Kinetic Study of the Nitrosation of 3-Substituted Indoles

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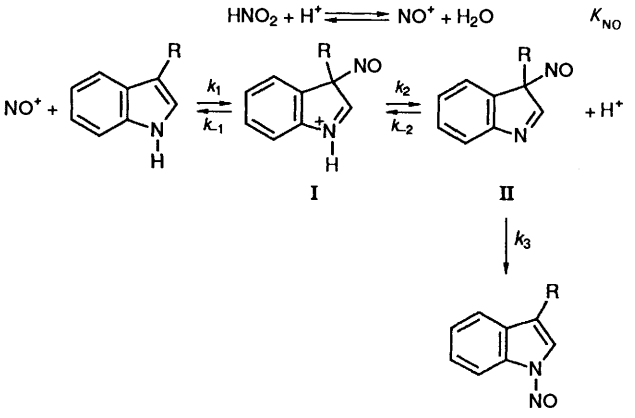
Kinetic studies of the nitrosation reaction of three 3-substituted indoles (3-methylindole, indol-3-yl acetate and indole-3-acetic acid) show that the final state is an equilibrium between the reactants (nitrous acid and indole derivative) and the 1-nitroso derivative. Values of the rate constants for the nitrosation of the three indoles and denitrosation of the corresponding nitrosoindoles as well as values of the equilibrium constants have been obtained. The almost complete insensitivity of the reaction rates to medium acidity, the absence of catalysis by the usual catalysts of nitrosation (halides) and the high reactivity at low acidities, are in contrast to the kinetic characteristics of other *N*-nitrosation reactions. This atypical behaviour is discussed in terms of possible reaction mechanisms.

The nitrosation of indoles with nitrous acid is a familiar organic reaction of some analytical use, the characteristics of the reaction products depending on the structure of the indole in a way that was established in 1945.[1] It is known, for example, that in indoles with the 3-position unsubstituted, nitrosation takes place at C-3 and the *C*-nitroso compound so formed tautomerizes quickly (where possible) to the oxime form. The kinetic characteristics of this reaction are well known.[2] If the 3-position is substituted and the 1-position free the product is the corresponding *N*-nitroso compound, whereas if the 1- and 3-positions are both substituted the indole is practically inert to attack by nitrous acid.

The first kinetic study on the nitrosation of a 3-substituted indole was carried out by Kurosky and Hofmann[3] who investigated the nitrosation of indole-3-acetic acid and α-*N*-acetyltryptophan: both substrates reacted at a similar rate and the rate equation was first-order with respect to both HNO_2 and the substrate. Although this direct dependency on nitrous acid concentration seems to suggest a mechanism whose slow step is NO^+ attack on the substrate, the lack of dependence of the second-order rate constant on the acidity is entirely out of keeping with this. These authors could not therefore propose any reaction mechanism. A reaction rate independent of acidity over the pH range 2–4 was later found in the denitrosation of *N*-acetyl-*N'*-nitrosotryptophan.[4] Since this is a denitrosation reaction the results can be generalized to the nitrosation reaction by applying the equilibrium condition. Subsequently, a kinetic study of the formation and decomposition reactions of this nitroso compound[5] has shown their rate constants also to be independent of acidity over the pH range 1–4. Consideration of the molecular structure of 3-substituted indoles leads to the conclusion that the most nucleophilic centre of the molecule should be C-3, which has been supported, for example, by the observation of protonation on this atom,[6] even if it is substituted. These considerations led us in earlier work[5] to propose the reaction mechanism shown in Scheme 1. When $k_{-2}[H^+] \gg k_3$ the slow step will be the internal transfer of the NO group from C to N leading eventually to the final *N*-nitroso product. This mechanism predicts a rate equation in full agreement with the experimental observations.

