Photolysis of *N*-2,4-Dinitrophenylamino-acids; Structural Requirements for the Formation of 6-Nitrobenzimidazole 1-Oxides

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6-Nitrobenzimidazole 1-oxides are formed from *N*-2,4-dinitrophenylamino-acids in optimum yields at very low pH and also at *ca.* pH 3. The reaction at pH 3 requires a hydrogen atom on the amino-group, whereas at low pH it does not. The mechanism of the reaction is discussed.

PHOTOLYSIS of *N*-2,4-dinitrophenylamino-acids (DNP-amino-acids) (I) in aqueous solution proceeds by two routes. One leads to the formation of carbon dioxide, 4-nitro-2-nitrosoaniline (NNA) (IIa), and the aldehyde with one less carbon atom than the parent amino-acid.[1] The other leads to the production of a substituted 6-nitrobenzimidazole 1-oxide (III).[2] The relative importance of these two routes depends on the pH of the solution.[3] In addition, there are structural requirements for the formation of the benzimidazole product. These requirements and related problems are examined in this paper.

[1] D. W. Russell, (*a*) *J. Chem. Soc.*, 1964, 2829; (*b*) *ibid.*, 1963, 894; (*c*) *Biochem. J.*, 87, 1.

[2] R. J. Pollitt, *Chem. Comm.*, 1965, 262.

[3] D. J. Neadle and R. J. Pollitt, *J. Chem. Soc.* (*C*), 1967, 1764.

We have previously shown [3] that DNP-α-amino-acids with free hydrogen on both the α-carbon and the amino-nitrogen give maximum yields of substituted 6-nitrobenzimidazole 1-oxides when photolysed either at pH values in the range 2—4 or at very low pH. DNP-α-aminoisobutyric acid (Ia) cannot give a benzimidazole 1-oxide in photolysis, since there is no hydrogen atom on the α-position. Hence, NNA is formed in good yield (assessed spectrophotometrically) at pH values from 0 to 10. Above pH 10 some additional decomposition occurs.

Confusing results were obtained when the photolysis of DNP-sarcosine (Ib) was examined by the techniques described previously.[3] A clearer pattern emerged when small quantities of solution were photolysed rapidly and the products were assessed spectrophotometrically. The immediate product at pH values in the range 0—10 was *N*-methyl-NNA (IIb).[1a] The yields were high at low pH but fell off somewhat at higher pH. When the solution from photolysis at pH 0 was set aside in the dark, a very slow increase in absorption at 230 nm. occurred, and the absorption at 354 and 440 nm. was decreased, until the spectrum resembled that of a 6-nitrobenzimidazole 1-oxide in acid solution.[3] A similar reaction was observed with DNP-α-alanine (Ic) although, in this case, some 2-methyl-6-nitrobenzimidazole 1-oxide was formed immediately even at pH 0. This secondary reaction is analogous to that described by Russell,[4] who obtained 2-aryl-6-nitrobenzimidazole 1-oxides by the reaction of aromatic aldehydes with NNA in boiling acetic acid. Our previous statement [3] that the secondary reaction is unimportant in dilute aqueous solution at room temperature thus requires modification.

The secondary reaction is acid catalyzed. In 9N-sulphuric acid, an equimolar mixture of formaldehyde and *N*-methyl-NNA (prepared by the photolysis of DNP-sarcosine at pH 6) was converted into the benzimidazole product by a first-order reaction (half-life 7·5 min. at 25°). Addition of excess of formaldehyde greatly increased the rate of reaction and initially gave zero-order kinetics for the removal of *N*-methyl-NNA. The first-order reaction between NNA and acetaldehyde (from the photolysis of DNP-α-alanine) in 9N-sulphuric gave a half life of 27 min. At pH 2·5, NNA and acetaldehyde did not react detectably during 18 hr.

Both DNP-sarcosine and DNP-α-alanine gave increased yields of benzimidazole products on rapid photolysis as the pH was decreased from 0 to −3, but this could not be accounted for solely in terms of the secondary reactions described above. In 9N-sulphuric acid, at least a 70% yield of the 6-nitrobenzimidazole 1-oxide was produced by a faster reaction associated directly with the photolysis.

