Studies on the Photochemical Behaviour of 2,4-Dinitrophenyl Derivatives of some Amino Acids and Peptides

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Photodecomposition of 2,4-dinitrophenyl-amino acids (DNP-amino acids) (Sanger, 1949; Blackburn, 1949; Mills, 1952) has been studied by Akabori, Ikenaka, Okada & Kohno (1953), who showed that several α -DNP-amino acids in aqueous solution were decomposed by light at about the same rate, but did not identify the products. They also found that ϵ -DNP-lysine was photostable. Pollara & Von Korff (1960), using ^{14}C -labelled DNP-amino acids, observed that many of these compounds in the solid state are decarboxylated by light to give the corresponding DNP-alkylamine.

For spectrophotometric determination, DNP-amino acids are commonly dissolved in dilute sodium hydrogen carbonate solution (Fraenkel-Conrat, Harris & Levy, 1955). This work was begun after such a solution of DNP-leucine had been left unintentionally in bright sunlight for several hours. The spectrum of the solution was not only different from that of a freshly prepared solution of DNP-leucine; it was also different from that of the product, DNP-isopentylamine, which would be expected to result from illuminating solid DNP-leucine, and indeed from that of any known compound. The identification of the products as 4-nitro-2-nitrosoaniline, 3-methylbutyraldehyde and carbon dioxide is reported by Russell (1963). Apart from the chemical interest of the reaction (eqn. 1), it seemed that it might be useful in structural studies of 'new' amino acids. Many DNP-amino acids, and a few DNP-peptides, were therefore examined for their behaviour towards light.

A brief account of this work has been given by Russell (1962).

MATERIALS AND METHODS

Materials. DNP-amino acids, except DNP- α -phenyl- α -alanine, were purchased from Mann Research Laboratories Inc., New York, N.Y., U.S.A. Before use they were checked for purity by paper chromatography in solvent

system *A* (see below). L-Prolyl-L-phenylalanine was prepared by D. F. Elliott and D. W. Russell (unpublished work). Other peptides and α -phenyl- α -alanine were purchased from Mann Research Laboratories Inc. DNP-peptides and DNP- α -phenyl- α -alanine were prepared by a standard method (Fraenkel-Conrat *et al.* 1955) and were chromatographically pure. 4-Nitro-2-nitrosoaniline was obtained by photolysis of DNP-leucine (Russell, 1963).

Paper chromatography. Two solvent systems were used: *A*, 2-methylpropan-2-ol-4-25 *N*-ammonia (4:1, v/v) (Vining & Taber, 1957), used with Whatman no. 3MM paper, and *B*, 1.5 *M*-phosphate buffer (Levy, 1954), used with Whatman no. 1 paper. All chromatograms were run in the dark at 25° by the descending technique.

Photochemical degradation. Solutions were placed in test tubes (2 cm. diam.) supported in a sloping position below a single Camplex plant irradiator (Simplex Dairy Equipment Co. Ltd.), or were exposed to bright sunlight. Spectral changes were followed with a Beckman model DU spectrophotometer. Photolysis was taken to be complete when no further change occurred.

Photochemical behaviour of dinitrophenyl-amino acids and -peptides. A solution (20 ml.) of the compound (50 μM) in aq. 1% (w/v) sodium hydrogen carbonate was illuminated, the distance between test tube and lamp being such that photolysis of DNP-leucine was complete in 15 min., (the 'standard' period). Other compounds were illuminated for 15 and 30 min., the solutions were cooled and their ultraviolet spectra were measured. To check that no part of the observed change was due to heating by the lamp, another portion of solution was exposed to bright sunlight until no further spectral change occurred.

When 4-nitro-2-nitrosoaniline appeared from the spectrum to be formed, but not quantitatively, the solution was extracted with ethyl acetate, the ethyl acetate was evaporated and the residue was chromatographed in system *A*. Authentic 4-nitro-2-nitrosoaniline (50 $\mu\text{g.}$) was used as a marker, and gave a single green spot with R_f 0.88.

RESULTS

The R_f values of some common DNP-amino acids in solvent system *A* are given in Table 1.

