165. *The Photochemical Degradation of* N-2,4-*Dinitrophenylamino-acids and Related Compounds. Part I. Formation of* 4-*Nitro*-2-*nitrosoaniline from* N-2,4-*Dinitrophenyl-leucine.*

By D. W. Russell.

The action of light on a neutral or alkaline aqueous solution of *N*-2,4-dinitrophenyl-leucine results in formation of carbon dioxide, 3-methylbutyraldehyde, and a green crystalline compound which, on physical and chemical evidence, is shown to be 4-nitro-2-nitrosoaniline (I).

Decomposition of 2,4-dinitrophenylamino-acids by light has been observed,[1-3] and the reaction in the solid state has been shown to be generally decarboxylation to the corresponding *N*-alkyl-2,4-dinitroaniline.[2] In a preliminary account [4] it was reported that the chief product formed in dilute, mildly alkaline aqueous solution is 4-nitro-2-nitrosoaniline (I). The present paper describes the isolation of this compound and the evidence for its structure.

When a dilute solution of *N*-2,4-dinitrophenyl-leucine in dilute sodium hydrogen carbonate solution was exposed to light from a mercury-vapour lamp, bright green needles,

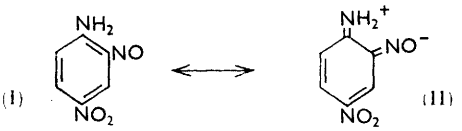
[1] Blackburn, *Biochem. J.*, 1949, **45**, 579; Mills, *ibid.*, 1952, **50**, 707.
[2] Pollara and Von Korff, *Biochim. Biophys. Acta*, 1960, **39**, 364.
[3] Akabori, Ikenaka, Okada, and Kohno, *Proc. Japan Acad.*, 1953, **29**, 509.
[4] Russell, *Biochem. J.*, 1962, **83**, 8P.

$C_6H_5N_3O_3$, crystallized. The ultraviolet ($\lambda_{max.}$ 284, 348 mμ) and infrared ($\nu_{max.}$ 3430, 3230, 1630, 1510, 1500, 1380, 1330 cm.$^{-1}$) spectra of this product, which also absorbed in the visible region ($\lambda_{max.}$ 700 mμ), were consistent with the presence of an aromatic nucleus bearing primary amino-, nitro-, and nitroso-substituents. When the compound was treated with a limited amount of stannous chloride, sufficient for reduction of one nitroso-group to an amino-group, 4-nitro-*o*-phenylenediamine was formed in high yield; partial oxidation with hydrogen peroxide in acetic acid gave 2,4-dinitroaniline. The compound was therefore a derivative of *p*-nitroaniline, substituted in the 2-position by a nitrogen function. The ultraviolet absorption at 284 mμ, together with the results of qualitative chemical tests, suggested that this function was a nitroso-group, and indeed, no other interpretation was consistent with the molecular formula. Confirmation was afforded by catalytic hydrogenation, in which 5 mol. of hydrogen were absorbed to give 1,2,4-triamino-benzene, isolated and characterized as its ditoluene-*p*-sulphonate.

Further evidence for the structure was obtained by examination of the other photolysis products. These were carbon dioxide and 3-methylbutyraldehyde. Reduction of the *o*-nitro-group was therefore accompanied by oxidation of the amino-acid residue at the α-carbon, and the reaction may be seen as a variation on the common mode of oxidation of α-amino-acids to ammonia, carbon dioxide, and the corresponding aldehyde.[5]

Experiments in which photolysis was conducted at different pH values showed that, while *N*-2,4-dinitrophenyl-leucine was rapidly decomposed by light at any pH, 4-nitro-2-nitrosoaniline was formed only at pH 6 or above. The reaction thus involves the anion, the un-ionized acid decomposing differently. The rate of reaction in dilute solution was independent of concentration, so that the process is intramolecular like similar photo-chemical rearrangements involving reduction of an *o*-nitro-group.[6]