Internal rearrangement of the NO group and, in particular, 1–3 rearrangements occur in the nitrosation of many substrates. Amides seem to undergo nitrosation initially on the oxygen atom, and the NO group is then transferred to the nitrogen atom;[7] similarly, in thioproline electrophilic attack starts on the sulfur atom followed by rearrangement to *N*-nitroso-thioproline.[8] A more recent example is that of the *C*-nitrosation



Scheme 1

of nitroalkanes,[9] where the NO group attaches to the oxygen atom and then during rearrangement moves to the carbon atom. It would seem to be the general case that in molecules with two nucleophilic centres nitrosation initially takes place at the most nucleophilic site, the nitroso group subsequently being transferred to yield the most thermodynamically stable product.

In the work reported here the reactivity of 3-substituted indoles in the presence of nitrous acid has been studied, and results are given specifically for the nitrosation reactions of 3-methylindole, indol-3-yl acetate and indole-3-acetic acid.

Experimental

Chemicals were from Merck or Aldrich and are of the greatest purity available commercially, employed without further purification. The low solubility of the indoles in water made it necessary to use water–dioxane mixtures (17.5% dioxane by volume). All kinetic work was carried out at 25 °C.

Kinetic measurements and UV–VIS spectroscopy were carried out in a Kontron-Uvikon 820 spectrophotometer with thermostatted cell holder. NMR and IR spectra were obtained using a Bruker MW250 and a Perkin Elmer spectrometer, respectively. ^1H NMR chemical shifts are relative to external Me_4Si. pH Measurements were performed on a Radiometer 82 pH meter equipped with a GK 2401C glass electrode.

Identification of the Reaction Products.—Nitrosation of 3-methylindole gives 3-methyl-1-nitrosoindole. This product was independently prepared following the procedure of Hodson and

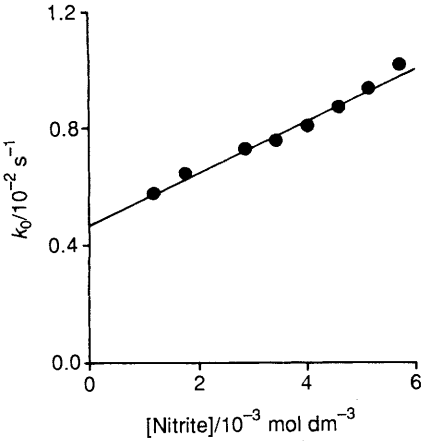


Fig. 1 Influence of $[Nitrite]_{tot}$ upon the pseudo-first-order rate constant, k_0, for nitrosation of indol-3-yl acetate. $[H^+] = 0.03$ mol dm^{-3}

Table 1 Values of the parameters a, b [eqn. (3)] and b' resulting from the kinetic study of nitrosation of indol-3-yl acetate at different acidities

$[H^+]$/mol dm^{-3}	$a/10^{-3}$ s^{-1}	b/dm^3 mol^{-1} s^{-1}	b'/dm^3 mol^{-1} s^{-1}
1.74×10^{-3}	3.90	0.78	0.92
0.01	5.26	0.78	0.80
0.0167	4.92	0.78	0.79
0.03	4.76	0.88	0.88
0.05	4.41	1.09	1.09
0.07	3.96	1.52	1.52
0.1	3.85	1.51	1.51

Smith,[10] giving a yellow liquid, m.p. 10–11 °C (lit.,[10] 10–12 °C). The UV–VIS spectrum in cyclohexane showed a peak at 265 nm (log $\varepsilon = 4.1$) and a broad band centred on 334 nm (log $\varepsilon = 3.8$), coinciding with those reported by Hodson and Smith in hexane at 264 nm (log $\varepsilon = 4.1$) and 329–334 nm (log $\varepsilon = 3.8$), respectively. The nature of this product was confirmed in the IR spectrum showing the absence of significant bands above 1500 cm^{-1}; a sign of the disappearance of the indolic N–H bond. The ^{1}H NMR spectrum in $CDCl_3$ showed a signal at 2.2 ppm (CH_3) and a complex absorbance between 7.2 and 8.4 ppm due to the aromatic protons.

