Nitro-heteroaromatic Derivatives of Amino-acids and Peptides. Part V.[1] Photochemical Formation of 2-Isobutylpyrido[2,3-*d*]imidazole 1-Oxide from 3-Nitro-2-pyridyl-DL-leucine †

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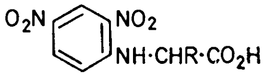
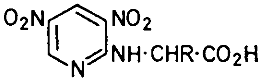
The photocyclization of 3-nitro-2-pyridyl-DL-leucine to give 2-isobutylpyrido[2,3-*d*]imidazole 1-oxide has been investigated spectrophotometrically. The quantum yields of the reaction brought about by irradiation at 366 nm in water have been determined at various hydrogen ion concentrations. The rate of photolysis depends on the degree of protonation of the pyridine nitrogen atom.

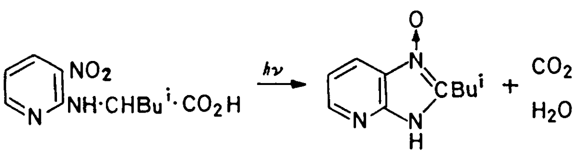
THE photolability of Dnp-amino-acids (I), involving loss of carbon dioxide, has been known for some time.[2] The nature of the products has been shown [3,4] to depend upon pH, low pH favouring the formation of a 6-nitrobenzimidazole *N*-oxide and higher pH giving 2-nitroso-4-nitroaniline and an aldehyde. The photochemical reaction of the analogous Dnpy-amino-acids (II) to give 2-amino-3-nitroso-5-nitropyridine, carbon dioxide, and an aldehyde has been reported;[5] however, the nitroso-derivative was partially photodecomposed during the reaction and this made it difficult to investigate the reaction mechanism. Furthermore, owing to the low basicity of the nitrogen heteroatom in the dinitropyridyl system,[6] it was difficult to substantiate any correlation between results of photolysis and pH. From this point of view, 3-Npy-derivatives seemed to be suitable substrates for investigating whether these heterocyclic compounds react as the free base or as the conjugate acid; in this case the higher basicity of the nitrogen heteroatom (pK_a *ca.* 0·5—1·0) would permit the easier determination of the relationship between the quantum yield of the photoreaction and the acidity of the medium.



(I)



(II)



(III) (IV)

We hoped to elucidate the mechanism of photodecomposition and to discover whether the kinetics of photolysis of 3-Npy-DL-leucine (III), over the pH range 0—4, gave evidence for the pH dependence of the reaction.

EXPERIMENTAL

Materials and Methods.—All chemicals used were of analytical reagent grade. 2-Fluoro-3-nitropyridine and 3-Npy-DL-leucine (III) were prepared as reported elsewhere;[7,8] the purity of the latter was tested by elemental analysis and by t.l.c. on silica gel G (Merck). Carbon dioxide evolved during the photolysis of compound (III) was estimated as previously described.[5] Compound (III) (0·056 g) in 0·75% (w/v) disodium hydrogen phosphate (100 ml) was irradiated for 2 h. The carbon dioxide was titrated with 0·0228M-sodium methoxide; 8·80 ml consumed, equivalent to 1·99 × 10^{-4} mol of carbon dioxide (yield 99·6%).

The previously described procedure[9] was used for measurements and calculation of the pK_a values of compound (III). The pH of buffered solutions (sodium acetate–hydrochloric acid for pH 0·65—5·0 and hydrochloric acid for lower pH values) was measured with a Sargent–Welch PBX pH meter previously calibrated at pH 4 and 7. All measurements were made at 27°.

Spectroscopy.—U.v. spectra were measured with Unicam SP 500 and Optica CF4 DR instruments, n.m.r. spectra with a Varian HA 100 spectrometer (tetramethylsilane as internal reference), and the mass spectrum with a Hitachi–Perkin-Elmer RMN-6E spectrometer.

Quantum Yield Measurements.—Light of wavelength 366 nm was obtained from an Osram HBO 200 high-pressure mercury lamp by use of a combination of a Balzers interference filter and a Corning glass filter (C.S. 052). The excitation conditions involved total absorption of light by compound (III), concentration *ca.* 3 × 10^{-4} mol l^{-1}, light path length 4·0 cm. The light intensity was measured with a ferrioxalate actinometer.[10] The amount of photoreaction (10—25%) was determined spectrophotometrically (0·5 cm cells), by measurements in the 370—450 nm region where the photolysis products show negligible absorption. Below pH *ca.* 0·5 a competitive dark reaction was observed; the change of optical density was therefore determined by differential analysis with as a blank an identical solution which was not irradiated.

2-*Isobutylpyrido*[2,3-d]*imidazole* 1-*Oxide* (IV).—A solution

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† The following abbreviations are used: Dnp, 2,4-dinitrophenyl; Dnpy, 3,5-dinitro-2-pyridyl; 3-Npy, 3-nitro-2-pyridyl.

[1] Part IV, C. Toniolo, D. Nisato, L. Biondi, and A. Signor, *J.C.S. Perkin I*, 1972, 1182.

