Denitrosation of *N*-Acetyl-*N*^{1}-nitrosotryptophan in Acid Solution

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The denitrosation of DL-*N*-acetyl-*N*^{1}-nitrosotryptophan has been studied kinetically in water in the acid range 4×10^{-2}–1M-H_2SO_4 and also at lower acidities in buffer solutions pH 2—6. The reaction gave DL-*N*-acetyltryptophan and nitrous acid quantitatively and was not significantly reversible under these conditions. First-order behaviour was found for both the nitrosamine and acid in the sulphuric acid reactions, and the reaction was also acid-catalysed in the pH region 2—6. The reaction rate constant was unaffected by the addition of *N*-acetyltryptophan. In 0.04M-H_2SO_4 the rate constant was unchanged by the addition of bromide ion, thiocyanate ion, iodide ion, and thiourea, and the kinetic solvent isotope effect k_{H_2O}/k_{D_2O} was 1.3 at 0.7M-H_2SO_4 and 1.1 at 0.1M-H_2SO_4. However at pH 6 catalysis was observed by chloride, bromide, thiocyanate, azide, and iodide ion with increasing efficiency along this sequence. As the concentration of the nucleophile was increased the reaction rate constant tended to become independent of the nucleophile concentration. Thus *N*-acetyl-*N*^{1}-nitrosotryptophan behaves as a typical nitrosamine at very low acidities around pH 6, whereas at higher acidities it shows a pattern of behaviour reminiscent of that shown by nitrosamides. The pH–rate profile for denitrosation shows clearly that there are two pathways associated with the acid-catalysed reaction, one predominant at around pH 4—7 and the other at acidities greater than pH 1. The former is associated with nucleophilic catalysis and the latter is not. The results are discussed in terms of two possible sites of protonation of the substrate (at O and C-3), and the changing effective rate limiting step, as the nucleophile concentration is changed. *N*-Acetyl-*N*^{1}-nitrosotryptophan can be used to nitrosate (or diazotise) other species such as 4-nitroaniline, but only in the absence of a nitrous acid trap, indicating that this nitrosamine is not a direct nitrosating agent towards amines under these conditions. A minor photochemical decomposition pathway has been established for *N*-acetyl-*N*^{1}-nitrosotryptophan at pH 6, but it was not examined in detail.

Previous work on the denitrosation of nitrosamines in aqueous acid solution has shown [1,2] that the kinetic results can be explained in terms of rate-determining attack by a nucleophilic species (Y^-) on the protonated form of the nitrosamine as outlined in Scheme 1. We have written the protonation as occurring in one stage at the amino-nitrogen atom, although conceivably *O*-protonation is also involved. For R′ = Ph and R″ = Ph or Me, the reaction was found to be very sensitive to the nature of Y^-, with iodide ion being *ca.* 15 000 times more reactive than chloride ion in one case. The normal reversibility of the reaction must be suppressed by the addition of a nitrite trap X, such as sulphamic acid or sodium azide, in sufficient concentration such that $k_3[X] \gg k_{-2}[R'R''NH]$. The observed solvent isotope effect k_{H_2O}/k_{D_2O} of 0.3 is consistent with Scheme 1, where there is rapid equilibrium formation of a low concentration of a protonated species.

$$R'R''NNO + H_3O^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R'R''\overset{+}{N}HNO + H_2O$$

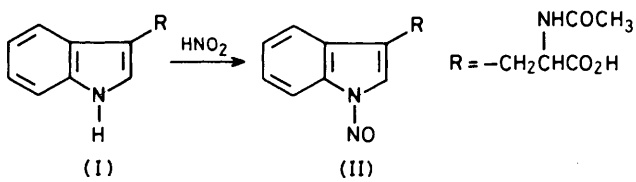
$$R'R''\overset{+}{N}HNO + Y^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R'R''NH + NOY$$

$$NOY + \text{Nitrite trap (X)} \xrightarrow{k_3} \text{Decomposition products}$$

