Kinetics and Mechanism of Nitrosation of Clonidine: a Bridge between Nitrosation of Amines and Ureas

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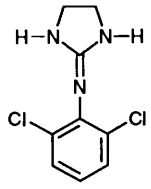
Nitrosation of clonidine has been studied kinetically both in acid medium (with nitrous acid) and in basic medium (with 2,2-dichloroethyl nitrite). The reactive form in acid medium was found to be the protonated clonidine (pK_a 8.18). The absence of catalysis by halides or thiocyanate, the existence of general base catalysis, and the measured solvent isotope effect all indicate that the reaction mechanism is different from that for the *N*-nitrosation of amines. Specifically, kinetic results indicate that the attack of the nitrosating agent on the substrate is not the rate determining step of the process, and suggest a mechanism that shows parallels with that found for ureas. However, in slightly basic medium, the reaction of clonidine with the alkyl nitrite occurs through the free base form of clonidine, as shown by the influence of acidity upon the reaction rate. In this case, the kinetic behaviour is similar to that exhibited by amines.

Guanidines can be considered nitrogenated analogues of ureas. However, their peculiar structure makes them compounds of great basicity, and in this sense, more similar to amines. The mechanisms of nitrosation of amides and ureas in acid medium have been exhaustively investigated in recent years;[1-4] despite the first impression of a mechanistic analogy between these compounds and amines when receiving the nitroso group, more recent studies have revealed substantial differences in their behaviour.[1-5] While for amines the attack of the nitrosating agent on the free (unprotonated) amine is the rate determining step of the process, in the case of the amides this first step is fast, the slow step being the transfer of a proton from an intermediate to the reaction medium. Moreover, in the case of the amides the reaction seems to occur initially on the oxygen atom,[1] probably the most nucleophilic centre of the molecule, where protonation[6] and alkylation[7] also take place, although a subsequent rearrangement leads to the thermodynamically more stable product, the *N*-nitrosamide. The reasons for this different pattern of behaviour may be related to the much lower basicity of amides in comparison to amines, and to the structural differences between both molecules.[1]

This situation makes the kinetic study of the nitrosation of guanidines, molecules that combine characteristics of both functional groups, very interesting. The reaction, that generally leads to the formation of the *N*-nitrosoguanidines, is known synthetically.[8] The decomposition of nitrosoguanidines was the subject of a number of kinetic studies, especially in the case of the powerful 'direct-acting' carcinogen 1-methyl-3-nitro-1-nitrosoguanidine.[9] Mirvish[10] has studied the possibility of the nitrosation of some alkylguanidines (arginine, methylguanidine and 1-methyl-3-nitroguanidine) with the aim of estimating the potential health risk to humans derived from their endogenous nitrosation. He was able to estimate rate constants for the process under pH conditions similar to those in the stomach. Nevertheless, as far as we know, there has not been any detailed kinetic study that would allow the knowledge of the nitrosation mechanism of this interesting group of compounds.

In the present work we report the results of a kinetic investigation in acid media (8×10^{-3}–0.15 mol dm^{-3} H^+) of the nitrosation of clonidine (structure **1**[11]), a guanidine with hypotensive properties.[12] The reaction product has been identified recently as *N*-nitrosoclonidine, which most probably has the structure **2**.[13] The possibility of carrying out nitrosation



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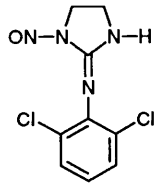

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in neutral or slightly basic media has also been investigated, using an alkyl nitrite as the nitrosating agent. Activated alkyl nitrites are known to readily transfer the nitroso group to amines, but the reaction is unknown for amides or ureas.

Experimental

Clonidine, in the form of its hydrochloride ($HCLO^+$), was obtained from Edol Laboratories, Portugal; D_2O (99.77%D) was supplied by CIEMAT (Spain). All other reagents were Merck products of the maximum purity commercially available and were used as received.

The monochloroacetic, dichloroacetic, trichloroacetic and trifluoroacetic acid buffers were prepared from the corresponding acids by neutralization with the corresponding fraction of NaOH.

For the reactions studied at $[H^+] > 0.01$ mol dm^{-3}, the reported acidities refer to the true concentrations of H^+ in the reaction medium, allowance having being made for the consumption of added protons for formation of nitrous acid from sodium nitrite (pK_a 3.4). For reactions at lower acidities, and also for reactions in the presence of buffers, the acidity was estimated from the pH readings.

The pH was measured with a Radiometer PHM 82 pHmeter, equipped with a GK 2401 C combined glass electrode.

