N-Nitroso Compounds. Part 2.[1] The Synthesis of *N*-Nitrosoclonidine and its Decomposition in Aqueous Acidic Media

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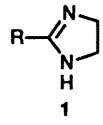
Clonidine, a 2-arylaminoimidazoline, undergoes nitrosation in aqueous nitrous acid to form *N*-nitrosoclonidine. ^{1}H NMR spectroscopy reveals that the nitroso group resides on one of the imidazoline nitrogen atoms. *N*-Nitrosoclonidine decomposes in aqueous acidic media to form quantitatively clonidine and nitrous acid. The reaction is acid catalysed and involves rate-limiting protonation of the substrate, as revealed by a solvent deuterium isotope effect, k_2^H/k_2^D, of 1.2 and the independence of the rate of denitrosation upon the presence of Cl^- or SCN^-. The Brønsted α exponent for general acid catalysis is 0.5. The mechanism is contrasted to that for analogous 2-arylimidazolines, which suffer acid-catalysed hydrolysis *via* a rapid pre-equilibrium protonation of the substrate followed by rate-limiting attack of the nucleophile to effect denitrosation or amidine hydrolysis. The present results are interpreted in terms of a protonation of the imidazoline nitroso nitrogen of *N*-nitrosoclonidine which results in an intermediate that decomposes by loss of the nitroso group.

The nitrosation of nitrogen-containing compounds has received considerable attention in recent years. This is due to the potentially cytotoxic nature of the species so formed. Primary amines form direct acting mutagenic diazo compounds,[2] secondary amines form *N*-nitrosamines which are carcinogenic upon metabolism by cytochrome P450 enzymes,[3] and secondary amides and peptides yield *N*-nitrosamides which are also direct acting mutagens.[4] Given the ubiquity of compounds that contain nitrogen-derived functional groups in both food and drugs, concern has been raised over the potential *in vivo*, especially gastric, nitrosation of these substances to form cytotoxic derivatives. This has led to many drugs being investigated for their ability to undergo nitrosation,[5–7] and *N*-nitroso derivatives have been reported as products from substrates as diverse as, for example, the antibiotic penicillins and tetracyclines, β-aminoalcohols that act as adrenergic receptors, heterocyclic antidepressants, and antidiabetic sulfonamides.

We are interested in the potential nitrosation of orally administered drugs that are taken chronically. In particular, the imidazoline class of compounds, *e.g.* **1**, has been little studied, though it contains a number of compounds that have a range of biological activity. For example, fenmetazole (**1**; R = 3,4-Cl_2-$C_6H_3OCH_2$) is an antidepressant, tolazoline (**1**; R = $C_6H_5CH_2$) is a vasodilator, and naphazoline (**1**; R = 1-$C_{10}H_7CH_2$) is a vasoconstrictor.[8]

In a previous study, we have shown that 2-arylimidazolines (**1**; R = aryl) could indeed form *N*-nitroso derivatives upon reaction with N_2O_4 or HNO_2. These *N*-nitrosoimidazolines are stable in basic media, but decompose in acidic media *via* two competitive pathways, *viz.* denitrosation or deamination *via* hydrolysis of the *N*-nitrosoamidine moiety.[1]

We have now turned our attention to the 2-aminoimidazolines, **1** (R = substituted amino), which are cyclic guanidines, and in particular to the antihypertensive agent clonidine, **1** (R = 2,6-$Cl_2C_6H_3NH$).[8] Clonidine has been reported previously to undergo nitrosation, though no attempt to establish the structure of the nitrosated product was made.[5] Several acyclic guanidines are known to form *N*-nitroso derivatives, the most celebrated being *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine[9] and the anti-ulcer drug cimetidine.[10] Herein we report upon the nitrosation of clonidine, the structure of the nitroso products so-formed, and the decomposition of the mononitrosated derivative in aqueous acidic media.



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Experimental

Clonidine was obtained from Edol Laboratories (Portugal) and was used as supplied.