Scheme 1

Interestingly, denitrosation studies of other *N*-nitroso-compounds have shown different characteristics. For example the denitrosation of *N*-n-butyl-*N*-nitrosoacetamide,[3] *N*-nitroso-2-pyrrolidone,[4] *N*-methyl-*N*-nitrosourea,[5,6] and *N*-nitrosotoluene-*p*-sulphonamide [7] all proceed with acid catalysis but without any kinetic dependence upon the concentration or nature of Y^-. Further, all show kinetic solvent isotope effects within the range 1.5—1.9. The results have been rationalised [6] using Scheme 1, in terms of the relative importance of the two terms k_{-1} and $k_2[Y^-]$. The change to the other pattern of behaviour shown by nitrosamides has also been achieved for nitrosamines [8] by reaction at high $[Y^-]$ for reactive nucleophiles such as SCN^- and $SC(NH_2)_2$, and also for reactions of nitrosamines in ethanol.[9]

Tryptophan is one of the essential naturally occurring amino-acids, the nitrosation reactions of which are at present under scrutiny.[10] To avoid complication due to possible attack at the α-amino-function, leading to deamination, we have worked throughout with the *N*-acetyl derivative. The



(I) (II)

nitrosation of DL-*N*-acetyltryptophan (I) yields [11] the *N*-nitroso-derivative (II), where substitution has occurred at the indole nitrogen atom. The methyl ester of (I) behaves similarly. These *N*-nitroso-compounds, which are model compounds for nitrosation studies of peptides and proteins,[10] have been shown [12] to be mutagenic, suggesting that nitrosation of side-chains of α-amino-acids may be important in the aetiology of cancer of the gastrointestinal tract. Further, in contrast to nitrosamines generally,[13] the *N*^{1}-nitrosotryptophan derivatives do not appear to require metabolic activation before becoming biologically active. It seems important and relevant to the possibilities of nitrosamine-induced carcinogenesis in humans, to examine *N*^{1}-nitrosotryptophan derivatives as potential nitrosating agents. We now present the results of a study of the denitrosation reactions of (II) in the presence of various nucleophiles and over a wide range of acid concentrations.

Table 1. Typical run for the denitrosation of (II) (6×10^{-5}M) in sulphuric acid (3.96×10^{-2}M) containing sodium azide (1.35×10^{-2}M) at 31°

t/s	Absorbance	$10^3 k_0$/s^{-1}
0	0.743	
20	0.701	3.08
40	0.664	2.98
60	0.628	2.97
80	0.592	3.02
100	0.560	3.01
120	0.529	3.02
140	0.500	3.02
160	0.473	3.02
180	0.449	3.00
200	0.427	2.98
220	0.401	3.02
240	0.381	3.01
∞	0.039	

Mean value $k_0 = (3.01 \pm 0.03) \times 10^{-3}$ s^{-1}

Experimental

DL-*N*-Acetyl-*N*^{1}-nitrosotryptophan (II) was prepared from DL-*N*-acetyltryptophan (I) and sodium nitrite under very mild conditions, avoiding an excess of acid, as has already been described.[11] The *N*-acetyltryptophan was of the highest purity grade available, and was used without further purification. Similarly the methyl ester of (II) was made by the nitrosation of *N*-acetyltryptophan methyl ester.[11] Potassium bromide, potassium thiocyanate, potassium iodide, thiourea, and citric acid were all of AnalaR grade. 4-Nitroaniline was recrystallised from aqueous ethanol.

Rate measusements were all carried out in aqueous solution at 31° using sulphuric acid solutions or a citric acid–disodium phosphate buffer,[14] prepared from 340 ml of 0.1M-citric acid and 660 ml of 0.2M-disodium phosphate, for the pH 6 work. The pH values of solutions were checked with a pH meter. All reactions were started by dissolving compound (II) in a small amount of AnalaR methanol, and this was added to the acid solution. Reactions were carried out in the cells of a recording spectrophotometer, noting the disappearance of the absorption at 335 nm due to the chromophore of (II). Good first-order plots were obtained from the normal integrated rate equation and the rate constants were reproducible to within ±5%. A typical run is quoted in Table 1 for the reaction of (II) (6×10^{-5}M) in sulphuric acid (4×10^{-2}M) containing sodium azide (1.35×10^{-2}M).