2,2-Dichloroethyl nitrite was synthesized from the corresponding alcohol and sodium nitrite in acid medium, following literature procedures,[14] and was stored in the refrigerator in the

Table 1 Influence of nitrous acid and clonidine hydrochloride concentrations upon the initial rate of nitrosation of clonidine

$[HCLO^+]_0$/mol dm^{-3}	$[H^+]_0$/mol dm^{-3}	$[HNO_2]_0$/mol dm^{-3}	$10^8\ r_0$/mol dm^{-3} s^{-1}
1×10^{-3}	0.065	0.01	2.14
2×10^{-3}	0.065	0.01	3.33
2.5×10^{-3}	0.065	0.01	4.07
4×10^{-3}	0.065	0.01	7.15
5×10^{-3}	0.065	0.01	9.46
6×10^{-3}	0.065	0.01	10.5
7.5×10^{-3}	0.065	0.01	11.9
8×10^{-3}	0.065	0.01	12.9
0.011	0.065	0.01	18.4
0.0125	0.065	0.01	21.1
0.015	0.065	0.01	26.5
0.016	0.065	0.01	29.0
0.01	0.07	2.5×10^{-3}	3.87
0.01	0.07	5×10^{-3}	9.47
0.01	0.07	7.5×10^{-3}	12.5
0.01	0.063	0.01	15.4
0.01	0.063	0.012	18.6
0.01	0.063	0.014	22.3

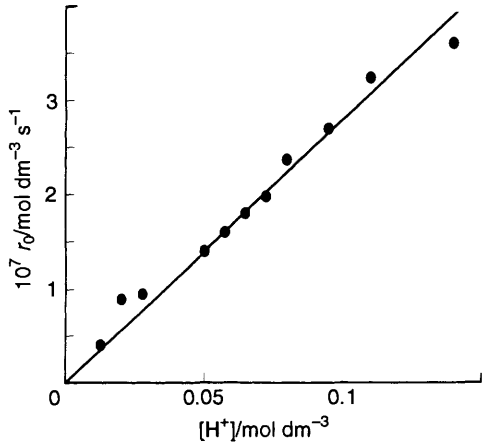


Fig. 1 Influence of $[H^+]$ upon the initial rate of nitrosation of clonidine. $[HCLO^+]$ = 0.01 mol dm^{-3}, $[HNO_2]$ = 0.01 mol dm^{-3}. Ionic strength = 0.5 mol dm^{-3}.

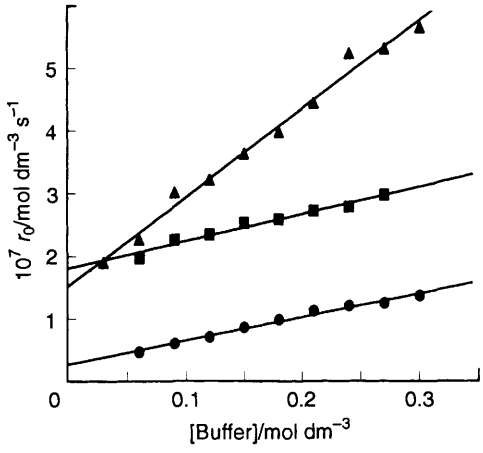


Fig. 2 Influence of the concentration of buffers upon the initial rate of nitrosation of clonidine. $[HCLO^+]$ = 0.01 mol dm^{-3}, $[HNO_2]$ = 0.01 mol dm^{-3}. Ionic strength = 0.5 mol dm^{-3}. (●) Monochloroacetate buffer, pH 2.05; (▲) dichloroacetate buffer, pH 1.25; (■) trichloroacetate buffer, pH 1.09. Ionic strength = 0.5 mol dm^{-3}.

presence of molecular sieves to prevent its hydrolysis. Due to its instability in water,[15] stock solutions were prepared in dioxane and small aliquots were added to initiate the reaction. The content in dioxane in all the experiments performed with alkyl nitrite was 3.6%.

Kinetic measurements were carried out at 25 °C, by continuously monitoring the increase in absorbance at 290 or 300 nm corresponding to the formation of nitrosoclonidine. The molar absorptivities of nitrosoclonidine at these wavelengths were measured using a sample available from earlier work,[13] and values of $(4.05 \pm 0.01) \times 10^3$ and $(2.67 \pm 0.01) \times 10^3$ mol^{-1} dm^3 cm^{-1} at 290 and 300 nm, respectively, were found. Kontron-Uvikon 930 or Perkin Elmer Lambda 2 spectrophotometers, both provided with thermostatted cell-holders, were used for the kinetic studies.

Kinetic analysis of the data was carried out using the initial rate method, to avoid problems derived from the decomposition of the product—presumably hydrolysis or a second nitrosation[13]—that competed with the nitrosation. Generally, the extent of reaction analysed did not exceed 5% of the reaction and the absorbance–time data resulted in a good straight line, the initial rates of the process being estimated from the slope of such plots. When a slight curvature of the trace was detected on that initial trace, the experimental data fitted a quadratic $A = a + bt + ct^2$ (A = absorbance, t = time) and the initial rate was estimated from parameter b. In the following, r_0 stands for the initial rate.