The bright green colour of the solid nitroso-compound indicates that it exists in the monomeric form.[7] No convincing support for the monomeric structure could be deduced from the infrared spectrum, although a shoulder at 1510 cm.$^{-1}$ is tentatively ascribed to the N=O stretching mode. If, however, the compound exists in the monomeric form in the solid state, some explanation is needed for the very high intensity of the ultraviolet absorption at 284 mμ when the spectrum is measured in dilute solution. Monomeric *C*-nitroso-compounds normally have a weak maximum in this region, whilst the dimeric forms absorb strongly.[7] That the intense absorption of 4-nitro-2-nitrosoaniline at 284 mμ is not due to the unlikely occurrence of dimerisation in solution is shown by the fact that the green solution in ethanol ($\lambda_{max.}$ 700 mμ) still absorbs strongly at 282 mμ. Rather it is suggested that the compound exists as a resonance hybrid to which the quinonoid form (II) makes a large contribution. The high value for $\varepsilon_{max.}$ of *NN*-dimethyl-*p*-nitrosoaniline has been similarly explained.[7]



(I) (II)

The photochemically active wavelength for the degradation of *N*-2,4-dinitrophenyl-leucine to 4-nitro-2-nitrosoaniline was not precisely determined. However, not only was the reaction carried out in vessels of thick Pyrex glass, which is opaque below 285 mμ, but also the lamp used had negligible emission below 360 mμ. The short-wavelength maximum in the spectrum of *N*-2,4-dinitrophenyl-leucine is thus photochemically inactive in this reaction. Some 18% of the total energy was emitted at *ca.* 364 mμ, according to graphs

[5] Greenstein and Winitz, " Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, 1961, Vol. II, p. 1318.
[6] De Mayo and Reid, *Quart. Rev.*, 1961, **15**, 393.
[7] Gowenlock and Lüttke, *Quart. Rev.*, 1958, **12**, 321.

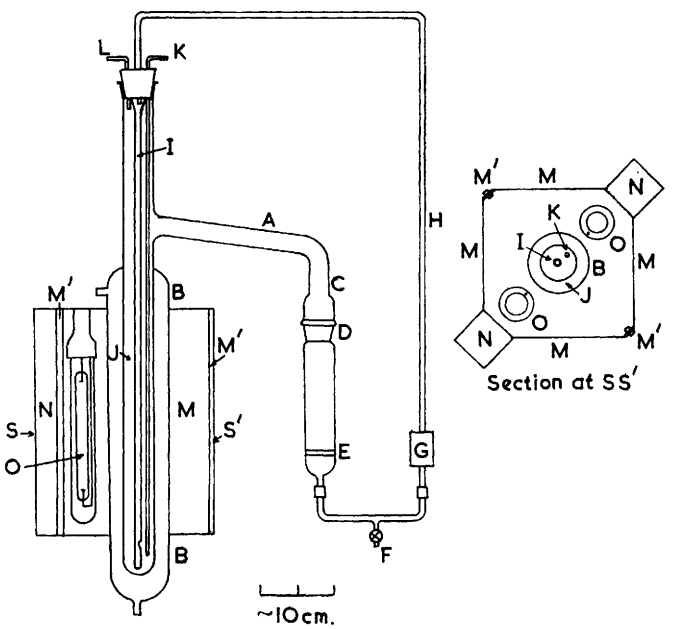
supplied by the manufacturers; the rest was emitted above 400 mμ. It is concluded that absorption in the high-intensity band of the leucine derivative at *ca.* 360 mμ is probably responsible for the transformation.

Experiments to be reported elsewhere have shown that the reaction is a fairly general one for α-2,4-dinitrophenylamino-acids, and that similar compounds in which the carboxyl group is not both free and in the α-position with respect to the dinitrophenylamino-group are relatively stable to light. Insufficient information is so far available for a mechanism to be postulated, but experiments bearing on this problem will be reported later.

Neither *o*-nitrosoaniline nor any of its derivatives have been described before in the literature, and the preparative scope of the reaction is therefore being explored.

