Radical-Nucleophilic Substitution ($S_{RN}1$) Reactions: Electron Spin Resonance Studies of Electron Capture Processes. Part 6.[1] Nitroimidazole Derivatives

Martyn C. R. Symons *
Department of Chemistry, The University, Leicester LE1 7HH
W. Russell Bowman *
Department of Chemistry, University of Technology, Loughborough, Leicestershire LE11 3TU

Exposure of dilute solutions of a range of nitroimidazole derivatives [(**1**)—(**5**), (**8**)—(**12**), and (**14**)] in methanol (CD_3OD) or methyltetrahydrofuran to ^{60}Co γ-rays at 77 K gave the corresponding radical anions, detected by e.s.r. spectroscopy. Analysis of the results shows that the radical anions of 4- and 5-nitroimidazoles [(**8**) and (**9**), and (**1**)—(**5**), respectively] have remarkably similar SOMOs, with *ca.* 46% of the SOMO localised on the nitrogen atom of the nitro group and *ca.* 20% on the adjacent CH units. Spin densities on the two ring nitrogen atoms and on C-2 are low. In contrast, the spin density on the nitro nitrogen of the radical anions of the 2-nitroimidazoles (**12**) and (**13**) is only *ca.* 34%, and is very low at both methine sites.

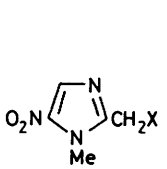
For the 4-nitro derivatives, when the *N*(1)-substituent was $-C(Me)_2NO_2$, the radical $Me_2\dot{C}NO_2$ was a major product. When the *N*(1)-substituent was *p*-nitrobenzyl, electron capture occurred preferentially at the aryl ring.

For the 2-bromomethyl-5-nitro derivative (**2**) dissociative electron capture occurred as a major pathway, and, in this case, a small hyperfine coupling to the bromine nucleus was detected for the radical anion. When the C(2)-substituent was CH_2Cl, CH_2OH, or $CH_2\overset{+}{N}Me_3$ [(**1**), (**2**), or (**4**)], only the parent radical anion was observed: there was no loss of Cl^-, ^-OH, or NMe_3, respectively. The significance of these, and related liquid-phase data, is discussed in terms of $S_{RN}1$ reactions observed for some of these compounds. The results are also discussed in terms of the large differences in antimicrobial activity of 2-, 4-, and 5-nitro derivatives.

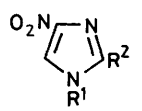
Nitroimidazoles are important antimicrobial drugs with a remarkable spectrum of activity.[2] They are now the main antibiotics used against anaerobic bacterial diseases and against a range of protozoal diseases such as giardiasis, trichomoniasis, and amoebiasis. They are also finding increasing use as radiosensitisers of hypoxic cell tumours.[2] The two market-leading derivatives are the 5-nitroimidazoles metronidazole (**14**) and tinidazole (**15**).

Despite intensive clinical studies, a detailed understanding of the antimicrobial activity of these and related derivatives is still lacking. It has been suggested[2-4] that the 5-nitroimidazoles are reduced by electrons donated by pyruvate/NADH *via* the hydrogenosomal enzyme pyruvate-ferrodoxin oxidoreductase, and that a reduced species attacks DNA causing loss of helical structure, strand damage, and consequent impairment of function as an enzyme template. Detection of the radical anions of nitroimidazoles by e.s.r. spectroscopy[5] in protozoal and anaerobic bacterial cells treated with nitroimidazoles suggests that the radical anions may be the reactive species. More information about the formation and properties of the radical anions of nitroimidazoles is therefore desirable.

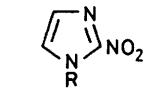
We are also interested in using e.s.r. spectroscopy to study intermediates in possible $S_{RN}1$ reactions. 2-Bromomethyl- and 2-chloromethyl-1-methyl-5-nitroimidazole [(**1**) and (**2**)] have been shown to undergo $S_{RN}1$ substitutions with nitronates[6] and nitroimidazole anions[7] [equation (1)]. These are of potential importance because 1-methyl-5-nitroimidazol-2-ylmethyl ($O_2NImidCH_2X$) derivatives exhibit activity against anaerobic bacteria.[2,8] Several derivatives, which include ronidazole (**6**) and pirinidazole (**7**), are used clinically. $S_{RN}1$ Reactions between nitronates and the analogous 'nitrobenzylic halides,' 2-halogenomethyl-5-nitrofurans,[9] *p*-nitrobenzyl and *p*-nitrocumyl halides,[10] and 2-(1-methyl-1-nitroethyl)-5-nitrothiophene[11] have also been reported.