Results and Discussion

Nitrosation of Clonidine in Acid Medium.—The pK_a of the clonidine hydrochloride was measured potentiometrically before carrying out the kinetic studies. The pK_a measured was 8.18, similar to literature values.[16] This means that at the working acidities (pH 1–3) clonidine will exist mainly in the protonated form, the percentage of free clonidine (unprotonated) being stoichiometrically insignificant.

The experimental rate equation was established using perchloric acid to regulate the acidity of the medium and sodium perchlorate to maintain a constant ionic strength of 0.5 mol dm^{-3}. Fig. 1 shows the influence of acidity upon the reaction rate at constant concentrations of nitrous acid and clonidine. The plot is a good straight line that passes through the origin, indicative of a first order term with respect to the concentration of H^+. Studies on the influence of the concentrations of nitrous acid and clonidine hydrochloride ($HCLO^+$) on the reaction rate were performed similarly (see Table 1). The reaction was also found to be of first order with respect to both reagents [eqn. (1)].

$$r_0 = k[HNO_2][H^+][HCLO^+] \quad (1)$$

The value of the third-order rate constant k obtained from the different experiments was: $(2.7 \pm 0.3) \times 10^{-2}$ mol^{-2} dm^6 s^{-1}

Table 2 Effect of added nucleophiles on the initial rate of nitrosation of clonidine[a]

$[Cl^-]$/mol dm^{-3}	$10^7 r_0$/mol dm^{-3} s^{-1}	$[SCN^-]$/mol dm^{-3}	$10^7 r_0$/mol dm^{-3} s^{-1}
0.01	1.31	3×10^{-3}	1.52
0.03	1.63	5×10^{-3}	1.34
0.05	1.45	7×10^{-3}	1.41
0.07	1.53	9×10^{-3}	1.49
0.1	1.33	0.01	1.39
		0.05	1.41

[a] Initial concentration $[HNO_2]_0 = 0.01$ mol dm^{-3}, $[HCLO^+]_0 = 0.01$ mol dm^{-3}, $[H^+]_0 = 0.065$ mol dm^{-3}.

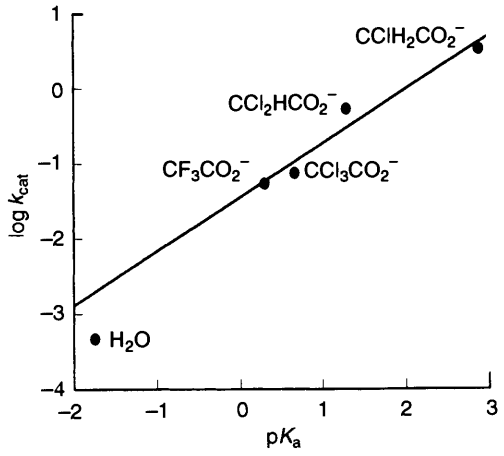


Fig. 3 Bronsted plot for the general base catalysis of the nitrosation of clonidine

when studying the order with respect to $HCLO^+$, $(2.55 \pm 0.1) \times 10^{-2}$ mol^{-2} dm^6 s^{-1} from the studies of the influence of nitrous acid and $(2.5 \pm 0.3) \times 10^{-2}$ dm^6 s^{-1} from the influence of acidity. The agreement is, therefore, very good, and allows a mean value of $(2.6 \pm 0.3) \times 10^{-2}$ mol^{-2} dm^6 s^{-1} to be established for k. Interestingly, this rate equation is similar to that found in the nitrosation of amides, ureas, carbamates, *etc.*[1a,2,4] Conversely, the nitrosation of amines at these pH values tends to occur by N_2O_3, leading to a rate equation of order 2 in nitrous acid. More significant is the dependence of the reaction rate on acidity, which is identical to the one found in the nitrosation of amides and ureas, substrates that, unprotonated at normal acidities, react with the nitrosating agent in their stoichiometric form. This behaviour differs from that of most amines, whose basicity causes them to be mostly protonated, so that the reactive species (free base) is only a minor proportion of the stoichiometric concentration, a fact that leads to the appearance of a term with $[H^+]$ in the denominator of the corresponding rate equation. In this case, the observed rate equation indicates, therefore, that protonated clonidine is the reactive species. This situation, which is impossible in the case of the amines, becomes possible for clonidine because it has more than one nucleophilic centre.

