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RADICAL ANIONS OF NITROSOIMIDAZOLES: PUTATIVE INTERMEDIATES IN THE MECHANISM OF ACTION OF NITROIMIDAZOLE ANTIBIOTICS

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Summary: The radical anions of 1-methyl-4-phenyl-5-nitrosoimidazole and 5-phenyl-4-nitrosoimidazole have been detected using temperature resolved e.s.r. spectroscopic techniques and are proposed as putative intermediates in the oxidation of thiols to disulphides by the nitrosoimidazoles.

5-Nitroimidazoles, *e.g.* metronidazole, are important antibiotics for the treatment of anaerobic bacterial and protozoal infections.[1,2] Evidence suggests that their activity is due to inhibition of DNA function and that reduction of the nitro group is required for the therapeutic activity of the drugs.[1,2,3] Some of the reduced species resulting from the reduction of 5-nitroimidazoles (RNO_2) are shown in equation 1. The nitro radical anions have been observed *in vitro*[4] and the structures have been studied.[5] Studies indicate that the radical anions do not interact with DNA,[6] and that the hydroxylamino- and amino-imidazoles are not active.[2,7] 5-Nitrosoimidazoles have been implicated in the mode of action and 1-methyl-5-nitroso-4-phenylimidazole has been shown to be as potent as the parent 5-nitroimidazole, 1-methyl-5-nitro-4-phenylimidazole.[8]

$$RNO_2 \xrightarrow{e^-} (RNO_2)^{-\cdot} \xrightarrow[-H_2O]{e^-,\ H^+} RNO \xrightarrow{e^-} (RNO)^{-\cdot} \longrightarrow RNHOH \longrightarrow RNH_2 \qquad (1)$$

We report our investigations of the reduction of nitrosoimidazoles to their radical anions and suggest that these anions may be important intermediates in the mechanism of action of nitroimidazoles. This step in the overall reduction of nitroimidazoles has not previously been studied.

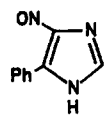
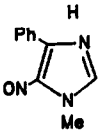
Detection of the radical anions of nitrosoimidazoles using e.s.r. spectroscopy

We have previously shown[5] that electron-capture by 2-, 4-, and 5-nitroimidazoles can be successfully studied using temperature resolved e.s.r. spectroscopy and the extension of this technique to nitrosoimidazoles is now reported. In these studies, electrons are generated using ionising radiation, and electron-capture resulting in stable adducts can be achieved using solid solutions at low temperature (77 K) to ensure that primary products are stabilised by immobility. Methanol (usually CD_3OD) and methyltetrahydrofuran (MeTHF) are ideal solvents for observing electron-capture by solutes. They give glassy solutions at 77 K, so that phase separation is avoided. The electron-loss centres are stabilised as solvent radicals, and electrons are able to migrate to solute molecules with little competition from the solvent. The technique is fully described in the literature.[9]

1-Methyl-5-nitroso-4-phenylimidazole and 4-nitroso-5-phenylimidazole undergo electron capture in both CD_3OD and MeTHF to yield stable radical anions at 77 K. Yellow coloured solutions were obtained indicating that the nitrosoimidazoles are good electron scavengers. In the absence of solute the glasses are deep violet from trapped electrons. On annealing to *ca.* 140 K no new features were observed in the e.s.r. spectra indicating stability between 77 and 140 K in solid matrices. The data for the radical anions are presented in Table 1 and a representative spectrum in figure 1.

These anions are expected to have a normal π-type SOMO with the O=N- unit coplanar with the ring. This structure is the same as that for $PhN\dot{O}^-$ radical anions,[10,11] and is expected on theoretical grounds. It contrasts with the SOMO for the cation $PhN\dot{O}^+$ which is largely confined to the in-plane NO orbital which, because of the non-linearity of the C-$\dot{N}^{\pm}$O unit has a lot of 2*s* character on nitrogen.[12]

TABLE 1: E.s.r. spectroscopic data for the nitrosoimidazole radical anions

	Solvent	Nucleus	A/G[a] $A_{//}$	$A_{\perp}$	A_{iso}[b]	$g_{\perp}$	$g_{//}$
	CD_3OD	^{14}N	26.5	≤ 7	8.8	ca. 2.0045	2.002
	MeTHF		25	≤ 7	8.3	ca. 2.0045	2.002
	CD_3OD	^{14}N	26	≤ 7	8.7	ca. 2.0045	2.002
	MeTHF		24.5	≤ 7	8.2	ca. 2.0045	2.002
$(PhNO)^{-\cdot}$ [c]	CD_3OD	^{14}N	26	≤ 7	≈ 8.1		
	MeTHF		26	≤ 7	≈ 8.1		

[a] 1 G = 10^{-4}T. [b] Using $A_{\perp} \approx 0$ which is normal for π-radicals.
[c] 3 H (ave) ≈ 3.8 G. [A_{iso} = 7.90 G and 4 H = 2.90, 2.83 (o), 1.01 (m), and 3.90 (p)][10]

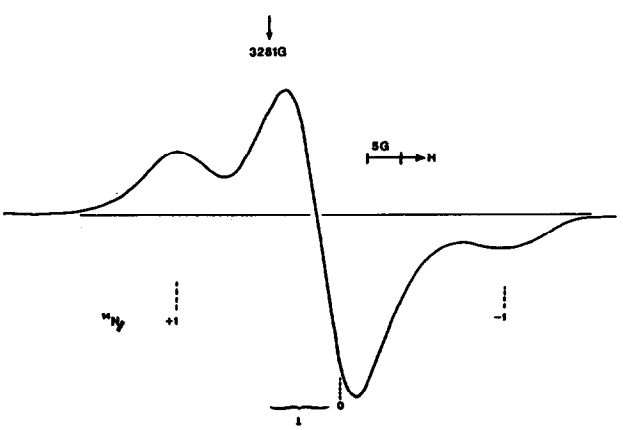


Figure 1
First derivative X-band e.s.r. spectrum for a dilute solution of the radical anions of 1-methyl-4-phenyl-5-nitrosoimidazole in MeTHF after exposure to ^{60}Co γ-rays at 77 K, showing features assigned to the radical anions.