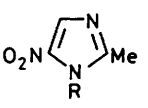
(**1**) X = Cl
(**2**) X = Br
(**3**) X = OH
(**4**) X = $\overset{+}{N}Me_3$
(**5**) X = H (dimetridazole)
(**6**) X = $OCONH_2$ (ronidazole)
(**7**) X = 2-pyridylthio (pirinidazole)



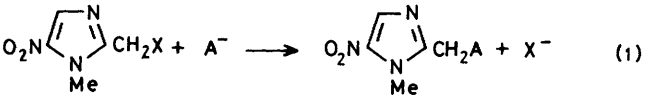
(**8**) R^1 = Me, R^2 = Me
(**9**) R^1 = CMe_2NO_2, R^2 = H
(**10**) R^1 = p-$O_2NC_6H_4CH_2$, R^2 = H



(**11**) R = H (azomycin)
(**12**) R = Me
(**13**) R = $CH_2CH(OH)CH_2OMe$ (misonidazole)



(**14**) R = CH_2CH_2OH (metronidazole)
(**15**) R = $CH_2CH_2SO_2Et$ (tinidazole)



(1)

X = Cl A = R_2CNO_2
X = Cl or Br A = 2- or 4-nitroimidazol-1-yl

$$RX + A^- \xrightarrow[h\nu]{\text{s.e.t.}} (RX)^{-\bullet} + A^\bullet \quad \text{Initiation} \quad (2)$$

$$(RX)^{-\bullet} \longrightarrow R^\bullet + X^- \quad (3)$$

$$R^\bullet + A^- \longrightarrow (RA)^{-\bullet} \quad \text{propagation} \quad (4)$$

$$(RA)^{-\bullet} + RX \xrightarrow{\text{s.e.t.}} RA + (RX)^{-\bullet} \quad (5)$$

Scheme. $S_{RN}1$ Mechanism (s.e.t. = single-electron transfer)

There is increasing evidence[10] supporting the $S_{RN}1$ mechanism (Scheme) for the substitution reactions of a wide range of compounds. E.s.r. spectroscopy has proved to be of use for detecting and identifying the radical anion and radical species postulated as intermediates. In initial studies using e.s.r. spectroscopy, one of us reported[12] electron capture by halogenoarenes to form the corresponding radical anions $(PhX)^{-\bullet}$, and their dissociation to phenyl radicals and halide anions, *i.e.* the first two steps of the aromatic $S_{RN}1$ mechanism [equations (2) and (3), with R = Ar and X = I or Br]. In subsequent papers we have extended the use of e.s.r. spectroscopy at low temperature to provide evidence for the $S_{RN}1$ reactions of 2-substituted 2-nitropropanes $[Me_2C(X)NO_2]$,[13] α-substituted 2-methyl-5-nitrofurans,[14] and *p*-nitrobenzyl and *p*-nitrocumyl derivatives (*p*-$NO_2C_6H_4CH_2X$ and *p*-$NO_2C_6H_4CMe_2X$).[15]

E.s.r. spectroscopy of matrix-isolated radical anions at low temperature has provided evidence for unstable species.[16] The low-temperature technique often allows the observation of first-formed species, and for strongly coupled nuclei anisotropic coupling constants are obtained which can be used to obtain reasonable estimates of spin densities. However, lines are broad and often smaller hyperfine splittings are not resolved. It has been established,[16] by using solvents such as CD_3OD and methyltetrahydrofuran (MeTHF), that the major reaction shown by solutes in dilute solution is electron capture.