In order to further explore the apparent differences between amines and clonidine, we studied the influence of the usual catalysts of the nitrosation process on the rate of the reaction. These catalysts (halides, thiocyanate, *etc.*) considerably accelerate the rate at which the amines nitrosate by providing important concentrations of new and effective nitrosating agents (ONCl, ONBr, ONSCN, *etc.*). However, nitrosation of amides and related compounds[5] is not susceptible to this type of catalysis. Table 2 shows the effect of the addition of important quantities of Cl^- (up to 0.1 mol dm^{-3}) and of the strong nucleophile SCN^- (up to 0.05 mol dm^{-3}) on the rate of the process. As can be observed, there is no trace of catalysis. This result seems to rule out a mechanism for the nitrosation of clonidine similar to that which operates in the case of the amines, *i.e.* a mechanism whose slow step is reaction between the nitrosatable substrate and the nitrosating agent.

To study the mechanism of the process in more detail, the possibility of the existence of general base catalysis, of the type found in the nitrosation of amides, was investigated. For this, buffers of monochloroacetic, dichloroacetic, trichloroacetic and trifluoroacetic acids were employed. The results obtained (see Fig. 2 for some examples) are indicative of significant buffer catalysis, the percentage of catalysis increasing upon increasing the proportion of basic form in the buffer. This finding strongly indicates that the reaction is subject to a general base catalysis, according to eqn. (2).

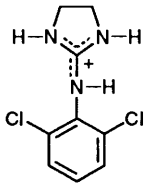
$$r_0 = [HCLO^+][HNO_2][H^+](k + k_{cat}[Base]) \quad (2)$$

Fig. 3 shows the Bronsted plot relating the catalytic efficiency ($\log k_{cat}$) and the pK_a of the catalysts (without statistical correction). Included in the plot is also the point corresponding to H_2O acting as a base—the non-catalytic term—obtained by dividing k by 55.5 mol dm^{-3}. The deviation of the point corresponding to H_2O is common in these types of representations,[2,4,17] although it could also be indicative of some curvature in the Bronsted plot. The existence of general base catalysis, which gives rise to a value for the Bronsted exponent of $\beta = 0.7$ (± 0.1), probably indicates that the transfer of a proton from an intermediate to the reaction medium takes place in the slow step of the reaction as in the case of the nitrosation of amides. The general acid catalysis observed when studying the denitrosation of *N*-nitrosoclonidine yielded $\alpha = 0.5$,[13] which implies a value of 0.5 for β in our nitrosation reaction. The difference is probably due to the restricted set of catalysts used in the construction of these plots, which causes a substantial uncertainty in their slopes. In fact, the more reliable points, where the extent of catalysis is larger (monochloroacetate and dichloroacetate), yield a slope of 0.5, which seems therefore a more reliable value. The small extent of catalysis exerted by trichloroacetate and trifluoroacetate makes their catalytic coefficients less reliable, especially since small solvent effects cannot be excluded. The practice of identifying the degree of proton transfer in the transition state with the value of the Bronsted slope allows us to estimate that the transition state of the slow process occurs neither very early nor very late along the reaction coordinate.

The reason why the behaviour of clonidine is more similar to that of amides and ureas than to the behaviour of amines can be understood if we consider that the reacting species is protonated clonidine, a species whose basicity will be much lower than that of the non-protonated clonidine. In fact, we were unable to measure potentiometrically the pK_a corresponding to a second protonation of clonidine, suggesting that the second protonation is very difficult, not occurring significantly at pH > 1.5. This idea places our true reactive substrate in the range of basicity of amides and ureas, and so it is not surprising that its reaction mechanism shows significant differences with that of the amines. It is easy to understand why reaction *via* free clonidine cannot be detected: its concentration

is so low that the reaction rate derived from this process would be much lower than that resulting from the less basic (and less reactive), but more abundant, protonated clonidine.

As in the case of other guanidines, protonation of clonidine occurs mostly on the iminic nitrogen (structure **3**). The structure resulting from this protonation seems intuitively the most



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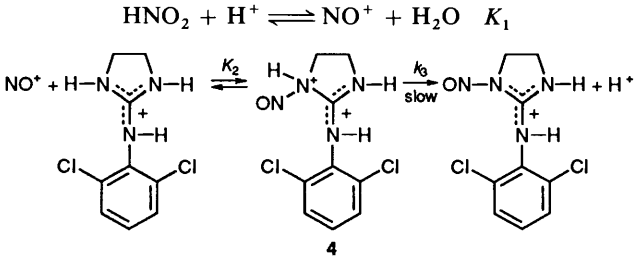
favourable of all possible tautomers and this is the structure which exists in the solid crystalline clonidine hydrochloride.[18] Several studies concerning charge distribution and bond orders in this structure can be found in the literature.[19]

Now, if we assume that structure **3** is the one that reacts with the nitrosating agents, it is tempting to propose the reaction mechanism in Scheme 1.