The release of nitrous acid was determined quantitatively, by running a typical reaction in the presence of 4-chloroaniline (and no other nitrite trap). After ten half-lives a portion was added to a solution of excess of 2-hydroxynaphthalene-3,6-disulphonic acid in borax. The resulting azo dye absorbance was measured at 510 nm (log ε 4.34). Based on the initial [(II)] the yield of nitrous acid was 101%.

The u.v. spectra of the reaction solutions after >10 half-lives were identical with those of (I) in acid solution.

Results and Discussion

Rate measurements were carried out in sulphuric acid over the range 0.04—1M and also in a buffer solution at pH 6. The effect of a number of additives on the rate constant for the reaction was noted for both sets of experimental conditions and will be discussed separately.

Table 2. Variation of the rate constant for denitrosation of (II) with acid concentration at 31°

10^2[H_2SO_4]/M	$10^4 k_0$/$^{-1}$
3.96	32.4
7.92	40.4
9.90	44.3
15.8	54.1
29.7	76.7
59.4	141
69.3 *	160
119	315

* In the presence of NaN_3 (7.4×10^{-3}M).

Table 3. Effect of added sodium azide upon k_0 for the reaction of (II) in 3.96×10^{-2}M-H_2SO_4 at 31°

10^3[NaN_3]/M	$10^4 k_0$/s^{-1}
0	32.4
3.38	29.2
6.75	30.9
13.5	30.1

Table 4. Effect of added nucleophiles upon k_0 for the reaction of (II) in 3.96×10^{-2}M-H_2SO_4 at 31°

Nucleophile		$10^4 k_0$/s^{-1}
		32.4
KBr	4×10^{-3}M	32.1
KBr	21×10^{-3}M	31.8
KBr	41×10^{-3}M	30.9
KSCN	8×10^{-3}M	34.6
KSCN	16×10^{-3}M	32.9
KSCN	24×10^{-3}M	33.7
$SC(NH_2)_2$	9×10^{-3}M	33.3 *
$SC(NH_2)_2$	19×10^{-3}M	33.2

* In the presence of NaN_3 (1.7×10^{-2}M).

Table 5. Effect of added *N*-acetyltryptophan (I) on the rate constant for the denitrosation of (II) in 3.96×10^{-2}M-H_2SO_4 at 31°

10^3[(I)]/M	$10^4 k_0$/s^{-1}
0	32.4
1.67	38.2
3.33	40.1
6.67	41.3

(a) *Reactions in Sulphuric Acid.*—Quantitative denitrosation of (II) giving nitrous acid and (I) was observed, and good first-order behaviour was found in all the kinetic experiments. The first-order rate constant k_0 is defined by $-d[(II)]/dt = k_0[(II)]$. Acid catalysis was established over the range 0.04—1M-sulphuric acid as is shown by the results in Table 2. The reaction is first order in H^+, or more strictly h_0 at the upper end of the acidity range. There is a small but significant intercept to the k_0–acidity plot indicating that the reaction has an uncatalysed pathway in addition to the acid-catalysed route. The second-order rate constant k_1 (defined by $k_0 = k_1[H_3O^+]$) has a value of 0.012 l mol^{-1} s^{-1}, which compares with values of 0.032 and 0.059 l mol^{-1} s^{-1} respectively for the reaction of *N*-methyl-*N*-nitrosoaniline [8] and *N*-nitrosotoluene-*p*-sulphonamide,[7] at the same temperature (31°).