The product from the photolysis of DNP-α-alanine in 11-N-hydrochloric acid has been isolated and its identity as 2-methyl-6-nitrobenzimidazole 1-oxide has been confirmed. The photolysis product from DNP-sarcosine has u.v. absorption spectra in acid and neutral aqueous solution resembling those of 6-nitrobenzimidazole 1-oxide, but the spectra at pH 10 and in ethanolic solution, resemble the spectrum of the neutral aqueous solution. This is in agreement with the expected 3-methyl-6-nitrobenzimidazole 1-oxide structure, and the assignments of the tautomeric structures made previously.[3] This structure was confirmed by the evidence of the n.m.r. spectrum, and the compound has has also been synthesized from *N*-methyl-NNA and formaldehyde. The product is very soluble in water, a property shown by 3-alkylbenzimidazole 1-oxides [5] and probably related to the presence of the semi-polar bond. In contrast, 1-methoxy-6-nitrobenzimidazole is more readily soluble in nonpolar solvents. In N-sodium hydroxide, 3-methyl-6-nitrobenzimidazole 1-oxide gives a substance ($\lambda_{max.}$, 399 nm.) which is slowly converted back to the benzimidazole oxide in neutral solution. The nature of this product has not been investigated.

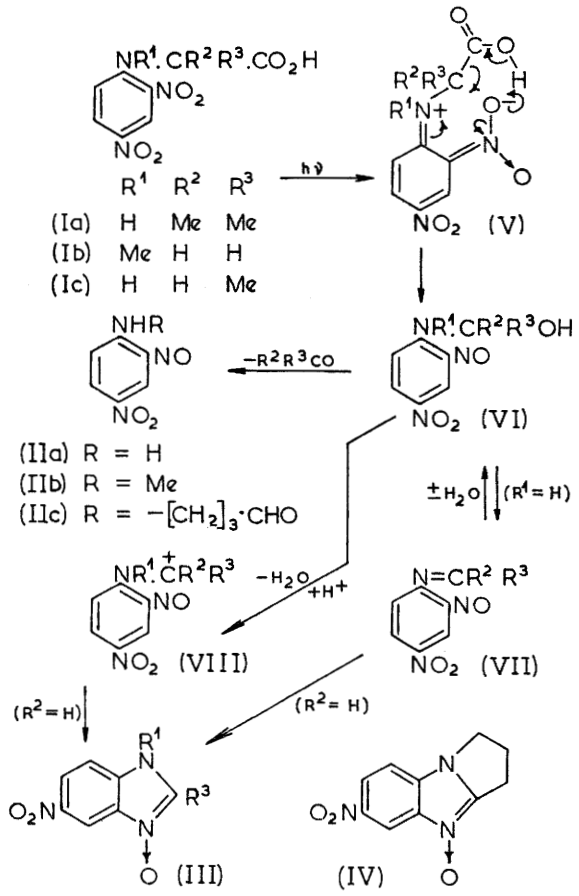
The photolysis products of DNP-proline vary with pH and time in an extremely complicated manner and we report the results only briefly. Rapid photolysis at pH 4·5 yields a product with the *N*-alkyl-NNA type of u.v. spectrum, which is probably compound (IIc). This, in acid solution, gives one spectroscopically recognizable intermediate (inflexions at 220 and 275 nm.) and then a benzimidazole-type of product which is probably 6-nitro-2,3-dihydro-1*H*-pyrrolo-[1,2-*a*]benzimidazole 4-oxide (IV). It has been isolated as the hydrochloride from preparative photolysis. At high pH, the initial product (IIc) gives, progressively, at least two other intermediates and finally a product which has a u.v. spectrum identical with that of a *N*-alkyl-2,4-dinitroaniline. This product is also formed, with others, by the action of alkali on compound (IV).

The effect of pH on the overall rate of photolysis has been briefly studied. With DNP-α-aminoisobutyric acid, where NNA is formed quantitatively, this was done spectrophotometrically; with DNP-sarcosine and DNP-α-alanine, a solvent-extraction method was used to determine the amount of unchanged DNP-amino-acid. In all three cases, there was a sharp fall in rate corresponding to the transition from a protonated to an ionized carboxy-group.[6] The rate of photolysis of DNP-α-alanine was thus largely independent of the nature of the products. A quantitative assessment of rates was not attempted since there is a slight shift of absorbance to longer wavelengths on ionization of the carboxy-group, and the emission of the tungsten lamp increases quite rapidly in this range.