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Scheme 1

This mechanism leads to rate eqn. (3) which coincides with

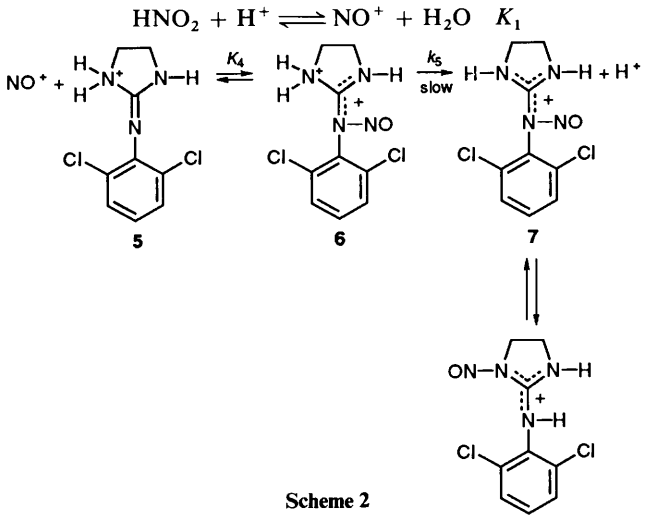
$$r_0 = K_1K_2k_3[HNO_2][HCLO^+][H^+] \qquad (3)$$

the experimental one [eqn. (1)]. The first step, the pre-equilibrium formation of the nitrosating agent through protonation of nitrous acid, is followed by a fast equilibrium reaction of the nitrosating agent with $HCLO^+$, leading to the formation of intermediate **4**. The final step is a limiting transfer of a proton from intermediate **4** to the reaction medium. For the sake of simplicity, NO^+ is proposed as the nitrosating agent, even though the exact nature cannot be ascertained, since the reaction rate is determined by the equilibrium concentration of intermediate **4**, which is independent of the specific way in which it may have been formed. This is why catalysis by halides is not detected, and this is, in general, one of the main criteria used to demonstrate that the attack of the nitrosating agent is a fast pre-equilibrium in nitrosation reactions. This mechanism explains, therefore, the experimental rate equation, the absence of catalysis by nucleophiles and the existence of general base catalysis, as being due to the existence of a proton transfer during the rate determining step of the process.

This simple mechanism, although apparently satisfactory, does not stand up to more detailed scrutiny. In particular, the proton abstracted from intermediate **4** is a proton belonging to a $N(NO)H^+$ group which must be extremely acidic. For example, it is known that protonated *N*-nitrosamines have pK_as usually some 10 units lower than those of the corresponding protonated amines,[20] but since the protonation of the *N*-nitrosamines probably takes place on the oxygen atom, the decrease in basicity of the amino-nitrogen brought about by the introduction of the nitroso group can be substantially lower. In fact, the pK_a for the protonation of the nitrosodimethylamine on the nitrogen atom has been estimated recently as *ca.* -12.[21] From these estimates, we can safely attribute to the proton that should be transferred from intermediate **4** (the nitrosated analogue of a twice protonated clonidine) a pK_a well below -10. According to Eigen's theory,[22] applicable to proton transfer between 'normal' acids and bases, such an acidic molecule would transfer the proton to water (pK_a of $H_3O^+ = -1.75$) by a diffusion-controlled process. In this situation, and since the concentration of water is far above that of any other base present, general base catalysis would not be expected to occur, in contrast with what is experimentally observed. One can say additionally that such an acidic intermediate would lose the proton to water in a process so fast that it would not be possible to talk of such an intermediate, its lifetime being so short that it would not be compatible with its diffusion through the solvent. Such a process should not, of course, show conventional base catalysis. Moreover, according to the Hammond postulate,[23] the transfer of a proton from intermediate **4** to water or to any of the bases added, being an energetically very favourable process, would have a transition state structurally very close to reagents and not intermediate between reagents and products as suggested by the observed Bronsted slope. It can be concluded both from the existence of general base catalysis and from the observed Bronsted slope (in spite of its uncertainty) that the pK_a of the intermediate that loses the proton cannot be widely different from that of the hydronium ion (-1.75) or the acid forms of the buffers used.

A somewhat similar situation has been detected in the case of the nitrosation of amides and related compounds,[1a] where the kinetic evidence found was seemingly incompatible with the acidities of the intermediates involved in the mechanism. In the case of the nitrosation of amines, the possibility that buffer catalysis arose as a consequence of a concerted or a pre-association mechanism[24] could be ruled out on the basis of two conclusive experimental facts:[1a] for 2-imidazolidone a change in rate-determining step could be observed at high buffer concentrations (the reaction rate becoming buffer independent) and an Eigen-type Bronsted plot (with a Bronsted slope changing from 1 to 0) could be observed for 1,3-dimethylurea. Both these facts required a stepwise mechanism with trapping of an intermediate by added bases. In the present case, we cannot absolutely discard other possibilities, but the similarities of behaviour between our substrate and amides make a stepwise mechanism with liberated intermediates more likely. In addition, the observed value of β and the measured solvent isotope effect (*vide infra*) seem to indicate that buffer catalysis does not arise from hydrogen-bonding stabilization of the transition state for formation of imtermediate **4** in a pre-association mechanism and both concerted and preassociation mechanisms would not easily explain the absence of catalysis by halides or SCN^-, since the actual nitrosating agent (ONX) would be present in the rate-determining step of the reaction. A tentative solution to this situation is found in the mechanism shown in Scheme 2, which shows similarities with that proposed for amides and ureas.[1] This mechanism differs from that in Scheme 1 in that it proposes attack of the nitrosating agent on the minor tautomeric structure of protonated clonidine, structure **5**.