A number of reports of e.s.r. spectra of 5-nitroimidazoles in liquid phase at room temperature have appeared.[5] These studies are largely concerned with the radical anions of metronidazole;[5a] only one paper has reported the e.s.r. spectra of nitroimidazole radical anions (metronidazole and misonidazole) at low temperature in solid matrices.[17]

Results and Discussion

Interpretation of E.s.r. Spectra.—In all cases, relatively well defined outer parallel features were observed, which were assigned as the $M_I(^{14}N) = \pm 1$ lines for the nitrogen atom of the nitro substituent [*e.g.* Figure 1 for the radical-anion of $O_2NImidCH_2OH$ (**3**)]. Generally, related perpendicular features (±1) were also observed though these were seriously overlapped by the more intense central line which comprises the $M_I(^{14}N) = 0$ component and, for CD_3OD systems, by solvent radical features. In several cases for the 4- or 5-nitro derivatives, the $M_I(^1H) = \pm 1/2$ components for the C(5) or C(4) ring protons were also resolved (*a ca.* 5 G).

For the $-CH_2Cl$ derivative (**1**) (Figure 2), the lines for the radical anions were broadened, but no coupling to chlorine nuclei was detectable. However, for the $-CH_2Br$ derivative (**2**) definite extra splittings of *ca.* 19 G were observed on the parallel $M_I(^{14}N) = \pm 1$ lines. This is certainly due to coupling to ^{81}Br and ^{79}Br, both of which have $I = 3/2$. We stress that this resolution arises primarily because of the larger magnetic moment for bromine relative to chlorine, though it may also imply greater delocalisation. This coupling is not the true 'parallel' coupling to the bromine since the ^{14}N coupling remains close to that for the other radical anions, and there is no requirement that $A_{||}(^{81}Br)$ lies along the $A_{||}(^{14}N)$ direction. Thus we are unable to estimate the extent of delocalisation onto

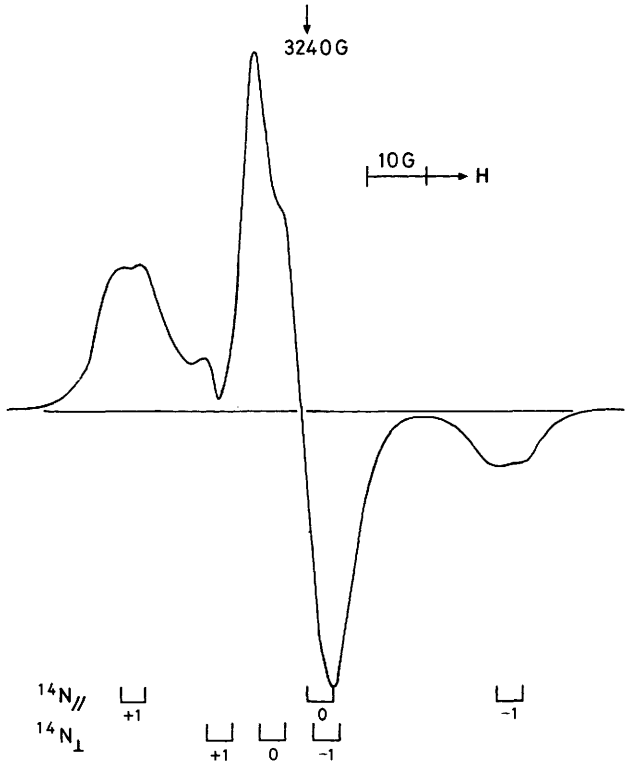


Figure 1. First-derivative *X*-band e.s.r. spectrum for a dilute solution of 2-(hydroxymethyl)-1-methyl-5-nitroimidazole (**3**) in MeTHF after exposure to ^{60}Co γ-rays at 77 K and annealing to remove solvent signals, showing features assigned to the corresponding radical anions. [Spectra for the nitroimidazoles (**4**), (**5**), (**8**), and (**14**) were almost indistinguishable from this spectrum.]