Russell has suggested [1b] that in the photolysis of DNP-α-amino-acids the formation of NNA is dependent on the ionization of the carboxy-group; this is clearly incompatible with more recent data. In any case, the formation of NNA decreases at pH values where the degree of ionization of the carboxy-group would scarcely

[4] D. W. Russell, *Chem. Comm.*, 1965, 498.
[5] S. Takahashi and H. Kano, *Chem. and Pharm. Bull.* (*Japan*), 1963, **11**, 1375.
[6] L. K. Ramachandran and L. V. S. Sastry, *Biochemistry*, 1962, **1**, 75.

be affected. Russell's suggestion [1a] that the initial step is the loss of the carboxy-group is still tenable, however, and receives support from the effect of pH on the overall photolysis rate described above. Photolysis in the absence of an α-carboxy-group is very slow,[1c] although the photolysis of *N*-methyl-2,6-dinitro-4-trifluoromethylaniline to give 2-nitro-6-nitroso-4-trifluoromethylaniline has been reported.[7] A scheme involving *N*-hydroxymethyl-2-nitro-6-nitroso-4-trifluoromethylaniline as an intermediate was postulated to account for this reaction. It seems reasonable to combine these two suggestions to give a concerted decarboxylation and oxygen transfer as the initial step. If the excited state for the DNP-amino-acid is written as structure (V) for simplicity, the transition to the intermediate (VI), now in the ground state, is easily envisaged.



It also seems reasonable that the protonated carboxy-group should react somewhat more readily than the ionized one.

The intermediate (VI) has the possibility of several routes. Direct hydrolysis would lead, in all cases, to the NNA. Reversible dehydration could occur (where $R^1 = H$) to give an anil (VII). Where $R^2 = H$, this anil could then cyclize to give the 6-nitrobenzimidazole 1-oxide. This route would not be open to DNP-sarcosine, and this suggests that for other DNP-amino-acids, this is the major route for benzimidazole formation between pH 2 and 5. The alternative, *via* the carbonium ion (VIII) would be available to DNP-sarcosine, and is probably the route which operates at very low pH. The reaction between formaldehyde and *N*-methyl-NNA to give the benzimidazole must proceed by an analogous route.

EXPERIMENTAL

No attempt was made to exclude oxygen from any of the photolysis mixtures.

Preparation of 3-Methyl-6-nitrobenzimidazole 1-Oxide.—By photolysis of DNP-sarcosine. DNP-sarcosine (0·6 g.) was photolysed in solution in 11N-hydrochloric acid (500 ml.). The solution was evaporated nearly to dryness under reduced pressure and was then passed down a short column of De-Acidite FF (quaternary ammonium) resin in the acetate form. The effluent was evaporated to dryness to give the crude product in the free form. Chromatography on alumina, with ethanol, gave the *benzimidazole* (0·17 g., 38%) as yellow crystals, m.p. 204° (decomp.) from aqueous acetone.

From formaldehyde and N-methyl-4-nitro-2-nitrosoaniline. *N*-Methyl-NNA (0·36 g.), prepared by the photolysis of DNP-sarcosine at pH 3·7, dissolved in 11N-hydrochloric acid (2 ml.) was cooled to 0°. Formaldehyde (40% solution) (0·25 ml.) was added and the mixture was allowed to stand for 10 min. Most of the hydrochloric acid was removed under reduced pressure, and the rest by passing the mixture through a short column of De-Acidite G (weak base) resin in the free base form. Chromatography on alumina with ethanol gave (together with other products) 3-methyl-6-nitrobenzimidazole 1-oxide (0·13 g., 35%) identical with that prepared by photolysis, $\lambda_{max.}$ (water) 202, 267, and 295sh nm. (ε 19,800, 15,000, and 5500); (0·5N-hydrochloric acid) 239 and 286 nm. (ε 17,000 and 7700); (pH 10 buffer) 267 and 300sh nm. (ε 14,700 and 5500); (0·5N-sodium hydroxide) 399 nm. (ε 12,000); (ethanol) 208 and 269 nm. (Found: C, 49·9; H, 3·8; N, 22·2. $C_8H_7N_3O_3$ requires C, 49·7; H, 3·65; N, 21·8%).