In principle, tautomer **5** should react at the most nucleophilic centre, the free iminic nitrogen. Such a reaction between the substrate and the nitrosating agent will be a fast pre-equilibrium step, followed by a slow proton transfer—susceptible to general base catalysis—from intermediate **6** to yield a structure more likely to receive the nitroso group in the endocyclic nitrogen. Intermediate **6** could have a pK_a much higher than that of intermediate **4** (probably not too far from 0), compatible with the existence of general base catalysis. An isomer of nitrosoclonidine, **7**, which could undergo a rapid internal rearrangement to produce the final product,[25] would thus be



Scheme 2

Table 3 Influence of clonidine hydrochloride concentration on the initial rate of its nitrosation in D_2O (99% D)[a]

[Clonidine hydrochloride]$_0$/mol dm^{-3}	$10^8 r_0$/mol dm^{-3} s^{-1}
1×10^{-3}	0.572
3×10^{-3}	1.80
5×10^{-3}	3.00
7×10^{-3}	4.28
9×10^{-3}	4.73
0.011	6.30
0.013	6.95
0.015	8.23
0.017	8.84
0.019	10.25

[a] $[D^+]_0 = 0.026$, $[DNO_2]_0 = 0.01$ mol dm^{-3}. Ionic strength = 0.5 mol dm^{-3}.

formed. This mechanism involving initial attack of the nitrosating agent at the most nucleophilic point of the molecule, followed by a rearrangement to a product of greater thermodynamic stability, is similar to that found for amides and ureas, which are initially nitrosated on the oxygen atom,[1] or 3-substituted indoles, which react initially at position 3 of the ring.[26] The advantage of these reaction pathways, followed by substrates of low basicity, seems to be the avoidance of highly unstable intermediates—intermediate **4** in this case. For substrates of very low basicity, but with only one nucleophilic centre in the molecule, such as alcohols, it is not possible to invoke mechanisms like the one in Scheme 2. In such cases, the attempt to avoid those highly energetic intermediates leads to a mechanistic change towards a concerted process.[15] The proposed mechanism also explains why the value of the measured rate constant for the reverse reaction is only[13] 1.4×10^{-2} mol^{-1} dm^3 s^{-1} at 35 °C. This value would be at odds with protonation by H_3O^+ of an intermediate whose pK_a is not far from zero.[22] This problem disappears in the mechanism of Scheme 2, according to which the measured overall rate constant in the reverse direction would include a presumably very low equilibrium constant for isomerization of *N*-nitrosoclonidine to structure **7**. This argument shows that the mechanism recently reported[13] for the denitrosation pathway must be revised in the light of the present results and the principle of microscopic reversibility. Definite validation of the mechanism in Scheme 2 is difficult since the pK_a for intermediate **6** is unknown. If we assume a pK_a in the range 0–1, a Bronsted slope of 0.5 would be expected for the series of buffers examined, which is not inconsistent with the experimental value of β measured (*vide supra*). The fact that β does not change abruptly from 1 to 0 in the vicinity of the pK_a of the intermediate (as in the simple Eigen's model) is not unusual in these type of proton transfers,[27a] especially if N-acids are involved.[27b]

One last indication about the nature of the slow step was obtained when the reaction was carried out in D_2O and the corresponding solvent isotope effect was measured. For this purpose, at constant acidity and nitrous acid concentration, the influence of the concentration of clonidine hydrochloride—which varied between 1×10^{-3} and 1.75×10^{-2} mol dm^{-3}—upon the reaction rate was studied (Table 3). The results obtained allow evaluation of the overall isotope effect for the uncatalysed reaction as $k(H)/k(D) = 1.3$. Once again, this outcome likens the behaviour of the clonidine to that of amides and ureas, but not of amines, which should show inverse solvent isotope effects (typically 0.3), due to the lower concentration of the species derived from the protonation of nitrous acid[28] in H_2O than in D_2O. If the mechanism in Scheme 2 is correct, the observed value for $k(H)/k(D)$ includes the influence of the isotopic substitution on the pre-equilibria constants K_1 and K_4 and on the rate constant for the slow step k_5. Replacement of water by deuteriated water increases the value of K_1 2.55 times.[28] Assuming that isotopic substitution does not have an appreciable effect on K_4, the value of the kinetic isotope effect on the slow step can be estimated as 3.3. This value is typical of a primary isotope effect, strongly pointing to a rate-determining proton transfer. The non-unity value of this effect is in agreement with a 'classical' interpretation of the isotope effect whose magnitude will be related to the degree of symmetry of the transition state.[17] Thus, both the value of the Bronsted slope β and of the solvent isotope effect on the slow step suggest a process with a transition state intermediate between reactants and products, but with a structure not very close to any of them. Once again, the value found for the isotope effect would be at odds with a proton transfer from a strongly acidic intermediate (intermediate **4**) to water. The value also seems rather high for a hypothetical pre-association or concerted mechanism, similar to that found in the nitrosation of alcohols.[15]