The derivative of indole-3-acetic acid was prepared as follows. Indole-3-acetic acid (1.3 g) was dissolved in a mixture of acetic acid (13 cm^3) and acetone or methanol (3 cm^3) at 0 °C. Sodium nitrite (2 g) in water was then added dropwise with stirring. The sample was left at room temperature for 10 min. and poured onto cold sodium hydrogencarbonate. The resulting solution had a strong yellow colour and its UV–VIS spectrum showed bands at 265 and 332 nm (broad band). These spectral characteristics coincide with those reported by Kurosky and Hofmann[3] for 1-nitrosoindole-3-acetic acid on the basis of a kinetic study. After slight acidification, the product was extracted with dichloromethane, washed repeatedly, dried ($MgSO_4$), and the solvent was removed under reduced pressure. The IR spectrum of the resulting yellow-brown solid confirmed that the reaction had occurred on the indolic nitrogen atom, the characteristic N–H band having disappeared. The NMR spectrum in $CDCl_3$ showed signals at 3.8 ppm (CH_2) and a very complex absorbance between 7.1 and 8.4 ppm. When dissolved in neutral or basic dioxane–water mixtures, the solid gave a yellow solution with the spectral characteristics mentioned above. At high acidity, however, the solution was red, and the change was detected spectroscopically by monitoring the appearance of bands at 534 and 290 nm. The addition of alkali regenerated the yellow colour and the original spectrum was observed. These acidity-induced colour changes are not instantaneous.

The *N*-nitroso compounds obtained are, in general, rather unstable and often undergo transformations, especially at high acidities, which were not studied and do not compete appreciably with the nitrosation reaction.

Results

Nitrosation of Indol-3-yl Acetate.—The kinetic study was carried out with total nitrite, $[Nitrite]_{tot}$, ($[HNO_2] + [NO_2^-]$) in large excess (>ten times) compared to the initial concentration of indol-3-yl acetate. The reaction was followed spectrophotometrically at 330 nm noting the increase in absorbance accompanying the formation of the *N*-nitroso derivative. The data showed a good fit in all cases to the integrated first-order eqn. (1).

$$\ln(A_\infty - A_t) = \ln(A_\infty - A_0) - k_0 t \qquad (1)$$

The influence of nitrite concentration on reaction rate was studied for concentrations between 1×10^{-3} and 6×10^{-3} mol dm^{-3} at constant pH. Plotting k_0 *vs.* $[Nitrite]_{tot}$ gave a straight line with an intercept at the origin (Fig. 1), thus obeying eqn. (2).

$$k_0 = a + b[Nitrite]_{tot} \qquad (2)$$

This rate equation is typical of reversible processes that after an infinite time reach equilibrium between reactants and products with significant amounts of each being present [eqn. (3)]. The

$$HNO_2 + \text{Indol-3-yl acetate} \rightleftharpoons \text{1-Nitrosoindol-3-yl acetate} \qquad (3)$$

term a represents denitrosation of the nitroso compound and b gives the nitrosation rate of the indole derivative. These two values were determined in a series of similar experiments performed over the acidity range 1.74×10^{-3} to 0.1 mol dm^{-3} $[H^+]$ (Table 1). The acidity was kept at one of these values by adding the necessary quantities of $HClO_4$ in each kinetic run, and controlled by measuring the pH. The existence of an equilibrium in the dissociation of nitrous acid [eqn. (4)] with a

$$HNO_2 \rightleftharpoons NO_2^- + H^+ \quad K_a \qquad (4)$$

pK_a of *ca.* 3.5 for our experimental conditions (17% dioxane), implies that at the lowest acidities not all the sodium nitrite added is found as nitrous acid. The relation between $[Nitrite]_{tot}$ and $[HNO_2]$ therefore becomes eqn. (5). To correct for this

$$[HNO_2] = [Nitrite]_{tot}[H^+]/(K_a + [H^+]) \qquad (5)$$

effect and to express the nitrosation rate in terms of the nitrous acid concentration a parameter $b' = b(K_a + [H^+])/[H^+]$ was introduced. Values for b' are also found in Table 1. Accepting that the process is reversible, the equilibrium constant K for eqn. (3) will be given by the quotient b'/a, which when calculated as the mean of the values obtained in the different experiments gave 250 dm^3 mol^{-1}. The most interesting aspect of these results is that they confirm the almost total independence of reaction rate (for nitrosation and denitrosation) on the acidity of the medium. In fact, the denitrosation rate constant a was constant for a 60-fold increase in proton concentration; the same change in acidity caused b' to change by a factor of only 1.6.