When dilute solutions of DNP-amino acids or -peptides were stored in the dark for 24 hr., their spectra did not change. When DNP-leucine was illuminated for the 'standard' period the spectrum of the photolysed solution was almost identical with that of a solution of 4-nitro-2-nitrosoaniline at the same concentration (Fig. 1); thus photolysis of

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Table 1. R_F values of dinitrophenyl-amino acids

Each DNP-amino acid (50 μ g.) was run separately in the solvent system 2-methylpropan-2-ol-4.25N-ammonia (4:1, v/v) on Whatman no. 3MM paper, in the dark and by the descending technique, at 25°.

DNP-derivative of	R_F
Aspartic acid	0.12
Glutamic acid	0.14
Cystine (bis)	0.35
Lysine (ϵ -mono)	0.35
Asparagine	0.44
Methionine sulfoxide	0.45
Glutamine	0.47
Threonine	0.54
Glycine	0.54
Serine	0.54
Tryptophan	0.66
(2,4-Dinitrophenol)	(0.66)
Proline	0.67
α -Alanine	0.68
Phenylalanine	0.69
Isoleucine	0.69
Valine	0.69
Lysine (bis)	0.74
Leucine	0.80
(4-Nitro-2-nitrosoaniline) (green)	(0.88)
(2,4-Dinitroaniline)	(0.88)

Fig. 1. Ultraviolet spectra of 4-nitro-2-nitrosoaniline, 50 μ M in 1% (w/v) sodium hydrogen carbonate (broken line), and of a solution of DNP-leucine, 50 μ M in 1% (w/v) sodium hydrogen carbonate, illuminated until no further spectral change occurred (continuous line). The light-path was 1 cm.

Table 2. Results of photolysis of dinitrophenyl-amino acids and -peptides

The results represent the ultraviolet absorption of solutions of the compounds after photolysis for the 'standard' period (see the text). NNA, 4-Nitro-2-nitrosoaniline.

DNP-derivative of	$\lambda_{\max.}$ ($m\mu \pm 2 m\mu$)		Ratio ($E_1:E_2$) of extinction at maxima 1 and 2	Product
	1	2		
α -Alanine	284	348	1.41	NNA
β -Alanine*	—	—	—	—
Asparagine	280	344	1.52	Unknown†
Aspartic acid	284	348	1.28	NNA‡
Arginine	284	348	1.38	NNA
Cystine (bis)	276	352	1.05	Unknown†
Cysteic acid	280	360	0.98	Unknown
Glutamic acid	284	348	1.40	NNA
Glutamine	284	348	1.36	NNA
Glycine	284	356	1.10	NNA‡
Isoleucine	284	348	1.41	NNA
Leucine	284	348	1.41	NNA
Lysine (ϵ -mono)*	—	—	—	—
Methionine sulfoxide	284	356	1.09	NNA‡
Methionine sulphone	284	352	1.20	NNA‡
Ornithine (δ -mono)*	—	—	—	—
α -Phenyl- α -alanine	284	348	1.32	NNA‡
Proline	280	—	—	Unknown
Serine	284	352	1.00	NNA‡
Threonine	284	352	1.20	NNA‡
Tryptophan*	—	—	—	—
Valine	284	348	1.31	NNA
(4-Nitro-2-nitrosoaniline)	284	348	1.41	—
α -Alanyl- α -alanine*	—	—	—	—
Glycylglycylglycine*	—	—	—	—
Leucylglycine*	—	—	—	—
Leucylglycylglycine*	—	—	—	—
Prolylphenylalanine§	—	—	—	—

* Compound little affected by illumination for twice the 'standard' period.

† Further illumination resulted in more decomposition, the spectrum becoming less like that of 4-nitro-2-nitrosoaniline.

‡ Presence confirmed by paper chromatography.

§ Compound partly decomposed by illumination for twice the 'standard' period.

DNP-leucine gave an almost quantitative yield of 4-nitro-2-nitrosoaniline. The same result was obtained, more slowly, with bright sunlight; photolysis of susceptible DNP-amino acids at midday and midsummer, at the concentration cited and at the latitude of this Laboratory (40° S), was usually complete within 30 min.