Because $A_{\perp}$ (^{14}N) is small and the lines are broadened by unresolved hyperfine splittings, we cannot estimate $A_{\perp}$ values except to say that they are relatively small. In Table 1 we have set $A_{\perp} = 0$, which is usually a fair approximation for ^{14}N in π-radicals. The resulting values for A_{iso} are close to those quoted for $PhNO^{\cdot-}$ radicals (8 G).[10]

These numbers give values of *ca.* 13 G for the anisotropic coupling, 2 B. Comparing that with an estimate for unit population (33 G)[13] gives a spin density on nitrogen of *ca.* 39% for these radical anions. It seems that the two types of anion may have very similar SOMOs as far as the NO unit is concerned.

It is noteworthy that the radical anions of 4- and 5-nitroimidazoles[5] also have almost equal spin-densities on the $-NO_2$ units. One difference between these compounds (RNO and RNO_2) is that on electron addition, the RNO molecule probably undergoes no major change in shape. However, the relaxed shape of $RNO_2^{\cdot-}$ radical anions is pyramidal at the $NO_2^{\cdot-}$ unit. This may help to explain why the rates of electron transfer with relatively poor donors is much slower for the nitro-derivatives.[11,14]

Redox reactions between nitrosoimidazoles and thiols

We also sought to discover which reactions involving the nitrosoimidazoles and their radical anions may be of significance to the mode of action. Thiols, *e.g.* glutathione, are known to inhibit the antimicrobial activity of, but react very slowly with, nitroimidazoles.[7] We have shown that sample thiols, with pK_a values similar to those for SH groups in biological systems, are rapidly oxidised to disulphides by nitrosoimidazoles (see Table 2). The redox reactions, monitored by t.l.c., were complete within 10 min at room temperature and exhibited a 1:1 stoicheiometry of thiol:nitrosoimidazole.

A putative mechanism for the redox reaction is shown in equations (2)-(4). The key step is the single electron transfer between thiolate and the nitrosoimidazole yielding a nitroso radical anion. The initial product of reduction, the nitroso radical anion, is probably further reduced. A number of coloured products were observed but not isolated. The redox reaction proceeds at similar rates for 4- and 5-nitrosoimidazoles. This links in with the similar e.s.r. spectra of the respective radical anions.

2-Methyl-3-nitrosoindole also efficiently oxidised thiols to disulphides, indicating that the redox reaction is general for aromatic nitroso compounds. The much higher rate of oxidation of thiols by nitrosoimidazoles than by nitroimidazoles was unexpected, but the data in the literature[11,14] clearly shows that nitrosobenzene is a much superior oxidant to nitrobenzene. Addition at the C-2 position, which takes place slowly for water and MeOH,[15] was not observed (*i.e.* $k_{s.e.t.} >> k_{addition}$).

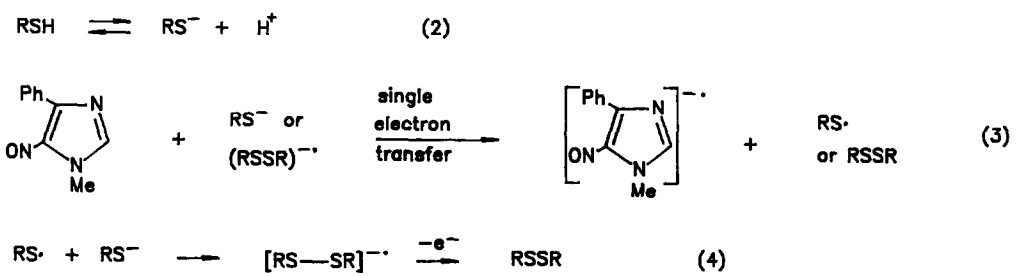


TABLE 2. Oxidation of thiols to disulphides by nitrosoimidazoles

Oxidant	Thiol	Solvent	% Yield of Disulphide[a]
1-Methyl-5-nitroso-	$HSCH_2CO_2Et$	Et_2O, MeOH	53, 84(36)
4-phenylimidazole	N-acetylcysteine	MeOH	79
	p-Cl-C_6H_4SH	Et_2O	b
4-Nitroso-5-phenylimidazole	$HSCH_2CO_2Et$	Et_2O, MeOH	(92); 61(61)
	N-acetylcysteine	MeOH	b
	p-Cl-C_6H_4SH	MeOH	b
1,2-Dimethyl-5-nitroimidazole	$HSCH_2CO_2Et$	Et_2O; MeOH	0[c]
2-Methyl-3-nitrosoindole	$HSCH_2CO_2Et$	MeOH	100
	N-acetylcysteine	MeOH	53

[a] % Yield based on thiol. The yields were calculated using n.m.r. spectroscopy with an internal standard. The yields in parenthesis are of pure isolated material. [b] % Yield not calculated, disulphide was the only RS-product. [c] No RSSR after 2 days.

Our results indicate that the radical anions of nitrosoimidazoles are "stable" and are possible reactive intermediates in the mechanism of action of the antimicrobial activity and the radiosensitisation in chemotherapy of nitroimidazoles. The rapid redox reaction between nitrosoimidazoles and thiols provides an explanation for the inhibition of nitroimidazole activity by thiols and may provide support to the theory that the mechanism of action is due to interaction with thiols rather than with DNA.[15]

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