[2] D. W. Russell, *Biochem. J.*, 1963, **87**, 1.

[3] D. W. Russell, (*a*) *Biochem. J.*, 1962, **83**, 8; (*b*) *J. Chem. Soc.*, 1963, 894; (*c*) *ibid.*, 1964, 2829; (*d*) *Chem. Comm.*, 1965, 498; (*e*) *J. Medicin. Chem.*, 1967, **10**, 984.

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

[5] E. Bordignon, G. G. Aloisi, and A. Signor, *Gazzetta*, 1970, **100**, 802.

[6] A. Signor and E. Bordignon, *J. Org. Chem.*, 1965, **31**, 3447.

[7] G. C. Finger and L. D. Starr, *J. Amer. Chem. Soc.*, 1959, **81**, 2674.

[8] A. Signor, L. Biondi, A. M. Tamburro, and E. Bordignon, *European J. Biochem.*, 1969, **7**, 328.

[9] B. J. Thamer and A. F. Voigt, *J. Phys. Chem.*, 1952, **56**, 225.

[10] C. A. Parker, *Proc. Roy. Soc.* (*A*), 1955, **220**, 104.

of compound (III) (1·0 g) in water (2 l) was placed in a 3 l cylinder. A Pyrex jacket containing a 1000 W high-pressure mercury arc (Hanovia Q1200/PL 357) and cutting off wavelengths lower than 300 nm was used. The resulting path-length of the system was 2·5 cm; water at constant temperature (20 ± 0·1°) was circulated through the jacket. After irradiation for 5 h no further change in the u.v. spectrum was observed. The solution was then evaporated and the solid residue was washed with anhydrous ether to give a microcrystalline *powder* (0·45 g, 50%), m.p. 188—190° (Found: C, 62·5; H, 6·9; N, 21·8. $C_{10}H_{13}N_3O$ requires C, 62·8; H, 6·8; N, 21·9%).

RESULTS AND DISCUSSION

When an aqueous solution of compound (III) was irradiated, carbon dioxide was evolved quantitatively and the pyridoimidazole *N*-oxide (IV) was isolated. Electronic absorption spectra at various times are shown in Figure 1. The products do not have appreciable absorption in the region of irradiation and analysis (350—450 nm). Compound (IV) showed $\lambda_{max.}$ *ca.* 290 nm, *m/e* 191 (M^+) and 175 (M – O, intense), τ (CF_3·CO_2H) 1·20 (q, *J* 1 and 8 Hz, H-4), 1·37 (q, *J* 1 and 6 Hz, H-6), 2·03 (q, *J* 6 and 8 Hz, H-5), 6·75 (d, *J* 7 Hz, CH_2), 7·45 (m, Me_2CH), and 8·83 (d, *J* 6 Hz, Me_2CH). The n.m.r. data are consistent with an electron-withdrawing effect by the imidazole ring at the 2-position. The 5-proton shows two *ortho*-couplings, as expected, the larger of which is assigned as $J_{4,5}$ by analogy with pyridine itself.[11,12] The 4-proton is apparently deshielded by the *N*-oxide function.

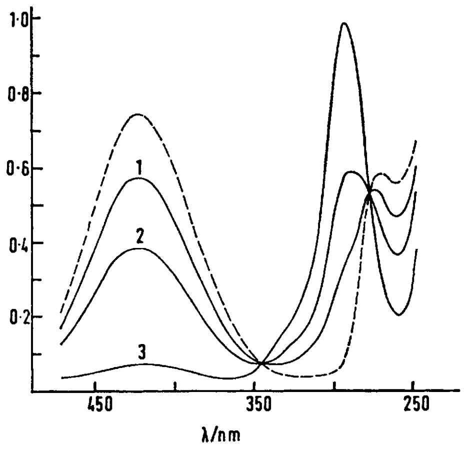


Figure 1 Spectral changes of an aqueous 10^{-4}M-solution of 3-Npy-DL-leucine irradiated at 366 nm; the dashed curve is the spectrum of the starting solution

When the photolysis of compound (III) in aqueous solution was followed at about pH 1, where both the free base and the conjugate acid are present (*cf.* Figure 2), with 366 nm light (in the region of absorption of the protonated substrate) and with 436 nm light (in the region of absorption of the free base), it was observed that the shorter wavelength light was much more efficient in causing photodecomposition. On this basis it appears that the quantum yield for the disappearance of the protonated compound is much larger than that for the free base. As far as the protonated form is concerned, the predominant ionic species that may be found as one goes from pH 0 to pH *ca.* 5 are shown in the Scheme. Therefore, the quantum yield of the photodecomposition could differ for the mono- and the diprotonated species and could depend on their concentrations, according to the relative pK_a values.