When solutions of other DNP-amino acids and -peptides were illuminated under the same conditions, almost quantitative formation of 4-nitro-2-nitrosoaniline could in many cases be deduced from the ultraviolet spectra. In others, although the compound was formed, the ratio of the extinctions at the two peaks, 284 and $348\text{ m}\mu$, was significantly less than that of pure 4-nitro-2-nitrosoaniline, and the higher wavelength maximum was shifted a few millimicrons bathochromically. Certain other DNP-amino acids, though decomposing rapidly on illumination, yielded products the spectra of which gave no evidence for the formation of 4-nitro-2-nitrosoaniline, whereas yet others were little affected by illumination for twice the 'standard' period (Table 2). No differences, except in the rate of photolysis, were observed between the effects of sunlight and those produced by the lamp employed.

DISCUSSION

The results show that, though α -DNP-amino acids are stable in sodium hydrogen carbonate solution in the dark, they are all decomposed at about the same rate by light. The only exception noted is DNP-tryptophan, which is decomposed extremely slowly; the reason for this is not known.

Although photolability is a general property of α -DNP-amino acids, not all decompose in the same way. A large group formed 4-nitro-2-nitrosoaniline in high yield. This group included DNP- α -phenyl- α -alanine, so that the extent of substitution at the α -carbon atom is not a factor in determining the course of the reaction. On the other hand, some other α -DNP-amino acids gave a lower yield of 4-nitro-2-nitrosoaniline, together with other (unidentified) products, and a few decomposed without formation of the nitroso compound. Though no precise conclusion can be drawn, it seems that the presence in the amino acid side chain of a group such as hydroxyl, sulphoxide etc. permits side reactions so that the characteristic product is formed to a smaller extent or not at all. The contrast between the almost quantitative formation of 4-nitro-2-nitrosoaniline from DNP-glutamine and its absence from photolysates of DNP-asparagine supports the contention (Steward & Thompson, 1952) that the two amides differ in structure. In none of the anomalous cases have the products been identified.

The behaviour of DNP-derivatives of compounds in which the amino group was in a position other than α was different, for they were little affected by illumination that destroyed α -DNP-amino acids. Although only a few DNP-peptides were studied, the results suggest that such compounds, although in some cases photolabile, are decomposed much more slowly than are α -DNP-amino acids. Photodecomposition of DNP-peptides apparently proceeds readily in certain organic solvents (Akabori, Sakakibara & Sakakibara, 1959).

Certain general conclusions may be drawn. First, if a DNP-derivative of an amino acid is stable to light, then the DNP group is almost certainly not located on an amino group which is in the α -position with respect to a free carboxyl group; conversely, marked photolability suggests strongly that the compound is an α -DNP-amino (or -imino) acid. The only exception to this generalization so far observed is DNP-tryptophan. Secondly, if illumination results in formation of 4-nitro-2-nitrosoaniline, the structure

is confirmed, but the converse cannot be assumed. Peptides with, for example, an *N*-terminal γ -glutamyl or a *C*-terminal ϵ -lysine residue are complex α -amino acids, and their DNP-derivatives should photolyse rapidly, probably with the formation of 4-nitro-2-nitrosoaniline.

The information given by experiments of this sort is no greater than may be gained by other means. However, the technique may prove useful in confirming structure. The discovery of numerous amino acids in plant extracts (Fowden, 1962) suggests caution in identifying any one of them solely by comparing R_f values in a few different paper-chromatographic systems. On the other hand, a classical chemical approach to identification is often precluded by shortage of material. In such cases the paper-chromatographic behaviour of a derivative, such as a DNP-compound, is useful for confirming identity. Since susceptibility of DNP-derivatives to photolysis, and the spectral properties of the products, may be determined simply and with no more of the derivative ($0.1\text{ }\mu\text{-mole}$) than may be eluted from a paper chromatogram, it is possible to get further confirmation from the same amount of material. Preliminary results suggest that formation of 4-nitro-2-nitrosoaniline is always accompanied by production of the aldehyde that would be obtained by oxidizing the parent amino acid. It should thus be possible to gain more information about the structure of an amino acid by photolysis of its DNP-derivative than is implied by the results reported here.