Nitrosation of Clonidine in Neutral/Basic Media.—Alkyl nitrites are capable of transferring the nitroso group to nucleophiles, in a process known as direct transnitrosation. This reaction facilitates the nitrosation of amines,[29] thiolates,[30] carbanions,[31] *etc.* in neutral or basic media.

The reactivity of alkyl nitrites is increased when the ability of the RO^- group as a leaving group improves, as is the case of the alkyl nitrites bearing electron-withdrawing substituents in the β-position.[29a,b,d] The reaction does not take place in acid medium, where alkyl nitrites hydrolyse at a rate higher than that of the nitroso group transfer.[15] The process has not been observed, however, for poor nucleophiles such as ureas or amides. The possibility that clonidine could be nitrosated in basic medium by means of alkyl nitrites has therefore been investigated. Since alkyl nitrites are used as vasodilators and muscular relaxants,[32] the reaction may have biological interest.

Nitrosation of clonidine at pH 7.8–8.8 has been achieved using 2,2-dichloroethyl nitrite as the nitrosating agent. Clonidine's own buffering ability was used to maintain the pH of the medium at these values, close to its pK_a. Table 4 shows that the initial rate of reaction, obtained at different pH values, is found to increase upon increasing pH, in a way that is indicative of the involvement of the non-protonated form of clonidine as the nitrosatable nucleophile. Such a mechanism[29b,d] would lead to eqn. (4), where k_n is the bimolecular rate constant

$$r_0 = \frac{k_n K_a [\text{Clonidine}_{\text{total}}][\text{Alkyl nitrite}]}{(K_a + [H^+])} \quad (4)$$

Table 4 Influence of pH on the initial rate of nitrosation of clonidine by 2,2-dichloroethyl nitrite[a]

pH	$10^8 r_0$/mol dm^{-3} s^{-1}
7.45	3.80
7.77	7.12
7.90	8.61
8.23	13.7
8.67	19.4

[a] $[\text{Clonidine}_{\text{total}}]_0 = 0.01$ mol dm^{-3}, $[\text{2,2-dichloroethyl nitrite}]_0 = 2 \times 10^{-3}$ mol dm^{-3}. Ionic strength = 0.5 mol dm^{-3}, 3.6% dioxane.

for reaction between free clonidine and alkyl nitrite and K_a is the acidity constant for protonated clonidine.

A plot of $1/r_0$ *vs.* $[H^+]$ results in a good straight line as predicted by eqn. (4). Moreover, a value of 8.25 for the pK_a of clonidine can be obtained from such a plot, in good agreement with that obtained experimentally (*vide supra*). A value of 1.4×10^{-2} mol^{-1} dm^3 s^{-1} is obtained for the bimolecular rate constant k_n between the alkyl nitrite and the non-protonated clonidine. Thus, in basic medium, the behaviour of clonidine in its nitrosation by alkyl nitrites is similar to that of amines.

It can be concluded that the basicity of the reactive form is the main factor determining the mechanistic behaviour of clonidine towards nitrosating agents. In acid media, when the protonated form predominates, it is this protonated form—of very low basicity—which reacts, showing a pattern of behaviour that is similar to that of other nitrogen nucleophiles of low basicity, such as ureas or amides, and different to that of the more basic amines. However, in basic or neutral media, where the more basic free form is abundant, reaction with alkyl nitrites occurs through this form, the kinetic behaviour being now akin to that of the amines of similar basicity.