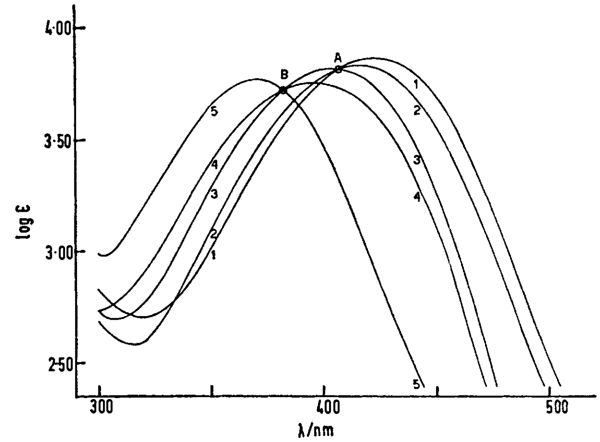
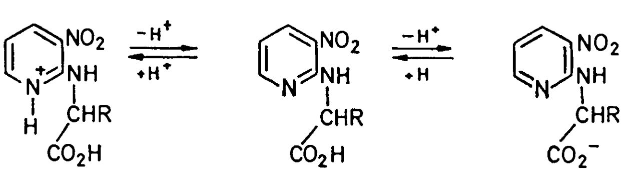


Figure 2 U.v. spectra of 3-Npy-DL-leucine at various pH values (7·5, 3·6, 2·2, 1·1, and –0·69 for curves 1, 2, 3, 4, and 5 respectively)



Scheme

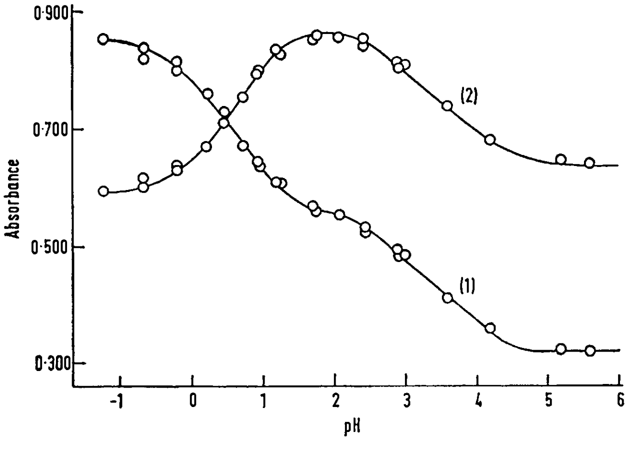


Figure 3 3-Npy-DL-leucine (2·82 × 10^{-4} mol l^{-1}; 0·5 cm cell); absorbance at 370 (1) and at 390 nm (2) against pH

To evaluate the possible connection between acidity of the acids and their respective rates of photolysis, we have studied in detail the protonation behaviour of compound (III). Figure 3 shows absorbance at 370 and

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[11] W. G. Schneider, H. J. Bernstein, and J. A. Pople, *Canad. J. Chem.*, 1957, **35**, 1487.

[12] W. G. Schneider, H. J. Bernstein, and J. A. Pople, *Ann. New York Acad. Sci.*, 1958, **70**, 806.

at 390 nm as a function of pH. The 370 nm curve shows the usual sigmoid shape with two inflections, corresponding to the dissociation of the pyridinium and carboxy-groups, respectively. The bell-shaped 390 nm curve exhibits a maximum at about pH 2; the same type of curve can be obtained in the wavelength region between the two isosbestic points A and B of Figure 2, as expected from the relative values of the molar extinction coefficients of the various species involved. The overlapping ionization constants were calculated from such bell-shaped absorbance–pH curves, by use of the method previously reported.[9] From such a calculation, the inflections in the 370 nm curve correspond to pK_a values of +3·5 and +0·6, respectively. Furthermore, the pK_a of the first excited singlet state, calculated from curves 1 and 5 of Figure 2,[13] is 5—6 units lower than that of the ground state.

On this basis, the dependence of the quantum yield of the photolysis of compound (III) on the acidity of the medium can be readily explained. The corresponding pH dependence sigmoid curve shows a single inflection in the pH range 0—2; the pK_a value obtained from such a curve is +0·7, in excellent agreement with the dissociation constant calculated for the pyridine group. The results are reported in the Table.

The results can be summarized as follows: (i) the photochemical reactivity of the free base is negligible; and (ii) the contribution to the overall quantum yield from the monoprotonated molecule is not important at pH values below 2. Thus the process may reasonably be considered as characteristic of the fully protonated molecule, and if the first excited singlet is involved it is conceivable that the protonated molecule, despite the much smaller basicity in its first excited singlet state, is not equilibrated with the conjugate base.

Quantum yields of the photochemical disappearance of 3-Npy-DL-leucine at 366 nm [a]

pH	Φ	pH	Φ	pH	Φ
5·20	0·003	1·75	0·041	0·44	0·180
4·20	0·004	1·25	0·073	0·22	0·214
3·60	0·007	1·20	0·077	−0·20 [b]	0·221
2·95	0·012	0·95	0·110	−0·20 [b]	0·225
2·90	0·011	0·93	0·120	−0·69 [b]	0·227
2·07	0·024	0·72	0·148	−0·69 [b]	0·240

[a] Potassium chloride added to make ionic strength 2·0 mol l^{-1}. [b] Data derived from M. A. Paul and F. A. Long, *Chem. Rev.*, 1957, **57**, 1.

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[13] A. Weller, *Z. Elektrochem.*, 1952, **56**, 662.