The effects of added sodium azide, potassium bromide, potassium thiocyanate, thiourea, and (I) are set out in Tables 3—5. For sodium azide there is no effect upon k_0 up to 1.35×10^{-2}M. This indicates quite clearly that under these conditions the rate of the reverse reaction [*i.e.* the *N*-nitrosation of (I)] is

insignificantly small compared with that of the denitrosation, since it is known that sodium azide in this concentration range and under these experimental conditions is an excellent trap for free nitrous acid.[15] This conclusion is also borne out by the failure of added (I) to reduce the observed values of k_0 at 3.96×10^{-2}M-H_2SO_4 (Table 5). [In fact there is a small increase in the rate constant over the range studied. This might be due to the change in the acidity of the medium on addition of (I): it is also possible that (I) itself acts as a general acid here, though the effect seems a little large for this. Exactly the same effect was noted at the higher acidity of 0.297M-H_2SO_4.]

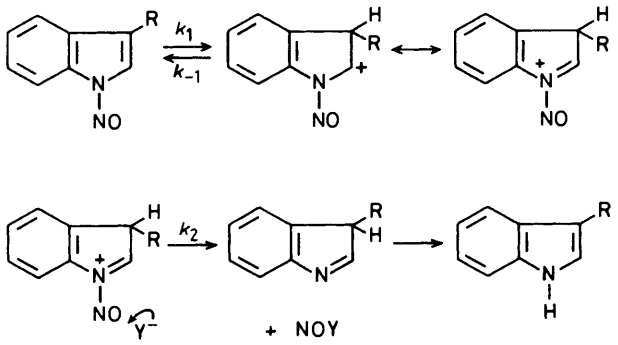
The rate constant for denitrosation at 3.96×10^{-2}M-H_2SO_4 is unaffected by the presence of the nucleophiles Br^-, SCN^-, and $SC(NH_2)_2$ up to *ca.* 0.02M as shown in Table 4. This contrasts with the behaviour of previous nitrosamines studied [1,2] but follows the pattern set for nitrosamides [3–6] and a nitrososulphonamide.[7] The kinetic solvent isotope effect k_{H_2O}/k_{D_2O} was found to be 1.3 at 0.7M- and 0.6M-H_2SO_4 and 1.1 at 0.1M-H_2SO_4. Again the results resemble those of the nitrosamides rather than those of the nitrosamines. It has been argued [6] that the different behaviour arises from the existence of the two limiting forms of the rate expression derived from Scheme 1 and which are given by equations (2) and (3), from the general expression, equation (1). These are based on the inequalities $k_{-1} \gg k_2[Y^-]$ and $k_{-1} \ll k_2[Y^-]$. It appears that for nitrosamines generally (except at high $[Y^-]$, and also in ethanol solvent), the former inequality applies, leading to a first-order dependence upon Y^- [equation (2)], whereas the introduction of the powerfully electron-withdrawing substituents >C=O and $-SO_2-$ increases the value of k_2 markedly so as to reverse the inequality, leading to the other limiting form, equation (3). It appears that the same change can be accomplished for nitrosamines by a change to a less polar solvent (ethanol) and also by working at high concentration of a powerful nucleophile [*e.g.* SCN^- or $SC(NH_2)_2$], thus increasing $k_2[Y^-]$ relative to k_{-1}. This explanation is consistent with the dependence of k_0 upon $[Y^-]$ or otherwise, and also with the simultaneous change in the kinetic solvent isotope effect. Since the overall reaction is not significantly reversible under these conditions ($k_3[X] \gg k_{-2}$-[R′R″NH]) we can ignore step k_{-2}. Thus in going from nitrosamines to nitrosamides (and their relatives) a change to an earlier rate-determining step is observed.