Nitrosation of 3-Methylindole.—The reaction was followed kinetically by noting the increase in absorbance at 350 nm

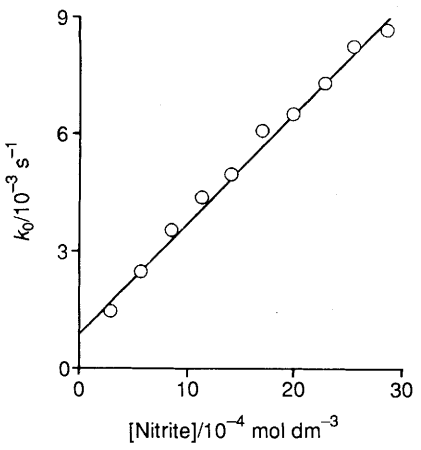


Fig. 2 Influence of $[Nitrite]_{tot}$ upon the pseudo-first-order rate constant, k_0, for nitrosation of 3-methylindole at pH 3

Table 2 Values of kinetic parameters after fitting data of the nitrosation of 3-methylindole at different acidities

$[H^+]$/mol dm^{-3}	$a/10^{-3}$ s^{-1}	b'/dm^3 mol^{-1} s^{-1}	C/dm^6 mol^{-2} s^{-1}
Low acidities			
3.5×10^{-4}	0.60	3.77	—
1×10^{-3}	0.98	3.72	—
2×10^{-3}	0.97	3.89	—
High acidities[a]			
5×10^{-3}	0.80	3.73	356
1×10^{-2}	0.81	4.17	340
1.67×10^{-2}	1.10	3.95	569
3.34×10^{-2}	1.32	4.23	686
6.67×10^{-2}	2.40	3.36	1383
0.1	2.86	4.39	1480

[a] The data for high acidities have not been corrected for partial dissociation of HNO_2, since this is negligible.

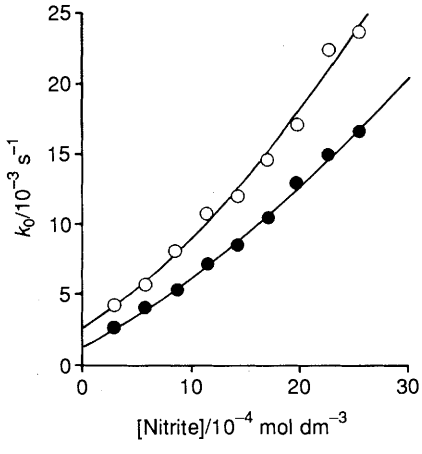


Fig. 3 Influence of $[Nitrite]_{tot}$ upon the pseudo-first-order rate constant, k_0, for nitrosation of 3-methylindole. ○, $[H^+] = 0.1$ mol dm^{-3}; ●, $[H^+] = 0.033$ mol dm^{-3}

accompanying formation of the *N*-nitroso compound. As in the earlier case [3-methylindole] ≪ $[Nitrite]_{tot}$ and the experimental data always showed a satisfactory fit to the first-order integrated equation. The influence of nitrite concentration on reaction rate was initially studied at low acidities. Series of experiments with $[H^+]$ varying from 3.5×10^{-4} to 2×10^{-3} mol dm^{-3} were carried out and resulted in a linear relation between the pseudo-first-order rate constant and $[Nitrite]_{tot}$ (Fig. 2). The intercept at the origin is again significant. This

Table 3 Influence of Br^- concentration on the pseudo-first-order rate constant k_0 for nitrosation of 3-methylindole[a]

$[Br^-]/10^{-2}$ mol dm^{-3}	$k_0/10^{-3}$ s^{-1}
—	4.04
2.86	4.23
5.71	4.36
8.57	4.15
11.4	4.13
13.6	4.45