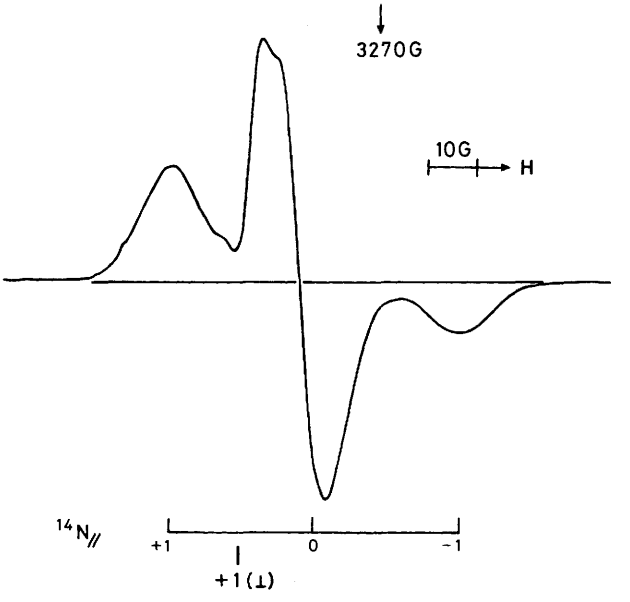


Figure 2. First-derivative *X*-band e.s.r. spectrum for a dilute solution of 2-(chloromethyl)-1-methyl-5-nitroimidazole (**1**) in MeTHF after exposure to ^{60}Co γ-rays at 77 K and annealing to remove solvent signals, showing features assigned to the corresponding radical anions

Table. E.s.r. parameters of radical anions formed by electron capture in the radiolysis of 2-, 4-, and 5-nitroimidazole solutions at 77 K

Substituents			Compound	Solvent	Nucleus	$A/G^{a,b}$ $A_{\parallel}$	$A_{\perp}$	A_{iso}	$2B^{c}$
5-NO_2	1-Me	2-Me	(5)	CD_3OD or MeTHF	^{14}N	31	8	15.7	15.3
5-NO_2	1-Me	2-CH_2Cl	(1)	CD_3OD	^{14}N ^{35}Cl	31.5	9	16.5	15.0
				MeTHF	^{14}N ^{35}Cl	30.5 0 ± 3	*d*		
5-NO_2	1-Me	2-CH_2Br	(2)	CD_3OD[e]	^{14}N ^{81}Br	31[f] 19[f]	*d*		
5-NO_2	1-Me	2-CH_2OH	(3)	CD_3OD	^{14}N ^{1}H	31.5 5	8	16.2	15.3
				MeTHF	^{14}N ^{1}H	31 5	*d*		
5-NO_2	1-Me	2-$CH_2\overset{+}{N}Me_3$	(4)	CD_3OD	^{14}N ^{1}H	32 5	9	16.7	15.3
				MeTHF	^{14}N ^{1}H	31 5	*d*		
5-NO_2	1-CH_2CH_2OH	2-Me	(13)	CD_3OD	^{14}N ^{1}H	32 5	7.5	15.7	16.3
4-NO_2	1-Me	2-Me	(8)	CD_3OD	^{14}N	32	8	16	16
				MeTHF	^{14}N	31	8	15.7	15.3
4-NO_2	1-CMe_2NO_2		(9)	CD_3OD[g]	^{14}N	32	*d*		
				MeTHF[g]	^{14}N	31	8	15.7	15.3
4-NO_2	1-(*p*-nitrobenzyl)		(10)	CD_3OD	^{14}N	29	*d*		
				MeTHF	^{14}N	28	*d*		
2-NO_2	1-H and 1-Me		(11), (12)	CD_3OD and MeTHF	^{14}N	25	9	14.3	
Liquid phase									
5-NO_2	1-CH_2CH_2OH	2-Me[h]	(14)	Pr^iOH	^{14}N ^{1}H	5.43		15.59	
4-NO_2[h]				Pr^iOH	^{14}N ^{1}H	5.37		15.97	
2-NO_2	1-$CH_2CH(OH)CH_2OMe$[h]		(13)	Pr^iOH	^{14}N ^{1}H			14.05	

[a] 1 G = 10^{-4} T. [b] In all cases, $g_{\parallel} = 2.002$, $g \simeq 2.006$. [c] $2B$ is the maximum value of the purely anistropic part of the hyperfine coupling. [d] Not resolved. [e] Only the outermost features detected for the radical anion; major species, $O_2NImid\dot{C}H_2$, $A(^1H)_{\parallel} \simeq 20$, $A(^1H)_{\perp} \simeq 12$, $A(^1H)_{iso} \simeq 15$. [f] If $A(^{14}N)_{\parallel} = 31$ then $A(^{81}Br)_{\parallel} \simeq 19$. [g] The radical $Me_2\dot{C}NO_2$ is also formed, $A_{Me} = 21$ G. [h] Ref. 20.

bromine. Nevertheless we conclude firmly that delocalisation onto bromine is significant.