$$k_0 = k_1k_2[H_3O^+][Y^-]/(k_{-1} + k_2[Y^-]) \quad (1)$$

$$k_0 = k_1k_2[H_3O^+][Y^-]/k_{-1} \quad (2)$$

$$k_0 = k_1[H_3O^+] \quad (3)$$

We have written the proton-transfer as occurring in one stage (step k_1). It is rather unusual for proton transfers of this type to be rate-determining, so it may well be that Scheme 1 is something of an oversimplification. Another possibility is that protonation occurs at the nitroso-oxygen atom followed by a rearrangement to nitrogen, which could become rate-limiting. In the case of the tryptophan derivative, an alternative site of protonation is C-3, as shown in Scheme 2, since indole systems generally protonate extensively at C-3 even with an alkyl substituent at this position.[16] Scheme 2 shows a possible mechanism for the denitrosation, with $k_2[Y^-] \gg k_{-1}$. It is not possible to discount a mechanism in which the NO^+ group is lost unimolecularly from the protonated nitrosamine. This could conceivably occur as the rate-limiting step and the kinetic relationship would be the same for both mechanisms. On the other hand we have no evidence to suggest why nucleophilic participation by Y^- might not be necessary in this, and



Scheme 2

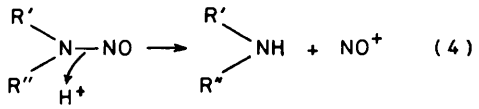


Table 6. Effect of changing the solvent to aqueous methanol on the rate constant for the denitrosation of (II) in 3.96×10^{-2}M-H_2SO_4 at 31°

% MeOH	$10^4k_0/s^{-1}$
5	31.4
14	30.5
24	23.6
43	19.8
62	12.8

others situations. There is also the kinetic solvent isotope effect which would be difficult to explain if NO^+ loss were rate limiting.

The kinetic evidence here is also in accord with a one-step mechanism, given by equation (4) in which NO^+ is expelled simultaneously with H^+ attack. It would, however, be difficult to explain a change over to this mechanism for nitrosamines at high concentrations of thiocyanate and thiourea,[8] where the same experimental characteristics are found as for nitrosamides and here now for the N^1-nitrosoindole system. It seems clear that for nitrosamines the rate-limiting step must change to an earlier one as a result of an increase in $[Y^-]$. This effect should be more pronounced for the most powerful nucleophiles as is observed.

The effect of change of solvent on the denitrosation of (II) was examined briefly using a series of methanol–water solvent mixtures, since in all the experiments a small quantity of methanol was added to dissolve the reactant. The results shown in Table 6 indicate that there is a small decrease in k_0 as the methanol component of the solvent is increased; the results are similar to that noted earlier [7] for the denitrosation of *N*-nitrosotoluene-*p*-sulphonamide, whilst contrasting with the 'normal' behaviour of nitrosamines where a large increase in rate occurs as the polarity of the solvent is lowered.[9]

Thus we have three experimental criteria which distinguish between the two limiting forms of the denitrosation mechanism. These are summarised in Table 7. Features in the left-hand column are generally shown by nitrosamines in aqueous media whilst those in the right-hand column are demonstrated by nitrosamides, nitrososulphonamide, and nitrosamines in ethanol, nitrosamines at very high $[Y^-]$, and by the N^1-nitrosoindole system. The reason why (II) follows the nitrosamide pattern rather than that of nitrosamines probably derives from the fact that here *C*-protonation occurs and the

Table 7. Summary of characteristics shown by nitrosamine and nitrosamide denitrosation reactions

Nitrosamine	Nitrosamide
Rate-limiting Y^- attack *i.e.* $k_{-1} \gg k_2[Y^-]$	Earlier rate-limiting step $k_2[Y^-] \gg k_{-1}$
1 Nucleophilic catalysis	No nucleophilic catalysis
2 $k_{H_2O}/k_{D_2O} \sim 0.3$	k_{H_2O}/k_{D_2O} 1.3—1.9
3 Large rate increase with decreasing solvent polarity	Small rate decrease with decreasing solvent polarity