[a] [3-Methylindole] = 5.57×10^{-5} mol dm^{-3}; $[H^+] = 0.01$ mol dm^{-3}, $[Nitrite]_{tot} = 8.57 \times 10^{-4}$ mol dm^{-3}

behaviour is, correspondingly, like that reported for the first compound, hence it can be described in terms of eqn. (2) and (3). The mean value of K calculated from the values of a and b' obtained (Table 2) was 4700 dm^3 mol^{-1}, greatly exceeding that obtained in the case of indol-3-yl acetate. When the reaction was studied at higher acidities ($[H^+]$ 5×10^{-3}–0.1 mol dm^{-3}) the behaviour was different: the graph of k_o *vs.* $[Nitrite]_{tot}$ (Fig. 3) has an upward curvature that becomes more pronounced with increasing acidity. This points to a second-order term in $[Nitrite]_{tot}$, with all the data fitting a second-order polynomial [eqn. (6)]. The intercept at the origin, A, corresponds to the rate

$$k_0 = A + B[Nitrite]_{tot} + C[Nitrite]^2_{tot} \quad (6)$$

of the denitrosation process and means the same as a (described previously in this paper). The appearance of a second-order term in $[Nitrite]_{tot}$ suggests that, in some way, two molecules of nitrous acid are involved in the nitrosation of 3-methylindole, and the results show that this effect is linked to the acidity of the medium. Invoking the principle of microscopic reversibility, this calls [see eqn. (7)] for a denitrosation term of rate constant

$$\text{3-Methylindole} + 2HNO_2 \rightleftharpoons \text{3-methyl-1-nitrosoindole} + HNO_2 \quad (7)$$

$\beta[Nitrite]_{tot}$ showing order one in nitrous acid and which will be contained in the factor B added to the term b that represents nitrosation involving a single molecule of nitrous acid.

To separate the terms b and β from the parameter B we remind ourselves that the condition for chemical equilibrium requires K (whose value has been determined at low acidity) = C/β. The values of b corresponding to each acidity level, which represent rate constants for the nitrosation that is first-order with respect to $[Nitrite]_{tot}$ were calculated using this procedure. The data for a, b' and C appear in Table 2. Studying the data for the entire acidity range reveals that while the proton concentration has changed by a factor of 290, a has varied by a factor of 3 (excluding the first point) or 5 (first point included) and b' by a factor of 1.16. These results are indicative of a very weak effect of acidity changes in the medium.

The appearance of a term that is second-order in $[Nitrite]_{tot}$ usually implies the intervention of a nitrosating agent that is not NO^+ but N_2O_3 reacting with the substrate in the limiting step of the process.[11] This potential explanation contradicts the experimental fact that the new term increases with proton concentration, since $[N_2O_3]$ is simply proportional to $[HNO_2]^2$, without any other influence of acidity. To find out more about the processes involved, experiments incorporating the addition to the medium of another effective nitrosating agent, NOBr, were performed. The results obtained (Table 3) show no kinetic effect for increasing the concentration of Br^- from 0–0.136 mol dm^{-3}. Furthermore, changing the ionic strength over a small margin (by adding 0–0.2 mol dm^{-3} $NaClO_4$) had no appreciable effect on reaction rate.

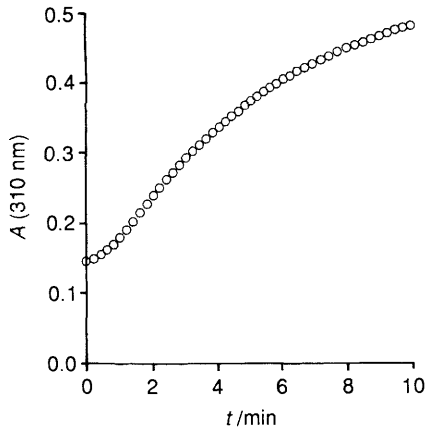


Fig. 4 Typical absorbance–time profile found in the nitrosation of indole-3-acetic acid at pH > 2. $[Nitrite]_{tot} = 1.16 \times 10^{-3}$ mol dm^{-3}, [Indole-3-acetic acid] = 8.66×10^{-5} mol dm^{-3}; $[H^+] = 0.092$ mol dm^{-3}