Experimental

Ultraviolet spectra were measured with a Unicam S.P. 500 or with a Beckman DU spectrophotometer. Unless otherwise stated, solutions for ultraviolet spectrophotometry were



Apparatus for photolysis. For key see text.

prepared by diluting a solution of the compound in ethanol (1 ml.) with 1% sodium hydrogen carbonate solution (99 ml.). Infrared spectra were measured with a Perkin-Elmer Infracord spectrophotometer; paraffin mulls were used except where otherwise stated. Microanalyses were by Dr. F. Pascher, Bonn.

Preparative Photolysis of N-2,4-*Dinitrophenyl-leucine.*—The apparatus (see Figure) was a liquid–liquid extraction vessel (upward displacement) of capacity *ca.* 1200 ml. to the level of the side-arm (A); it was modified by provision of a jacket (B) through which cold water could be circulated during illumination. The vertical end (C) of the side-arm led *via* a B34 ground-glass joint (D) to a wide-bore tube through which passage of solid was prevented by a sintered-glass disc (E) (porosity 2). Provision was made for withdrawing samples of the reaction mixture by means of the tap (F). A pump (G) served to return solution *via* tubes (H) and (I) to the bottom of the reaction vessel (J). In operation, solution was circulated through the reaction vessel, thence *via* the side-arm through the sintered-glass disc, and then back to the reaction vessel. The solution in the main vessel was stirred by a stream of air or nitrogen

admitted through tube (K) and passed out through tube (L), after which it was passed through a solution of 2,4-dinitrophenylhydrazine (0·5%) in 4N-hydrochloric acid (2 l.) (not shown in the diagram).

Illumination was provided by two Camplex plant-irradiators (Simplex Dairy Equipment Company Limited), of which only one is shown in the main diagram for reasons of clarity. The reflectors (M) of the irradiators were bolted together by their free margins (M′) to form a reflecting box which was mounted about the reaction vessel. The magnetic deflectors were removed from the lamp housings (N) by the manufacturers to permit operation in a vertical position. The light source (O) was two Osram mercury lamps of type MA, the energy of which was emitted in five bands, at approximately 364, 404, 437, 544, and 580 mμ. A small amount of continuous radiation was emitted throughout the spectrum.

A solution of *N*-2,4-dinitrophenyl-DL-leucine (5·95 g.) and sodium hydrogen carbonate (20 g.) in water (2 l.) was placed in the apparatus, circulated, and stirred overnight in the dark. No spectral change occurred. The lamps were switched on for 3 days, after which there was little further change in the spectrum. The crystals (2·70 g.) which had collected on the sintered-glass disc were recrystallized to give 4-*nitro*-2-*nitrosoaniline* (I) (2.40 g.), green needles (from 70% ethanol), m. p. 183—184° (decomp.), raised by further recrystallization to 185—186° (decomp.), $\lambda_{max.}$ 284, 348, 428 mμ (log ε 4·24, 4·09, 3·72) and, in ethanol, $\lambda_{max.}$ 282, 340, 422, 700 mμ (log ε 4·16, 4·10, 3·67, 1·74), $\nu_{max.}$ 3430m, 3230m (N–H), 1630s (N–H), 1510sh (N–O?), 1500m, 1380m (NO_2), 1330s (N–H) cm.$^{-1}$ (film) [Found: C, 43·1, 43·2; H, 2·8, 3·1; N, 24·6, 24·8; O, 28·8, 28·9%; *M*, 158, 165 (lowering of vapour pressure in acetone), 178 (micro-Rast). $C_6H_5N_3O_3$ requires C, 43·1; H, 3·0; N, 25·2; O, 28·8%; *M*, 167].

The compound was moderately soluble in most common organic solvents to give green solutions, sparingly soluble in water to give a yellow solution, and insoluble in light petroleum (b. p. 60—80°). The ultraviolet spectrum was qualitatively unchanged on addition to an aqueous solution of mineral acid to pH 1 or of sodium hydroxide to pH 11. The compound was extracted by organic solvents from aqueous solutions in the pH range 1—11. It dissolved in warm 2N-sodium hydroxide to give a red solution from which ammonia was evolved on boiling. Qualitative tests for the presence of a nitroso-group were positive. Thus, with a solution of *NN*′-diphenylbenzidine in sulphuric acid the compound gave a blue colour;[8] its solution in concentrated sulphuric acid was red, changing to green when phenol was added;[9] it dissolved in glacial acetic acid to give a green solution which, when it was warmed with a few drops of aniline, became bright red.[10]