On annealing to 170 K the radical anions for the $-CH_2Br$ derivative (**2**) gave way to features assignable to the corresponding radical, $O_2NImid\dot{C}H_2$. This loss of halide anions was not detected prior to total loss of radicals for the chloro derivative, despite the higher solvation energy for Cl^-. This again suggests that delocalistion of the SOMO onto chlorine is less than that onto bromine.

The radicals $O_2NImid\dot{C}H_2$ were clearly identified by their characteristic triplet e.s.r. features $A(^1H)_{\parallel} \simeq 20$ G, $A_{\perp} = 12$ G, whence $A_{iso} \simeq 15$ G. These results are very close to those for the analogous nitrofuran derivatives.[14]

The e.s.r. spectrum of the radical anion of 1-(1-methyl-1-nitroethyl)-4-nitroimidazole (**9**) (Figure 3) closely resembles that of 1,2-dimethyl-4-nitroimidazole (**8**). However, detection of the radicals $Me_2\dot{C}NO_2$ in the spectrum ($A_{Me} = 21$ G) suggests that electron addition to both nitro groups occurs, the latter giving only the dissociation product. No coupling to the second nitro group was observed.

In contrast, the spectrum of the radical anion of 1-(*p*-nitrobenzyl)-4-nitroimidazole (**10**) exhibits parallel features [$A(^{14}N) = 29$ (CD_3OD) or 28 G (MeTHF)] which correlates with coupling constants observed for the radical anions of nitrobenzene[18] and *p*-nitrobenzyl derivatives rather than 4-nitroimidazole radical anions. Thus the unpaired electrons reside on the aryl nitro groups rather than the imidazole nitro groups.

Only two derivatives with the nitro group on C(2) have been studied, *viz.* the 2-nitroimidazoles (**11**) and (**12**) (Table). However, the results show that there is a definite reduction in $A(^{14}N)_{\parallel}$, and to a lesser extent in $A_{\perp}$. No other splitting was observed.

Aspects of Structure.—Approximate 2*s* and 2*p* spin densities have been calculated from the isotropic and anisotropic ^{14}N coupling constants in the usual way[19] (Table), for those radical anions in which the perpendicular ^{14}N components are resolved. The 2*s* and $2p_z$ populations for 4- and 5-nitroimidazoles are very similar, ranging between 2.8 and 3.0% 2*s* character and between 45 and 48% 2*p* character, giving a total of nearly 50% spin density on nitrogen for both derivatives.

Similarly, when resolved, coupling to the adjacent C*H* was *ca.* 5 G, in agreement with liquid-phase data. Coupling to the ring nitrogen nuclei was not resolved, nor could we detect coupling to the C(2)-substituent nuclei except for α-bromine. Thus the SOMO is largely confined to the $-CH{=}C(NO_2)-$ moiety, the

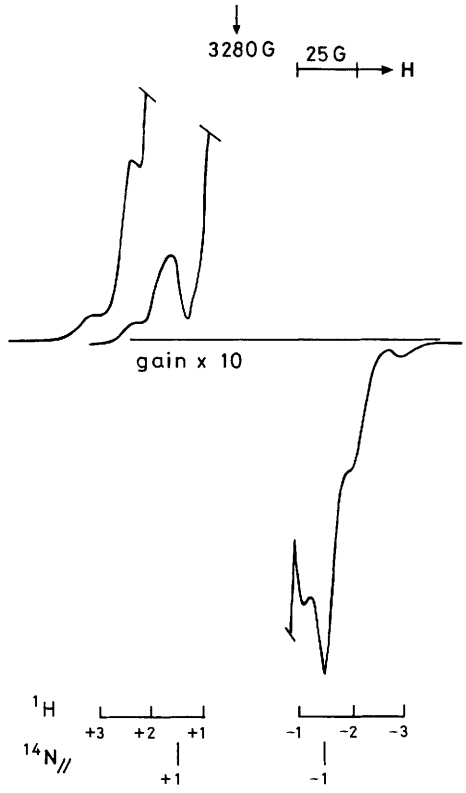


Figure 3. First-derivative *X*-band e.s.r. spectrum for a dilute solution of 1-(1-methyl-1-nitroethyl)-4-nitroimidazole (**9**) in CD_3OD after exposure to ^{60}Co γ-rays at 77 K showing outer features (^{1}H) assigned to $Me_2\dot{C}NO_2$ and parallel features ($^{14}N_{||}$) for the radical anion. The central region (not shown) is dominated by solvent radicals

estimated spin density on C(4) or C(5) being *ca.* 20%, and most of the remaining spin density (*ca.* 30%) being presumably on the two oxygen atoms.