Nitrosation of Clonidine.—Clonidine (0.35 g, 1.5 mmol) in DMSO (2 cm^3) was added with stirring to a solution of sodium nitrite (1.05 g, 15 mmol) in aqueous HCl (0.1 mol dm^{-3}, 10 cm^3) at room temperature. After 20 min the reaction mixture was extracted into CH_2Cl_2 (2 × 10 cm^3), the organic extract dried and evaporated, and the residue chromatographed on silica gel using diethyl ether: light petroleum (40–60 °C) (7:3) as eluent. Two compounds were isolated in this way. The first, isolated in a yield of 30%, was identified as *N*-nitrosoclonidine on the basis of the following physical data: δ_H($CDCl_3$) 3.50 (2 H, t, *J* 7 Hz), 3.92 (2 H, t, *J* 7 Hz), 5.9 (1 H, br, exch.), 7.02 (1 H, t, *J* 9 Hz), 7.39 (2 H, d, *J* 9 Hz); *m/z* (relative intensity) 262 (1)/260 (7)/258 (11) (M^+; isotope pattern for two Cl atoms), 233 (6)/ 231 (39)/229 (61) ($M^{\bullet+}$ + H − NO), 232 (7)/230 (20)/228 (23) ($M^{\bullet+}$ − NO), 205 (5)/203 (23)/201 (47) ($M^{\bullet+}$ + H-NO-C_2H_4), 195 (40)/193 (100) ($M^{\bullet+}$-NO-Cl), 172 (35) ($C_6H_3Cl_2NHC^{\bullet+}$), 160 (11) ($C_6H_3Cl_2NH^{\bullet+}$), 145 (15) ($C_6H_3Cl_2^{\bullet+}$); ν_{max}/cm^{-1} 3000, 1700, 1280 and 1140 (Found: C, 41.6; H, 3.7; N, 21.1. Calc. for $C_9H_8N_4OCl_2$: C, 41.63; H, 3.32; N, 21.58%). This compound had m.p. 98–100 °C and r_f 0.3 [ether/light petroleum (1:1)].

The second, which was much less stable, was isolated in 10% yield and identified as a dinitroso derivative on the basis of the following physical data: δ_H($CDCl_3$) 3.90 (4 H, s), 7.07 (1 H, t, *J* 9 Hz), 7.40 (2 H, d, *J* 9 Hz); *m/z* 291 (1)/289 (6)/2287 (10) ($M^{\bullet+}$,

Table 1 Pseudo-first-order rate constants for the hydrolysis of **2** in acidic solutions of ionic strength 1 mol dm^{-3}

Acid	[Acid]/ mol dm^{-3}	T/K	$k_0/10^{-3}$ s^{-1}	$k_2/10^{-2}$ cm^3 mol^{-1} s^{-1}
HCl	0.1	308	1.45	1.43
	0.2	308	2.9	
	0.3	308	3.8	
	0.4	308	6.2	
$HClO_4$	0.2	308	4.1	1.9
	0.3	294	1.74	
	0.3	298	2.87	
	0.3	302	4.61	
	0.3	308	6.3	
	0.4	308	7.7	
	0.5	308	9.0	
$DClO_4$	0.22	308	3.9	1.72
	0.29	308	5.5	
	0.44	308	7.7	
	0.56	308	8.5	

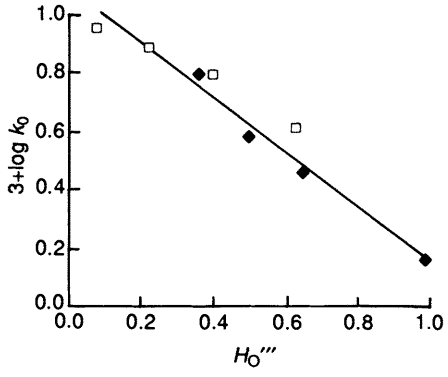


Fig. 1 Dependence of log k_0 upon H_0''' for the HCl, ◆, and $HClO_4$, □, catalysed decomposition of **2**

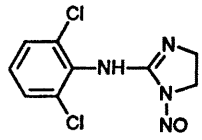
isotope pattern for two Cl atoms). This compound had r_f 0.6 [ether/light petroleum (1 : 1)].