Table 8. The rate constant for denitrosation of (II) as a function of pH at low acidity at 31°

pH	$10^4 k_0/s^{-1}$
2.41	28.0
3.12	27.7
3.96	23.3
4.93	17.1
5.45	12.1
5.88	7.38
6.15	5.88
6.82	2.16

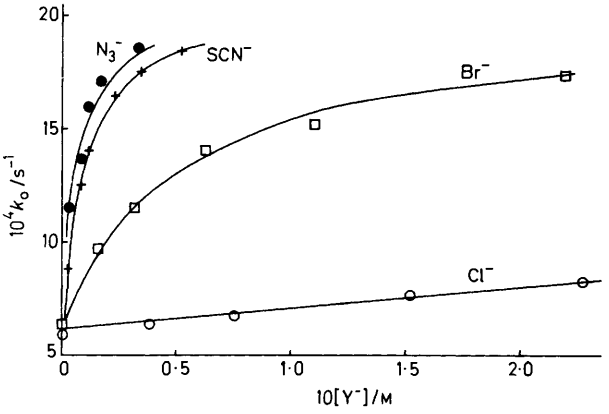


Figure 1. The extent of nucleophilic catalysis in the denitrosation of *N*-acetyl-N^1-nitrosotryptophan (II) at pH 6 and at 31°

resulting rate constants are such as to favour the other limiting form, with $k_2[Y^-] \gg k_{-1}$.

(b) *Reactions at pH* 6.—We have also examined the kinetics of the denitrosation of *N*-acetyl-N^1-nitrosotryptophan at much lower acidities, over the pH range 2—6 in a critic acid–phosphate buffer since these conditions are similar to those encountered *in vivo*. Denitrosation again occurred readily and irreversibly as shown by the complete disappearance of the absorbance at 335 nm, and the excellent first-order kinetic behaviour over greater than two half-lives. The reaction is also acid catalysed as shown by the data presented in Table 8. The situation regarding nucleophilic catalysis is, however, surprisingly quite different at pH 6 compared with the earlier results noted for reaction at 3.96×10^{-2}M-H_2SO_4 (pH *ca.* 1), in that very definite catalysis was observed for the following nucleophiles, in increasing order of effectiveness: Cl^-, Br^-, SCN^-, N_3^-, and I^-. The results are shown graphically in Figure 1, for all these nucleophiles except I^-, where the graph is very close to that of N_3^-. For chloride ion, over the concentration range studied, k_0 is linearly dependent upon $[Cl^-]$,

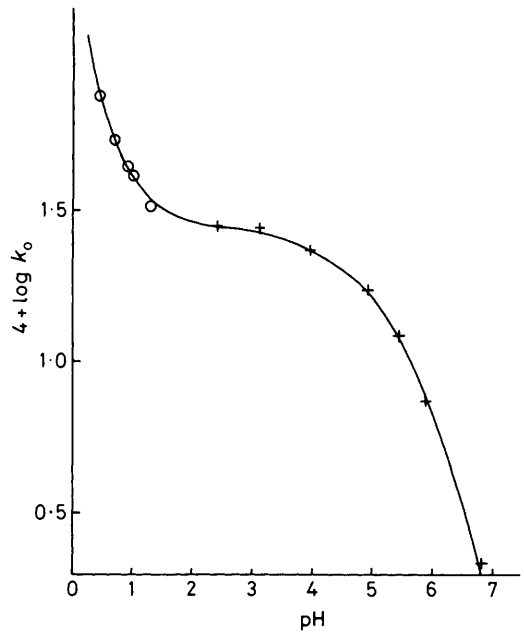


Figure 2. The log k_0–pH profile for the denitrosation of *N*-acetyl-N^1-nitrosotryptophan (II) at 31°: ○, H_2SO_4; +, buffer

but for the more powerful nucleophiles, the first-order dependence is soon lost with increasing concentration, and k_0 tends to a limiting value for each nucleophile at *ca.* 19×10^{-4} s^{-1}. This contrasts quite markedly with the situation at pH *ca.* 1, where there is no evidence of any nucleophilic catalysis. The order of reactivity of nucleophiles found here (Figure 1) is as expected. This is the first time that nucleophilic attack by the azide ion has been detected in denitrosation reactions. Previous work in this area has been at much higher acidities so that the azide ion has been virtually totally protonated. This is not the case at pH 6, and the azide ion appear to have reactivity comparable with that of the iodide ion.

