetrachloride saturated with 1N-hydrochloric acid. In both cases only one deep spot due to glycine (almost in the same density) was obtained, but a distinct difference was observed in the ethyl acetate soluble by-products.

Two milligrams of DNP-glycyl-L-leucine (sintered at 75~78°, resolidified and then decomposed at 110~115°) were dissolved in a small amount of ether and diluted with 20 ml. of chloroform or carbon tetrachloride, and treated exactly as in the case of DNP-leucyl-glycine. As expected, a clear single leucine spot was obtained in both solvents.

Application of these results to DNP-tri or higher peptides would be more interesting and the method may be utilizable as a new route for step-wise degradation of peptides from their *N*-terminal amino acid residues. Studies along this line are being pursued in this laboratory.

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