6-Nitro-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole 4-Oxide.—DNP-proline cyclohexylammonium salt (0·96 g.) dissolved in 0·5% aqueous acetic acid (4 l.) was photolysed for several hours. The solution was then acidified further with hydrochloric acid, extracted with ether; the aqueous residue was evaporated to small volume and neutralized with sodium hydroxide. The solution was then passed down a short column of Dowex 50 (sulphonic acid) resin in the H^+ form and the product was eluted with 2N-ammonia. The eluate was rapidly taken to dryness and the residue was dissolved in the minimum quantity of warm ethanol; dry hydrogen chloride was passed into the solution to give the *benzimidazole hydrochloride* as buff needles (0·105 g., 16%) which decomposed slowly above 180° (hot stage), $\lambda_{max.}$ (water) 203 and 265 nm. (with shoulders at 300 and 340 nm.) unchanged at pH 10; (0·5N-hydrochloric acid) 235 and 290 nm.; (ethanol) 208 and 270 nm. (Found: C, 47·0; H, 4·15; N, 16·4. $C_{10}H_{10}ClN_3O_3$ requires C, 47·0; H, 3·9; N, 16·4%).

1-Methoxy-6-nitrobenzimidazole.—This was prepared from 6-nitrobenzimidazole 1-oxide by a method similar to that used by Takahashi and Kano for the ethoxy-compound.[8]

[7] R. E. McMahon, *Tetrahedron Letters*, 1966, 2307.
[8] S. Takahashi and H. Kano, *Chem. and Pharm. Bull.* (*Japan*), 1964, **12**, 282.

The product had m.p. 120—122°, $\lambda_{max.}$ (water or 0·5N-sodium hydroxide) 240 and 306 nm.; (0·5N-hydrochloric acid) 233 and 283 nm.; (ethanol) 238 and 296 nm. (Found: C, 50·0; H, 3·7; N, 21·8. $C_8H_7N_3O_3$ requires C, 49·7; H, 3·65; N, 21·8%).

N.m.r. Spectra.—To confirm the structure of the photolysis product of DNP-sarcosine, the spectra of this and a number of related derivatives were taken on a Varian A60 spectrometer in 2N-D_2SO_4 in D_2O with either tetramethylsilane or tetramethylammonium chloride as internal reference. 6-Nitrobenzimidazole 1-oxide gave τ 0·37 (2-H), 1·22 (7-H), 1·55 (5-H), and 1·99 (4-H); 5-H was coupled to 7-H (J 3 c./sec.) and to 4-H (J 9 c./sec.). The other compounds gave similar spectra for 4-H, 5-H, and 7-H although with slightly differing τ values. 2-Methyl-6-nitrobenzimidazole 1-oxide gave additionally τ 7·8 (CH_3). 1-Methoxy-6-nitrobenzimidazole gave τ 0·10 (2-H) and 5·52 (OCH_3). 3-Methyl-6-nitrobenzimidazole 1-oxide gave τ 0·42 (2-H) and 5·94 (N-CH_3). These results agree with analogous ones of Takahashi and Kano [9] for non-nitrated benzimidazolium salts.

Rapid Photolysis.—Two 400-w medium-pressure mercury arc lamps with glass jackets were used. The solutions, in the spectrophotometer cell, was placed between these at a distance of *ca.* 5 cm. Photolysis was usually complete within 30 sec. and the solution warmed up only slighty in this short time. Spectra were taken (Beckmann DB spectrophotometer) as soon as possible after photolysis.

Rate Studies.—For these, tungsten spot lamps were used as the discrete emission bands of the mercury-vapour lamps gave misleading results where there was a change of $\lambda_{max.}$ of the DNP-amino-acid with the ionization of the carboxy-group. The solutions to be photolysed were placed in optically matched test tubes, which were then suspended in a cold water bath in a circular rack. The rack was then rotated at *ca.* 10 revolutions/min. while the tubes were illuminated through the glass sides of the bath. In this way, each tube was exposed to the same optical environment. After photolysis, the tubes were placed in the dark and the contents examined as soon as possible.

We are grateful to Dr. A. J. Waring for help in obtaining the n.m.r. spectra.

[9/809 *Received, May 15th,* 1969]

[9] S. Takahashi and H. Kano, *Chem. and Pharm. Bull.* (*Japan*), 1966, **14**, 375.