The R_f values of several common DNP-amino acids are recorded in Table 1 for the solvent system

2-methylpropan-2-ol-4·25N-ammonia (4:1, v/v). Many solvent systems for these compounds have been proposed (see, for example, Kubota, 1961); the information is given because the system seems to be a very versatile one. It was used (Vining & Taber, 1957) for α -amino acids and α -hydroxy acids, and later (Russell, 1960) for *N*-methyl-amino acids. It is a stable one-phase system, and gives compact spots with DNP-derivatives, the R_f values lying between 0·1 and 0·8 approximately. The DNP-derivatives of leucine and isoleucine are particularly well separated.

SUMMARY

1. Solutions of DNP-amino acids and -peptides in dilute aqueous sodium hydrogen carbonate were examined photochemically.

2. The α -DNP-amino acids were all rapidly decomposed by light with the exception of DNP-tryptophan; β -, δ - and ϵ -DNP-amino acids were stable, and DNP-peptides were either stable or decomposed much more slowly than α -DNP-amino acids.

3. Formation of 4-nitro-2-nitrosoaniline on photolysis appeared to be a property exclusively of derivatives possessing the structure

but not all compounds possessing this structure yield 4-nitro-2-nitrosoaniline.

4. The solvent system 2-methylpropan-2-ol-4·25N-ammonia (4:1, v/v) was useful for the paper chromatography of DNP-amino acids.

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REFERENCES

- Akabori, S., Ikenaka, T., Okada, Y. & Kohno, K. (1953). *Proc. imp. Acad. Japan*, **29**, 509.
 Akabori, S., Sakakibara, S. & Sakakibara, K. (1959). *Bull. chem. Soc. Japan*, **32**, 311.
 Blackburn, S. (1949). *Biochem. J.* **45**, 579.
 Fowden, L. (1962). *Endeavour*, **21**, 35.
 Fraenkel-Conrat, H., Harris, J. I. & Levy, A. L. (1955). *Meth. biochem. Anal.* **2**, 539.
 Kubota, Y. (1961). *Chem. Pharm. Bull.* **9**, 666.
 Levy, A. L. (1954). *Nature, Lond.*, **174**, 126.
 Mills, G. L. (1952). *Biochem. J.* **50**, 707.
 Pollara, B. & Von Korff, R. W. (1960). *Biochim. biophys. Acta*, **39**, 364.
 Russell, D. W. (1960). *J. Chromat.* **4**, 251.
 Russell, D. W. (1962). *Biochem. J.* **83**, 8F.
 Russell, D. W. (1963). *J. chem. Soc.* p. 894.
 Sanger, F. (1949). *Biochem. J.* **45**, 563.
 Steward, F. C. & Thompson, J. F. (1952). *Nature, Lond.*, **169**, 739.
 Vining, L. C. & Taber, W. A. (1957). *Canad. J. Chem.* **35**, 1109.

Biochem. J. (1963) **87**, 4

An Enzyme from Rat Liver Catalysing Conjugations with Glutathione

2. REPLACEMENT OF NITRO GROUPS*

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Booth, Boyland & Sims (1961) and Coombes & Stakelum (1961) showed that the soluble supernatant fraction of rat liver contained an enzyme catalysing the formation of glutathione *S*-conjugates with replacement of the active halogen of aromatic compounds. Booth *et al.* (1961) found this enzyme to be specific for glutathione although Coombes & Stakelum (1961) state that a small reaction occurred with cysteine and cysteinylglycine. Booth *et al.* (1961) also showed that several compounds that are known to be excreted as mercapturic acids by the body can act as the

acceptor (or second substrate) for the glutathione in the enzymic reaction. The enzyme has been called 'glutathio kinase' (Al-Kassab, Boyland & Williams, 1962; Boyland, 1962).

In the metabolism of some polychloronitrobenzenes the nitro group is displaced during metabolism, not the halide (Bray, Hybs, James & Thorpe, 1953; Betts, James & Thorpe, 1955; Betts, Bray, James & Thorpe, 1957). Evidence is now presented that the enzyme that catalyses the replacement of halogen will also displace the nitro group in these compounds. It also increases the rate of reaction of 4-nitropyridine *N*-oxide and of the carcinogen 4-nitroquinoline *N*-oxide with

* Part 1: Booth, Boyland & Sims (1961).