Products of the Acid-catalysed Hydrolysis of N-*Nitrosoclonidine.*—*N*-Nitrosoclonidine was subjected to acid-catalysed hydrolysis using a variety of acid concentrations. Immediately the substrate was consumed (between 30 and 60 min), the formation of nitrite was determined by the modified Shinn method using a value of log ε_{max} = 4.53 at λ = 541 nm, as described previously.[1] The organic co-product was isolated by extraction and identified as clonidine. No other organic product was detected.

Kinetic Studies.—Kinetic runs were carried out in thermostatted cuvettes, and were initiated by injecting a small aliquot (75 mm^3) of a dioxane solution of *N*-nitrosoclonidine (4 × 10^{-3} mol dm^{-3}) into the reaction medium. Initial substrate concentrations were therefore *ca.* 10^{-4} mol dm^{-3}. Ionic strength was maintained by the use of appropriate additions of sodium perchlorate. Reactions were monitored by UV spectrophotometry and exhibited clean isosbestic points. Kinetic data were obtained by monitoring the reactions at λ_{max} 255 nm. Pseudo-first-order rate constants were determined from the slopes of straight line plots of log $(A_t - A_\infty)$ *versus* time. Reactions were first order up to 10 half lives, and the pseudo-first-order rate constants, k_0, were reproducible to ± 5%. At the end of each reaction, the concentration of NO_2^- liberated was determined by the modified method of Shinn, allowing for the slight decomposition of NO_2^- in acidic solutions, as previously described.[1]

Results and Discussion

Nitrosation of Clonidine.—Clonidine is nitrosated using 1.25 mol dm^{-3} HNO_2 yielding two products. These can be separated by chromatography and identified as the mono- and di-nitroso compounds, **2** and **3**, respectively.



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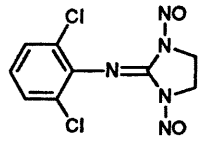

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The identity of these products derives from their physical and spectroscopic data. Thus, the mononitrosated product can be assigned the structure **2** on the basis of the following. First, elemental analysis is consistent with the structure, and the mass spectrum clearly shows the molecular ion and its associated chlorine isotope pattern. Moreover, fragments involving the loss of NO, (NO + C_2H_4) and (NO + Cl) are as expected for such a structure and peaks at m/z = 145, 160 and 172 identify the presence of the 2,6-dichlorophenylamino fragment. Nitrosation does not occur, therefore, at the *para* position of the aryl ring, a reaction that could potentially take place due to the enhanced nucleophilicity of an aryl ring attached to a nitrogen atom. Second, the ^1H NMR chemical shifts of the ring CH_2 protons of clonidine exhibit one resonance at δ3.74, indicating their equivalence due to rapid proton tautomerism. In contrast, in the ^1H NMR spectrum of the mononitroso derivative, the two ring CH_2 groups resonate at quite distinct chemical shifts, δ 3.50 and 3.92, indicating that they experience two chemically different environments. Nitrosation thus occurs at one of the ring nitrogen atoms rather than at the exocyclic nitrogen.

The ^1H NMR spectrum of the dinitroso product exhibits only one peak for the ring CH_2 protons, as would be expected if the product has the symmetrical structure **3**.

Hydrolysis of N-Nitrosoclonidine, **2**.—Dinitrosoclonidine **3** is rather unstable, rapidly forming the mononitrosoclonidine, **2**. *N*-Nitrosoclonidine itself is stable in solutions of pH > 5, but yields clonidine and nitrous acid in quantitative yield in solutions of pH < 3.4. The reactions follow a first-order dependence on substrate concentration, and the pseudo-first-order rate constants, k_0 also depend upon the acid concentration (Table 1). Plots of k_0 *versus* $[H^+]$ are straight lines passing through the origin. Thus, the rate of reaction is governed by eqn. (1), where k_2 is the second-order rate constant, and $k_2 = k_0/[H^+]$. Values of k_2 are obtained from the slopes