It is difficult to see why the limiting form of equation (3) should apply at higher acidities whereas the other form, equation (2), appears to apply at pH 6 at least for chloride ion and low concentration of the other nucleophiles. The difference between these two forms depends on the relative magnitudes of the terms k_{-1} and $k_2[Y^-]$ (see Scheme 1), and it is not evident that this should be pH dependent. A more likely explanation of the different behaviour is that different protonation sites are involved at the two different acidities. This explanation is borne out by examination of the log k_0–pH profile which is given in Figure 2. Here we have included all the results for the experiments in the buffer solution and some of the results for the sulphuric acid work. Even though both sets of results do not correspond to exactly the same experimental conditions, the trend is quite clear, that there are indeed two different acid-catalysed pathways, one operative in the pH range 5—7 and the other below pH 2. Between pH 2 and 4 there is little change in rate constant corresponding to the formation of one fully protonated form. Taking the extrapolated limiting value for that mechanism as $4 + \log k_0 = 1.45$, it is possible to calculate the pK_a value of *ca.* 5.5 for this protonation. This value is much larger than that expected for any protonation site in (II) which would lead to denitrosation. Hinman and Lang [16] have measured the pK_a value of 3-methylindole [a good model for (I)] as −4.55, probably for C-3 protonation. It seems unlikely that the protonation equilibrium on the ring nitrogen

$$>N-NO + H^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} >N-N=\overset{+}{O}H$$

$$>N-N=\overset{+}{O}H + Y^- \xrightarrow{k_2} >NH + NOY$$

Scheme 3

atom (with a nitroso-group attached) has a pK_a value as high as 5.5, so alternative sites must be examined. The methyl ester of (II) gave a pH–rate profile (Figure 2) virtually identical to that of the free acid, so any intramolecular proton transfer from the carboxylic acid group can be ruled out. However, the experimental evidence points clearly to the existence of two separate pathways, with different dependencies upon added nucleophiles. We propose that at the higher acidities, protonation occurs at C-3 with the limiting condition $k_2[Y^-] \gg k_{-1}$ (from Scheme 2) always applicable. At the lower acidities, in the pH range 3—7, it is suggested that protonation occurs at the nitroso-group oxygen atom (as in Scheme 3), which becomes virtually complete at pH 2. In this range $k_2[Y^-]$ and k_{-1} are of comparable magnitude, so that nucleophilic catalysis is observed at low $[Y^-]$. Step k_2 here in Scheme 3 may well involve more than one stage, *e.g.* possible O ⟶ N hydrogen rearrangement.

N-Acetyl-*N*^{1}-nitrosotryptophan methyl ester has been used to nitrosate other amines such as diphenylamine,[17] under somewhat different experimental conditions. From our present findings it seems likely that, at least at the higher acidities, this occurs by prior hydrolysis to nitrous acid which (probably in its protonated form) is the likely reagent for amine nitrosation rather than the *N*-nitroso-derivative itself. We have tested this futher with (II) using 4-nitroaniline as the receptor amine. This was chosen (a) because of its high reactivity towards diazotisation,[18] and (b) for spectral reasons so that the product diazonium ion (maximum absorbance at 310 nm) and the amine itself (maximum absorbance at 360 nm) could be observed spectrophotometrically without much interference from the indole chromophores. When the reaction was carried out in the absence of sulphamic acid (a good nitrite trap) an increasing absorbance at 310 nm was noted together with a decreasing absorbance at 360 nm, indicating that diazotisation was indeed taking place. However, when (II) was added, in acid solution (0.4M-H_2SO_4), to 4-nitroaniline with a slight excess of sulphamic acid present, there was no evidence of any diazonium ion formation. This confirmed that in this acidity region (II) does not act *directly* as a trans-nitrosating species, but initially yields free nitrous acid.