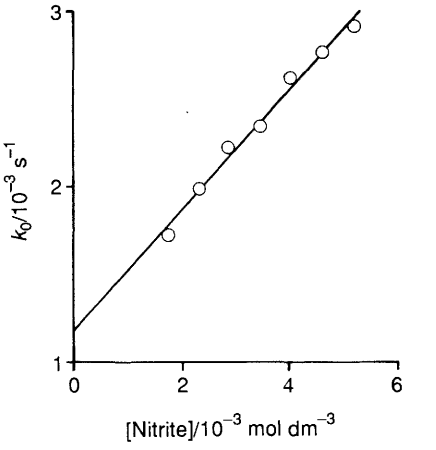


Fig. 5 Influence of $[Nitrite]_{tot}$ upon the pseudo-first-order rate constant, k_0, for nitrosation of indole-3-acetic acid; pH = 3.14

Table 4 Values of the rate constant b' and the equilibrium constants K corresponding to nitrosation of various 3-substituted indoles

Indole	b'/dm^3 mol^{-1} s^{-1}	K/dm^3 mol^{-1}
3-Methylindole	4	4700
Indol-3-yl acetate	1	250
Indole-3-acetic acid[a]	*ca.* 0.5	*ca.* 430
N-Acetyltryptophan[b]	1	850

[a] Result obtained at a single value of pH. [b] Ref. 5.

Study of Indole-3-acetic Acid.—The nitrosation reaction of this substrate was followed at 310 nm. In this case, the kinetic characteristics depended to a large extent on the experimental conditions. The indole was always in great deficiency with respect to $[Nitrite]_{tot}$. At moderate acidities (pH 2–4) the data did fit the integrated first-order equation and the final solution had the pale yellow colour (in fact, almost colourless at our working concentrations) typical of *N*-nitroso compounds. On increasing the acidity this was no longer the case and the absorbance–time profiles assumed in their early stages a strange, sometimes slightly sigmoidal form (Fig. 4). Under these conditions the final solutions showed a reddish colour. This behaviour is in keeping with the properties of 1-nitrosoindole-3-acetic acid discussed in the Experimental section. It would seem that this behaviour is the result of two consecutive reactions, the first process being the formation of 1-nitrosoindole-3-acetic acid which evolves to the unidentified red product of greater molar absorptivity causing the sigmoidal form of the absorbance–time curve. This problem probably affects some of the kinetic data we have previously published on this substrate,[12] although the fact that ethanol was added in that work to overcome the low solubility of the indole-3-acetic acid might change the situation because of the presence of ethyl nitrite in the medium. This difficulty means that our kinetic studies have been centered on the region of moderate acidity in which the *N*-nitroso compound seems stable; at pH 3.14, a series of experiments were carried out varying $[Nitrite]_{tot}$ between 1.71×10^{-3} and 5.14×10^{-3} mol dm^{-3}. The intercept at the origin in the graph of the results (Fig. 5), as before implies the reaction is reversible. From the data represented in Fig. 5 the values of b' and a were calculated as 0.5 dm^3 mol^{-1} s^{-1} and 1.18×10^{-3} s^{-1}, respectively, and this led to a predicted value for the equilibrium constant K of *ca.* 420 dm^3 mol^{-1}.

Discussion

For comparative purposes some of the results, including values obtained for *N*-acetyltryptophan in a previous study,[5] are collected in Table 4. Similar values can be seen for the rate of nitrosation of all substrates. Although the greater reactivity of the methylated derivative might be due to its greater basicity ($pK_a = -4.55$)[13] compared to that of indole-3-acetic acid ($pK_a = -6.13$),[13] a further likely explanation could be related to the small size of the methyl group in comparison with the other substituents. This would sterically favour the reaction, particularly if (as seems likely) the reaction begins on C-3. Without exception, the reactions were reversible, with equilibrium constants (Table 4) of similar magnitude in every case apart from the somewhat higher value obtained for 3-methylindole.