The data from the e.s.r. spectra of the 2-nitroimidazoles (**11**) and (**12**) in solid matrices, coupled with the liquid-phase data for misonidazole,[20] show that there is a major reduction in spin density on the nitro nitrogen, but that this is dominated by a loss of 2*p* character. The spin densities at the two methine centres is small, but there is a small increase in spin densities on the two ring nitrogen atoms relative to those for the 4- and 5-nitro derivatives.

The coupling to bromine in $(O_2NImidCH_2Br)^{-\bullet}$ is relatively small, as was the case for $(p\text{-}O_2NC_6H_4CH_2Br)^{-\bullet}$.[15] For any coupling to be observed, the C–Br bond must overlap hyperconjugatively with the π-system, and bromine probably occupies the 90° out-of-plane site. As we have indicated for the analogous $(p\text{-}O_2NC_6H_4CH_2Br)^{-\bullet}$ and $(O_2NFurCH_2Br)^{-\bullet}$, this delocalisation is probably due to electron donation into the C–Br σ* molecular orbital rather than from the C–Br bond into the ring as observed for benzyl bromide radical cations.[21] The results (Table) suggest that the spin-density at C(2) is low in both the 4- and 5-nitroimidazole radical anions. Even the positive charge on the $\overset{+}{N}Me_3$ substituent makes very little difference to the SOMO. These results accord with liquid-phase data for the related radical anions. They suggest that for the 5-nitro radical anions the C(2) spin-density is only *ca.* 2% based on C–^{1}H coupling, whereas for the 4-nitro radical anions it is *ca.* 8% as judged from the C–CH_3 proton coupling. These results suggest that C(2)-halogenomethyl derivatives of the 5-nitro radical anions should undergo loss of halide anions even less efficiently than for the 4-nitro radical anions.

The results for the radicals $O_2NImidCH_2$ give a spin density on the methylene group of *ca.* 0.65 as judged from the isotropic proton hyperfine coupling. The values for analogous radicals are $O_2NFur\dot{C}H_2$ 0.56,[14] and $Ph\dot{C}H_2$ *ca.* 0.69. Thus these resemble benzyl radicals more than the closely related nitrofuran derivatives. ^{14}N Coupling was not resolved and therefore information about the structure of this radical could not be deduced.

The values obtained from the e.s.r. spectrum of the radical anion of 1-(*p*-nitrobenzyl)-4-nitroimidazole (**10**) indicated that this has a structure similar to those of other $(p\text{-}O_2NC_6H_4\text{-}CH_2X)^{-\bullet}$ systems,[15] with the spin density residing on the aryl *p*-nitro group and not the imidazole 4-nitro group.

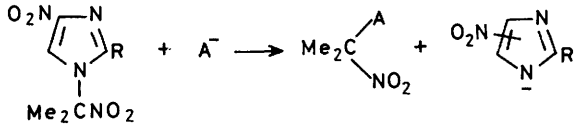
Dissociation of the Intermediate Radical Anions.—One of the key steps in the $S_{RN}1$ mechanism is the dissociation of the intermediate radical anions [Scheme, equation (3)]. Therefore any data obtained from the e.s.r. studies pertaining to this step provide useful evidence for this stage of the reaction. However, as in some of our previous work [1,14,15] the halogen derivative radical-anions are remarkably stable in the solid state despite the ease with which they dissociate in the liquid state. Thus in solid matrices only the bromo derivative undergoes dissociation. In the case of the corresponding nitrofuran derivatives, only the iodo radical-anion dissociated under our conditions. Also, we were unable to detect dissociation of $(p\text{-}O_2NC_6H_4CH_2Br)^{-\bullet}$ or $(p\text{-}O_2NC_6H_4CH_2I)^{-\bullet}$. These results contrast strongly with results for $Me_2C(X)\dot{N}O_2^-$, which was found to dissociate readily in the solid state. Indeed a proportion was already partially dissociated at 77 K.[13] These differences, which reflect the changes in spin density on the carbon atom bound to the $-CH_2Hal$ substituent, must also occur in the liquid-phase reactions, but we know of no evidence in support of this.