The precipitate which had formed in the 2,4-dinitrophenylhydrazine solution was dissolved in warm ethanol (200 ml.) and poured into 2N-hydrochloric acid (600 ml.). The precipitate (4·46 g.), recrystallized, gave 3-methylbutyraldehyde 2,4-dinitrophenylhydrazone (4·12 g.), needles (from ethanol), m. p. 123—124°. The infrared and ultraviolet spectra were identical with those of an authentic sample, m. p. 123—124°, prepared by oxidizing leucine with ninhydrin;[11] the mixed m. p. was 123—124° (Found: C, 49·5; H, 5·3. Calc. for $C_{11}H_{14}N_4O_4$: C, 49·6; H, 5·3%).

Similar results were obtained when sodium hydrogen carbonate was replaced by disodium hydrogen phosphate (15 g.). In this case carbon dioxide-free nitrogen was used to remove the aldehyde. After collection of the nitroso-compound the filtrate was acidified by 6N-sulphuric acid, and nitrogen was passed through the solution. Carbon dioxide was detected in the effluent gas.

When the solvent was a 0·1M-phosphate buffer of pH 6·0, in place of the sodium hydrogen carbonate solution, 4-nitro-2-nitrosoaniline (2·0 g.) and 3-methylbutyraldehyde 2,4-dinitrophenylhydrazone (4·0 g.) were obtained.

4-*Nitro*-o-*phenylenediamine from* 4-*Nitro*-2-*nitrosoaniline*.—The nitroso-compound (0·50 g.) was suspended in boiling ethanol (5 ml.), and a solution of stannous chloride dihydrate (1·35 g.) in concentrated hydrochloric acid (1·5 ml.) was added cautiously. The mixture was boiled for 5 min., cooled, and poured into saturated sodium hydrogen carbonate solution (100 ml.). The mixture was extracted with ethyl acetate, and the extract dried (Na_2SO_4) and filtered. Removal of the solvent left a residue of almost pure 4-nitro-*o*-phenylenediamine (0·42 g.), red needles (from

[8] Anger, *Mikrochim. Acta*, 1959, 387; 1960, 58.
[9] Liebermann, *Ber.*, 1874, **7**, 247.
[10] Baeyer, *Ber.*, 1874, **7**, 1639.
[11] Virtanen and Rautanen, *Biochem. J.*, 1947, **41**, 101.

water), m. p. 205—206°. The ultraviolet spectra at pH 1 and pH 8 were identical with those of an authentic sample,[12] m. p. 204—206°, and the mixed m. p. was 204—205°. The infrared spectra of the two samples were also identical (Found: C, 46·8; H, 4·6; N, 27·0. Calc. for $C_6H_7N_3O_2$: C, 47·0; H, 4·6; N, 27·5%).

2,4-*Dinitroaniline from* 4-*Nitro*-2-*nitrosoaniline*.—The nitroso-compound (0·50 g.) in glacial acetic acid (10 ml.) and 30% hydrogen peroxide (5 ml.) was boiled for 10 min., then cooled, diluted with water (35 ml.), and left at 0° overnight. The precipitate of almost pure 2,4-dinitroaniline (0·39 g.) formed yellow prisms (from acetone–water), m. p. and mixed m. p. 177—179°. The infrared and ultraviolet spectra were identical with those of a reference sample, m. p. 177—179°.