$$\text{Rate} = k_2[\mathbf{2}][H^+] \quad (1)$$

of plots of k_0 *versus* $[H^+]$ (Table 1). The data in Table 1 give rise to the following observations. First, the lack of an intercept in plots of k_0 *versus* $[H^+]$ indicates the absence of a reaction that is not acid catalysed. Second, the second-order rate constant, k_2, for acid catalysis in $HClO_4$ is apparently different to that obtained in HCl. However, the acidity functions for $HClO_4$ and HCl are somewhat different in the regions of acidity employed in the present study. When a plot of log k_0 *versus* H_0''', the acidity function derived for tertiary aromatic amines,[11,12] is constructed, the graph is a common line of slope −0.9 (Fig. 1) which substantiates the use of the H_0''' acidity function. Thus, the apparent difference in k_2^{HCl} and $k_2^{HClO_4}$ is due to the differing acidities of solutions of the two acids. Third, the rate constants in $DClO_4$ are smaller than those in $HClO_4$, and the solvent deuterium isotope effect for acid catalysis, k_2^H/k_2^D = 1.2 (± 0.1). Fourth, the temperature dependence of k_0 gives rise to values of the activation parameters $\Delta H^\ddagger$ and $\Delta S^\ddagger$ of 72 (± 5) kJ

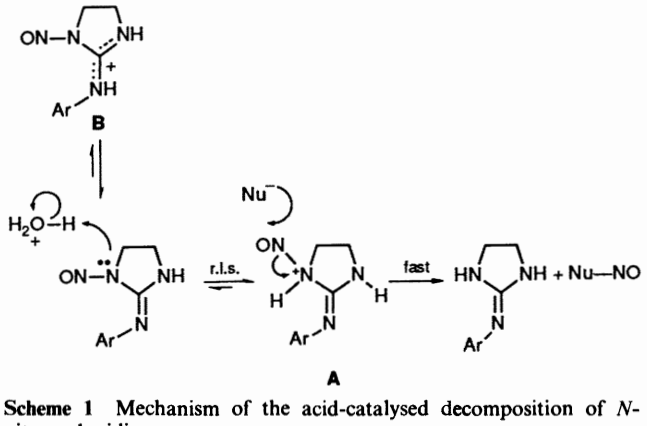
Table 2 Effect of the counter cation on the 0.3 mol dm^{-3} $HClO_4$ catalysed decomposition of **2** at 35 °C in solutions of ionic strength 1 mol dm^{-3}

Cation	[Cation]/mol dm^{-3}	$k_0/10^{-3}$ s^{-1}
Cl^-	0	6.3
	0.3	6.1
	0.4	5.57
	0.5	6.07
	0.6	5.75
SCN^-	0	6.3
	0.002	6.14
	0.004	5.91
	0.007	6.14

mol^{-1} and -52 (± 15) J mol^{-1} K^{-1}, respectively. With the exception of the activation parameters, these observations are quite unlike those made previously of the corresponding *N*-nitroso-2-arylimidazolines, **1** (R = aryl), where a solvent deuterium isotope effect for acid catalysis of *ca.* 0.3 is obtained, and the denitrosation reaction is competitive with amidine hydrolysis.

Next, we turned our attention to the effect of the counter anion on the rate of denitrosation of **2**. Drastic catalytic effects can be observed upon the rate of denitrosation of various *N*-nitroso compounds, including *N*-nitrosamines[13] and *N*-nitrosoimidazolines,[1] but not *N*-nitrosoamides,[14] or *N*-nitrososulfonamides.[15] Such catalysis has been correlated to the nucleophilicity of the counter anion in the reaction medium and interpreted in terms of a rate-limiting nucleophilic attack of the anion at the nitrogen atom of the NO group. The data for *N*-nitrosoclonidine, **2**, are contained in Table 2, and these show conclusively that the rate is independent of the counter anion.

Thus, *N*-nitrosoclonidine, which is an *N*-nitroso-2-arylaminoimidazoline, undergoes an acid-catalysed decomposition reaction by a mechanism that is significantly different from the analogous *N*-nitroso-2-arylimidazolines. The hydrolysis of the latter involves rapid equilibrium protonation of the substrate followed by rate-limiting denitrosation or hydrolysis of the *N*-nitrosoamidine moiety. The data for *N*-nitrosoclonidine, especially the solvent deuterium isotope effect and lack of a dependence on the counter anion lead us to propose a mechanism, outlined in Scheme 1, which involves rate-limiting proton transfer to the nitrogen atom bearing the NO group in the neutral substrate. Independent titration experiments have shown that nitrosoclonidine is not extensively protonated between pH 3–12.5. Subsequent denitrosation of the protonated substrate, whether catalysed by nucleophiles or not, is rapid and thus kinetically independent of the anion present. Our results are unable to determine whether the loss of the NO group is unimolecular or catalysed by nucleophiles.