Studies[5b] of the e.s.r. spectrum of the radical anion of ronidazole (**6**) in liquid phase at room temperature indicate that the radical-anion is reasonably stable, suggesting that dissociation to $O_2NImid\dot{C}H_2$ and anions ($^-OCONH_2$) does not take place. The aminocarboxylate anion ($^-OCONH_2$) is a poor nucleofuge and therefore this result is not surprising. Also hydroxide anion or NMe_3 is not lost from the radical anion of the hydroxy (**3**) or $\overset{+}{N}Me_3$ derivative (**4**).

The lack of dissociation observed for the radical anion of 1-(*p*-nitrobenzyl)-4-nitroimidazole (**10**), $^-O_2\dot{N}C_6H_4CH_2X$ with X = 1-(4-nitroimidazole) is in keeping with the lack of dissociation observed[15] under these conditions for $^-O_2\dot{N}C_6H_4CH_2X$.

The 4-nitroimidazole analogue, the radical anion of 1-(1-methyl-1-nitroethyl)-4-nitroimidazole (**9**), does, however, exhibit dissociation to $Me_2\dot{C}NO_2$. We suggest that this observation can be explained by two different radical-anions being formed on electron capture by the imidazole (**9**): (*a*) a radical anion with the spin density on the imidazole 4-nitro group, which has little if any overlap into the aliphatic nitro moiety (dissociation of this radical anion is neither expected nor observed); and (*b*) a radical anion $Me_2C(X)\dot{N}O_2^-$ with X = 1-(4-nitroimidazole) in which the π* MO of the aliphatic nitro group overlaps with the C–N(1) σ* MO as observed in related species $Me_2C(X)\dot{N}O_2^-$.[12] This radical-anion was not observed in the e.s.r. spectrum but the detection of $Me_2\dot{C}NO_2$ suggests that the radical anion *is* formed, but dissociates even at 77 K to give $Me_2\dot{C}NO_2$. These results are in accord with the observed $S_{RN}1$ reactions[7] [equation (6)] for the 1-(Me_2CNO_2) derivative, *i.e.* loss of 4-nitroimidazolide anions rather than nitrite anions from the intermediate radical anions [(**9**)$^{-\bullet}$].

None of the 2-, 4-, or 5-nitroimidazole radical-anions observed by e.s.r. spectroscopy dissociated to give species which suggested that loss of nitrite anions had occurred. In fact, the matrix containing the radical anions of 1,2-dimethyl-4-



(6)

(9) R = H, A = Me_2CNO_2 R = Me, A = Me_2CNO_2 or 4-nitroimidazol-1-yl

nitroimidazole retained the signals even after being warmed to liquid phase at room temperature and then refrozen to 77 K; this is a very unusual observation in irradiation studies. To our knowledge that are no reports of the dissociation of the radical anions $ArNO_2^{-\bullet}$ to aryl radicals and nitrite anions, supporting our observations for 2-, 4-, and 5-nitroimidazoles. This contrasts with the ready loss of nitrite anions from certain aliphatic nitro derivatives.

Greater dissociation is observed in CD_3OD, as compared with MeTHF, as reported for related systems.[1,13–15] This is readily understood in terms of the greater anion-solvating ability of the alcohol.

As already reported, the results establish that there is significant overlap between C–X σ* orbitals and the imidazole/nitro π* orbitals in $(O_2NImidCH_2X)^{-\bullet}$. As for the related $(O_2NFurCH_2X)^{-\bullet}$ and $(O_2NC_6H_4CH_2X)^{-\bullet}$ systems,[1,14,15] we suggest that dissociation of the radical anions $O_2NImidCH_2X)^{-\bullet}$ proceeds *via* smooth reorganisation of molecular orbitals to the required transition state for loss of halide anions; and that two separate species, a σ* species $(O_2NImidCH_2^{\bullet}X)^-$, which dissociates directly, and a π* species ($^-O_2\dot{N}ImidCH_2X$), which is more stable, are simply valence-bond forms of the actual structure.