(c) *Photochemical Reaction.*—Nitrosamines in general undergo a photochemical decomposition particularly in acid solution, when it is thought that nitric oxide and the corresponding aminium radical ions are first formed.[19] A small rate enhancement was observed when (II) was subjected to denitrosation at pH 6 irradiated with a 500 W lamp at 50 cm, compared with the control experiment in subdued light. This indicated that there is a minor photochemical pathway to the reaction. Oxygen is known to be involved in the photochemical decomposition of tryptophan itself,[20] so we have examined briefly the decomposition of (II) at pH 6 in the presence of oxygen and a sensitiser (Rose Bengal). A 50% increase in the rate constant was observed compared to the reaction carried out under nitrogen. The products were not investigated for the oxygen reaction, but it is clear from the change in rate constant that there is, under these conditions, a new, more favourable route to the decomposition. Although the photochemical reaction is a minor pathway, it is clearly advantageous in general to conduct rate measurements on the ground state reactivity of *N*-nitrosamines in subdued light.

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References

1 I. D. Biggs and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1975, 107.
2 J. T. Thompson and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1977, 1932.
3 C. N. Berry and B. C. Challis, *J. Chem. Soc., Perkin Trans. 2*, 1974, 1638.
4 B. C. Challis and S. P. Jones, *J. Chem. Soc., Perkin Trans. 2*, 1975, 153.
5 J. K. Snyder and L. M. Stock, *J. Org. Chem.*, 1980, **45**, 1990.
6 G. Hallett and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1372.
7 D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1976, 1838.
8 G. Hallett, S. S. Johal, T. A. Meyer, and D. L. H. Williams, '*N*-Nitroso-compounds: Analysis, Formation and Occurrence,' I.A.R.C. Scientific Publication No. 31, International Agency for Research on Cancer, Lyon, 1980, p. 31.
9 S. S. Johal, D. L. H. Williams, and E. Buncel, *J. Chem. Soc., Perkin Trans. 2*, 1980, 165.
10 R. Bonnett and P. Nicolaidou, *Heterocycles*, 1977, **7**, 637.
11 R. Bonnett and R. Holleyhead, *J. Chem. Soc., Perkin Trans. 1*, 1974, 962.
12 S. Venitt, C. Crofton-Sleigh, S. L. Ooi, and R. Bonnett, *Carcinogenesis*, 1980, **1**, 523.
13 D. F. Heath, *Biochem. J.*, 1962, **85**, 72.
14 C.R.C. Handbook of Biochemistry, 3rd edn., p. 362.
15 D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1975, 655.
16 R. L. Hinman and E. B. Whipple, *J. Am. Chem. Soc.*, 1962, **84**, 2534; G. Berti, A. de Settimo, and D. Segnini, *Gazz. Chim. Ital.*, 1961, **91**, 571; R. L. Hinman and J. Lang, *J. Am. Chem. Soc.*, 1964, **86**, 3796.
17 R. Bonnett and P. Nicolaidou, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1969.
18 M. R. Crampton, J. T. Thompson, and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1979, 18.
19 Y. L. Chow, *Acc. Chem. Res.*, 1973, **6**, 354.
20 Z. I. Yoshida and M. Kato, *J. Am. Chem. Soc.*, 1954, **76**, 311; W. E. Savige, *Aust. J. Chem.*, 1971, **24**, 1285; C. A. Benassi, E. Scoffone, G. Galiazzo, and G. Iori, *Photochem. Photobiol.*, 1967, **6**, 857; M. Nakagawa, S. Kato, S. Kataoka, and T. Hino, *J. Am. Chem. Soc.*, 1979, **101**, 3136.

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