Scheme 1 Mechanism of the acid-catalysed decomposition of *N*-nitrosoclonidine

Table 3 Dependence of k_2^{HA} for the acid-catalysed decomposition of **2** upon pK_a of the general acid

Buffer	pK_a	pH	$k_2^{HA}/10^{-4}$ dm^3 mol^{-1} s^{-1}
CF_3CO_2H	0.22	0.83	139
		1.14	121
$CHCl_2CO_2H$	1.37	1.14	26.1
		1.49	26.9
CH_2ClCO_2H	2.87	1.65	5.11
		2.25	6.25

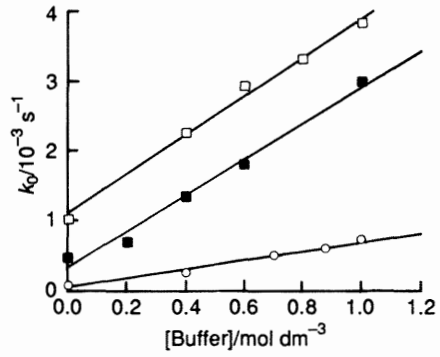


Fig. 2 Dependence of k_0 upon [Buffer] for the general acid-catalysed decomposition of **2**: □, $CHCl_2CO_2H$, pH 1.14; ■, $CHCl_2CO_2H$, pH 1.49; ○, CH_2ClCO_2H, pH 2.25

But why should proton transfer to **2** be rate limiting, when for the corresponding *N*-nitrosoimidazolines proton transfer occurs in a rapid pre-equilibrium step? We propose that *N*-nitrosoclonidine can exist in acid solutions as several protonated forms of which the most likely is structure **B** (Scheme 1), but that it is only protonation of the nitrosoamino nitrogen atom, structure **A**, that activates the substrate sufficiently to undergo denitrosation. Since this nitrogen is relatively electron deficient, proton transfer to it might be expected to be slow, as it is in nitrosoamides, nitrososulfonamides and nitrosoureas. The protonated form **B**, arising from fast proton transfer, resembles that of the corresponding nitrosoamidines, but the extra delocalisation of charge due to the presence of the third nitrogen atom must render this form of the protonated substrate less reactive. Other protonated structures which arise from fast proton transfer, would not be expected to denitrosate because the *N*-nitroso group is not in a suitable relationship to either of the protonated amino groups.

A mechanism involving rate-limiting proton transfer should display general acid catalysis, and confirmation of such an effect can be found from Fig. 2, where the value of k_0 is observed to increase with increasing concentration of the general acid catalyst. The second-order catalytic rate constant, k_2^{HA}, for general acid catalysis is obtained from the slope of the plots in Fig. 2, and can be seen to be independent of the pH of the medium but dependent upon the nature of the buffer acid species. Values of k_0 and k_2^{HA} for three general acids are contained in Table 3. The values of k_2^{HA} vary with the pK_a of the acid, and a plot of $\log k_2^{HA}$ *versus* pK_a gives a straight line from which a value of the Brønsted coefficient $\alpha = 0.5$ may be obtained. This value is similar to the value of 0.64 observed for rate-limiting proton transfer to nitrosamides.[14a] It is unclear, however, why the value of the solvent deuterium isotope effect is so low for such a value for α, when values of 1.5 and 1.8 have been determined for nitrososulfonamides[15] and amides,[14b] respectively.

In conclusion, our results show that clonidine can be

nitrosated by nitrous acid to form *N*-nitrosoclonidine, **2**. This compound decomposes in acid solutions, to yield clonidine and nitrous acid, by a mechanism that involves rate limiting proton transfer to *N*-nitrosoclonidine. No evidence was found for a decomposition pathway that involves hydrolysis of the guanidino moiety, a pathway that could potentially form an alkylating agent. Thus, it would appear that *N*-nitrosoclonidine is unlikely to prove a direct acting mutagen.

Acknowledgements

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