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References

1 (*a*) A. Castro, E. Iglesias, J. R. Leis, M. E. Peña and J. Vázquez Tato, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1725; (*b*) J. Casado, A. Castro, F. Meijide, M. Mosquera and J. Vázquez Tato, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1759; (*c*) C. Bravo, P. Hervés, J. R. Leis and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2*, 1991, 2091.
2 J. Casado, A. Castro, M. Mosquera, M. F. Rodríguez Prieto and J. Vázquez Tato, *Ber. Bunsenges. Phys. Chem.*, 1983, **87**, 1211.
3 G. Hallett and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1372.
4 J. Casado, A. Castro, J. R. Leis, M. Mosquera and M. E. Peña, *Monatsh. Chem.*, 1984, **115**, 1047.
5 D. L. H. Williams, *Nitrosation*, Cambridge University Press, 1988.
6 R. B. Homer and C. D. Johnston, in *The Chemistry of Amides*, ed. J. Zabicky, Wiley, New York, 1970, p. 187.
7 B. C. Challis, J. N. Iley and H. S. Rzepa, *J. Chem. Soc., Perkin Trans. 2*, 1983, 1037.
8 See, *e.g.*, A. F. McKay and G. F. Wright, *J. Am. Chem. Soc.*, 1947, **69**, 3028.
9 C. L. Galtress, P. R. Morrow, S. Nag, J. L. Smalley, M. F. Tschautz, J. S. Vaughn, D. N. Wichems, S. K. Zigler and J. C. Fishbein, *J. Am. Chem. Soc.*, 1992, **114**, 1406 and references therein.
10 S. S. Mirvish, *Toxicol. Appl. Pharmacol.*, 1975, **31**, 325.
11 L. M. Jackman and T. Jen, *J. Am. Chem. Soc.*, 1975, **97**, 2811.
12 See, *e.g.*, P. A. von Zwieten, *Prog. Pharmacol.*, 1975, **1**, 1.
13 J. Iley, F. Norberto, E. Rosa, V. Cardoso and C. Rocha, *J. Chem. Soc., Perkin Trans. 2*, 1993, 591.
14 W. A. Noyes, *Org. Synth. Coll. Vol. II*, 1943, p. 108.
15 E. Iglesias, L. García-Río, J. R. Leis, M. E. Peña and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1673.
16 P. B. M. W. M. Timmermans and P. A. van Zwieten, *Arzneim.-Forsch.*, 1978, **28**, 1676.
17 R. P. Bell, *The Proton in Chemistry*, Chapman and Hall, London, 1973.
18 G. Byre, A. Mostad and C. Romming, *Acta Chem. Scand. Ser. B*, 1976, **30**, 843.
19 A. Carpy, J. M. Leger, G. Leclerc, N. Decker, B. Rouot and C. G. Wermuth, *Mol. Pharmacol.*, 1982, **21**, 400.
20 B. C. Challis and J. A. Challis, in *The Chemistry of Amino, Nitroso and Nitro Compounds and their Derivatives*, ed. S. Patai, Wiley, New York, 1982, ch. 26.
21 L. K. Keefer, J. A. Hrabie, B. D. Hilton and D. Wilbur, *J. Am. Chem. Soc.*, 1988, **110**, 7459.
22 M. Eigen, *Angew. Chem., Int. Ed. Engl.*, 1964, **3**, 1.
23 G. S. Hammond, *J. Am. Chem. Soc.*, 1955, **77**, 334.
24 W. P. Jencks, *Chem. Soc. Rev.*, 1981, **10**, 345.
25 M. E. Kletskii, R. M. Minyaev and V. I. Minkin, *Zh. Org. Khim.*, 1980, **16**, 686.
26 C. Bravo, P. Hervés, J. R. Leis and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2*, 1992, 185.
27 (*a*) See, *e.g.*, F. Hibbert, *Adv. Phys. Org. Chem.*, 1986, **22**, 113; (*b*) see, *e.g.*, C. H. Arrowsmith, A. Awwal, B. A. Euser, A. J. Kresge, P. P. T. Lau, D. P. Onwood, Y. C. Tang and E. C. Young, *J. Am. Chem. Soc.*, 1991, **113**, 172.
28 A. Castro, M. Mosquera, M. F. Rodríguez Prieto, J. A. Santaballa and J. Vázquez Tato, *J. Chem. Soc., Perkin Trans. 2*, 1988, 1968.
29 (*a*) B. C. Challis and D. E. G. Shuker, *J. Chem. Soc., Chem. Commun.*, 1979, 315; (*b*) L. García-Río, E. Iglesias, J. R. Leis, M. E. Peña and A. Ríos, *J. Chem. Soc., Perkin Trans. 2*, 1993, 29; (*c*) S. Oae, N. Asai and K. Fujimori, *J. Chem. Soc., Perkin Trans. 2*, 1978, 1124; (*d*) J. Casado, A. Castro, F. M. Lorenzo and F. Meijide, *Monatsh. Chem.*, 1986, **117**, 335.
30 H. M. S. Patel and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1990, 37.
31 J. R. Leis, M. E. Peña and A. Ríos, *J. Chem. Soc., Perkin Trans. 2*, 1993, 1233.
32 L. J. Ignarro, H. Lippton, J. C. Edwards, W. H. Baricos, A. L. Hyman, P. J. Kadowitz and C. A. Gruetter, *J. Pharmacol. Exp. Ther.*, 1981, **218**, 730.

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