Catalytic Hydrogenation of 4-*Nitro*-2-*nitrosoaniline*.—The nitroso-compound (0·50 g.) and 10% palladium–charcoal (0·2 g.) in ethanol (20 ml.) were shaken with hydrogen (Found: H_2 absorbed, 347 ml. One nitro- and one nitroso-group together require 336 ml.). The mixture was rapidly filtered into a solution of toluene-*p*-sulphonic acid (1·03 g.) in ethanol (5 ml.). After some hours at 0° the precipitate of 1,2,4-*triaminobenzene ditoluene*-p-*sulphonate* (1·01 g.) was collected, washed with water and acetone, and dried *in vacuo* (H_2SO_4) (Found: C, 51·6; H, 5·1; N, 9·1; S, 14·5. $C_{20}H_{25}N_3O_6S_2$ requires C, 51·4; H, 5·4; N, 9·0; S, 13·7%). The compound darkened but did not melt below 320°. It decomposed with formation of purple material when recrystallization was attempted. A reference sample, prepared by catalytic hydrogenation of 4-nitro-*o*-phenylenediamine, behaved in the same way (Found: C, 51·6; H, 5·3; N, 9·1; S, 14·2%). The ultraviolet spectra (in N-hydrochloric acid) of the two samples were identical, as were their infrared spectra.

Effect of pH *on the Photochemical Decomposition of* N-2,4-*Dinitrophenyl-leucine*.—The apparatus was a Liebig condenser sealed at one end to form a jacketed tube of *ca.* 40 ml. capacity. This was clamped in a sloping position below a single Camplex plant-irradiator operated horizontally.

A 5×10^{-3}M-solution (1 ml.) of *N*-2,4-dinitrophenyl-leucine in ethanol wasdiluted with a buffer solution (99 ml.) and illuminated, cold water being circulated through the jacket. Rapid changes in the ultraviolet spectra took place at all pH values. When no further change, or only slow further change, took place, the ultraviolet spectra of the solutions were measured (see Table).

The behaviour of 10^{-5}M-solutions of 4-nitro-2-nitrosoaniline was also investigated in the same way. Only very slow changes were observed in the ultraviolet spectra of the solutions on illumination.

Buffer (0·1M)	pH	$\lambda_{max.}$ (±2 mμ) in photolysed soln. 1	2	E_1/E_2	Buffer (0·1M)	pH	$\lambda_{max.}$ (±2 mμ) in photolysed soln. 1	2	E_1/E_2
HCl	1·0	236	284	1·7	Phosphate ...	6·0	284	348	1·5
HCl–citrate...	2·0	~236	284	—	,, ...	7·0	284	348	1·4
,, ...	3·0	260	—	—	,, ...	8·0	284	348	1·4
Phosphate ...	4·0	260	—	—	KOH–glycine	10·0	284	348	1·3
,, ...	5·0	268	—	—	,,	12·0	284	348	1·1

Rate of Photochemical Decomposition of N-2,4-*Dinitrophenyl-leucine*.—Solutions (5×10^{-4}, 10^{-3}, and $2 \cdot 10^{-3}$M) of the compound in 1% sodium hydrogen carbonate solution were illuminated in the apparatus used for the previous experiment. The geometry and concentrations satisfied approximately the condition of " infinite " optical thickness of the solution. At intervals, samples were withdrawn and diluted with water, and the ratios of the absorbances at 284 and 348 mμ measured. The proportions of *N*-2,4-dinitrophenyl-leucine and 4-nitro-2-nitrosoaniline were calculated, and the validity of the calculation checked by comparison of the complete spectrum with that of a solution of a mixture of the two compounds in the calculated proportions. No significant differences were found. Spectra of partly photolysed solutions did not alter when solutions were removed from the apparatus and stored in the dark.

For each concentration a linear relation was found between time of illumination and percentage decomposition. The rate of decomposition (moles $hr.^{-1}$) was the same at all three concentrations. At still higher concentrations, precipitation of 4-nitro-2-nitrosoaniline began before useful spectral measurements could be made.

[12] Griffin and Peterson, *Org. Synth.*, Coll. Vol. III, p. 242.

The technical assistance of Mrs. M. E. Carruthers, Mrs. A. S. R. Davies, and Mr. G. Cox is gratefully acknowledged. This work, carried out mainly in these laboratories, was begun at Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand, and I am indebted to the Director, Dr. A. T. Johns, for his interest and encouragement.

TWYFORD LABORATORIES, TWYFORD ABBEY ROAD,
LONDON, N.W.10.

[*Received, August 3rd,* 1962.]