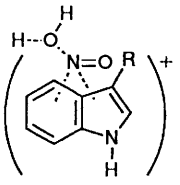
With respect to the characteristics of the kinetics, the near lack of influence of acidity on the constants b' (nitrosation) and a (denitrosation) supports the mechanism of Scheme 1, with the migration of the nitroso group from C to N being the slow step. This mechanism is further supported by the absence of catalysis by Br^-, since it predicts that the rate will only be proportional to the equilibrium concentration of intermediate **II**, which will be independent of the way in which it was formed (NO^+, NOBr, N_2O_3, *etc.*). According to this mechanism, if the slow step is that of rate constant k_3, the rate equation will be that shown in eqn. (8), where $K_1 = k_1/k_{-1}$ and $K_2 = k_2/k_{-2}$. Values of $K_{NO}k_1$[14]

$$r = K_{NO}K_1K_2k_3[HNO_2][Indole] \quad (8)$$

between 2000 and 6000 dm^6 mol^{-2} s^{-1} have been reported for a large number of substrates (mainly amines) independently of their basicity.[14] This value is thought to correspond to diffusion-controlled attack by NO^+ because of this lack of influence of the basicity or other structural considerations on the range of values.[14] It is not possible to obtain the values of the bimolecular constant of reaction k_1 which would enable this hypothesis to be confirmed because there are no reliable values available for K_{NO}. According to our mechanism, for the internal migration to be the rate limiting step at the working acidities the following conditions must be satisfied: $k_3 \ll k_{-2}[H^+]$ and $k_2 \ll k_{-1}$ (or of similar magnitude if intermediate I were in steady-state conditions). For the lowest acidity values studied (say pH 4, the pH value at which the rate constants are still independent of acidity) and for a typical value of b' (1 dm^3 mol^{-1} s^{-1}), one can easily confirm that these conditions imply that $K_{NO}k_1 > 1 \times 10^5$ dm^6 mol^{-2} s^{-1}, which exceeds, by more than 20 times, the limit usually associated with the maximum reactivity of NO^+, the diffusion-controlled limit. For this

observation various explanations can be invoked. Substrates reacting at atypically high rates with NO^+ are known. For example, for the attack of NO^+ on 2,3-diaminonaphthalene a value of 7.5×10^4 has recently been proposed.[15] Values of $K_{NO}k_1$ up to 6×10^8 dm^6 mol^{-2} s^{-1} (at 2 °C) have been associated with the diazotization of certain pyridines.[16] The constancy of values found for anilines and aliphatic amines (over a large range of pK_a) and for several α-naphthylamines,[17] 3-aminoquinoline[18] as well as some processes of aromatic *C*-nitrosation,[19] might represent a diffusion limit. The limit can be greater in certain substrates, perhaps because of larger collision cross sections in these cases. There is nothing novel in suggesting different diffusion limits depending on the structure of the substrate which undergoes nitrosation. Such is the case of the reactivity of N_2O_3[20] and of NOBr[21] which show different reaction rates for aromatic and aliphatic amines, although diffusion controls the reactions in either case.

Another possible explanation could be a type of initial pre-association[22] between the indole, the nitrous acid and the proton giving rise to a transition state such as that shown in Scheme 2 in which the aromatic ring by donating charge would stabilize formation of the nitrosating agent through protonation of nitrous acid. Loss of water would give an NO^+–indole complex; this then reacts quickly—within the solvent cage—following the steps shown in Scheme 1. The possibility of evolu-



Scheme 2

tion mediated by the initial transfer of a single electron, *i.e.*, by a radical pair, instead of an electrophile–nucleophile reaction, cannot be ruled out, since it seems to take place in a considerable number of reactions.[23,*]

In the case of 3-methylindole, the appearance at high acidities of a second-order term in nitrous acid that increases with acidity suggests the intervention of a second NO^+ ion in the nitrosation. Unless this ion somehow assists the intramolecular migration of the nitroso group from C to N, it is not easy to understand its role in a conventional mechanism.

Finally, it is worth pointing out that the indoles are a group of compounds with a particularly complex chemistry and the anomalies occurring extensively throughout their chemical behaviour also become apparent when studying the kinetics of the nitrosation reaction of their 3-substituted derivatives. The acidity independence of the reaction rate, the absence of catalysis by halides and the atypically high reactivity shown at low acidities, *etc.* set them apart from the rest of the substrates that also undergo *N*-nitrosation reactions.

* Although one might think of the involvement of N_2O_3 as being responsible for this abnormally high reactivity, a simple calculation shows that for this to be the case N_2O_3 should react with the indoles at a rate which is not compatible with its being in equilibrium. In addition, under the experimental conditions used for the nitrosation of *N*-acetyltryptophan,[5] the observed reaction rates are higher than the rates of formation of N_2O_3,[11] therefore allowing us to discard this possibility.

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References

1 P. E. Verkade, J. Lieste and E. G. G. Werner, *Recl. Trav. Chim. Pays-Bas.*, 1945, **64**, 289.
2 B. C. Challis and A. J. Lawson, *J. Chem. Soc., Perkin Trans. 2*, 1973, 918.
3 A. Kurosky and T. Hofmann, *Can. J. Biochem.*, 1972, **50**, 1282.
4 T. A. Meyer, D. L. H. Williams, R. Bonnett and S. L. Ooi, *J. Chem. Soc., Perkin Trans. 2*, 1982, 1383.
5 A. Castro, E. Iglesias, J. R. Leis, M. E. Peña, J. Vázquez Tato and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1165.
6 R. L. Hinman and E. B. Whipple, *J. Am. Chem. Soc.*, 1962, **84**, 2534; R. L. Hinman and J. Land, *J. Am. Chem. Soc.*, 1964, **86**, 3796.
7 A. Castro, E. Iglesias, J. R. Leis, M. E. Peña and J. Vázquez Tato, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1725.
8 A. Castro, E. Iglesias, J. R. Leis, J. Vázquez Tato, F. Meijide and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2*, 1987, 651; T. Tahira, M. Tsuda, K. Wakabayashi, M. Nagao and T. Sugimura, *Gann.*, 1984, **75**, 889.
9 E. Iglesias and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1988, 1035.
10 H. F. Hodson and G. F. Smith, *J. Chem. Soc.*, 1957, 3546.
11 D. L. H. Williams, *Nitrosation*, Cambridge University Press, 1988.
12 A. Castro, P. Hervés, E. Iglesias. J. R. Leis and M. E. Peña, *J. Chem. Res. (S)*, 1988, 76.
13 N. C. Marziano, G. M. Cimino and R. C. Passerini, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1915.
14 J. H. Ridd, *Adv. Phys. Org. Chem.*, 1978, **16**, 1.
15 S. M. N. Y. F. Oh and D. L. H. Williams, *J. Chem. Res. (S)*, 1989, 264.
16 E. Kalatzis and P. Papadopoulos, *J. Chem. Soc., Perkin Trans. 2*, 1981, 239.
17 J. Casado, A. Castro, E. Iglesias, M. E. Peña and J. Vázquez Tato, *Can. J. Chem.*, 1986, **64**, 133; A. Castro, E. Iglesias, J. R. Leis and M. E. Peña, *Ber. Bunsenges. Phys. Chem.*, 1986, **90**, 891.
18 C. Bravo, A. Castro, E. Iglesias, J. R. Leis and M. E. Peña, *Bull. Soc. Chim. Fr.*, 1987, 573.
19 A. Castro, E. Iglesias, J. R. Leis, M. Mosquera and M. E. Peña, *Bull. Soc. Chim. Fr.*, 1987, 83.
20 J. Casado, A. Castro, J. R. Leis, M. A. López Quintela and M. Mosquera, *Monatsh. Chem.*, 1983, **114**, 639.
21 A. Castro, J. R. Leis and M. E. Peña, *J. Chem. Res. (S)*, 1986, 216.
22 W. P. Jencks and J. M. Sayer, *Faraday Symp. Chem. Soc.*, 1975, **10**, 41.
23 See, *e.g.*, R. Taylor, *Electrophilic Aromatic Substitution*, Wiley, Chichester, 1990.

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