Biological Activity.—The e.s.r. spectra of the radical-anions of the 4- and 5-nitroimidazoles indicate that they have similar structures and distributions of spin-density, whereas the e.s.r. spectra of the radical anions of the 2-nitroimidazoles indicate a different structure and spin-density distribution. Similar biological activity might therefore be predicted for 4- and 5-nitroimidazoles and different activity for the 2-nitroimidazoles, if the radical anions are key intermediates in their radiosensitising and antimicrobial activity.

In fact, the compounds exhibit large differences in antimicrobial activity against anaerobic organisms.[2] The 2-nitroimidazoles are most active but are not significantly more active than the 5-nitroimidazoles. The 4-nitroimidazoles, however, are almost inactive. Our observations therefore suggest that the differences in biological activity cannot be readily explained by differences in radical anion structure, and that other factors should be considered. Differences in reduction potentials have been widely proposed[2,22] to explain the low reactivity of the 4-nitroimidazoles relative to the 2- and 5-nitro derivatives. Representative data[2,22] support these proposals: *e.g.* $E_{\frac{1}{2}}$ values for misonidazole (**13**) −272 mV, azomycin (**11**) −375 mV (2-nitroimidazoles), metronidazole (**14**) −382 mV (a 5-nitroimidazole), and 4-nitroimidazole −540 mV. Also, if the intermediates responsible for the antimicrobial activity are the hydroxylamines[2] as opposed to the radical anions, the difference in activity may be due to differing DNA reactivity amongst 2-, 4-, and 5-hydroxyamino-imidazoles.[2–4]

2-Nitroimidazoles are more efficient radiosensitisers than the 5-nitroimidazoles for both hypoxic bacterial and mammalian cells.[20] It has been suggested[20] that the difference in activity is due to the stronger electron affinity of 2-nitroimidazoles relative to 5-nitroimidazoles. The difference in structure and spin density of the radical anions of the 2- and 5-nitroimidazoles as indicated by the e.s.r. spectra and the difference in reduction potentials support this hypothesis. Metronidazole (**14**) and misonidazole (**13**) appear to act as protection agents of DNA exposed to γ-rays *in vitro*,[17] suggesting that some further reduced species (*e.g.* the hydroxylamine) is responsible for the radiosensitisation, or that there is a more specific interaction involving the repair mechanism.

The $O_2NImidCH_2X$ analogues (**1**)—(**7**) show similar antimicrobial activity[8] and their radical-anion structures are also similar. Thus the nature of the substituent X is not important. The ability of $(O_2NImidCH_2X)^{-\bullet}$ to dissociate does not appear to be important to the reactivity, suggesting that the 5-nitroimidazole radical anion is the biological intermediate, rather than the radical $(O_2NImid\dot{C}H_2)$ formed by loss of halide ion. These observations are important to the understanding of the mode of action of ronidazole (**6**) and pirinidazole (**7**), which have clinical application.

In conclusion, our results provide evidence for the structures of the radical anions of a range of nitroimidazoles. These radical anions are intermediates in both the radiosensitizing and the antimicrobial activity of these compounds.

Experimental

E.s.r.—Degassed samples were irradiated in dilute solution (*ca.* 1% v/v) in CD_3OD or MeTHF. Samples were frozen to small beads in liquid nitrogen and irradiated at 77 K in a Vickrad ^{60}Co γ-ray source with doses up to 1 Mrad. E.s.r. spectra were measured with a Varian E109 spectrometer. Samples were annealed to selected temperatures or until changes occurred in the e.s.r. spectra, and recooled to 77 K for study. For the CD_3OD systems solute radicals were detectable at 77 K, but for the MeTHF systems it was necessary to anneal until the solvent radicals features were lost before well defined solute features were observed.

Materials.—Metronidazole was purchased from Aldrich Chemical Co. 1,2-Dimethyl-4-nitroimidazole and 1,2-dimethyl-5-nitroimidazole were prepared by *N*-methylation of 2-methyl-4(5)-nitroimidazole.[22] 2-(Chloromethyl)-1-methyl-5-nitroimidazole, 2-(bromomethyl)-1-methyl-5-nitroimidazole, 2-(hydroxymethyl)-1-methyl-5-nitroimidazole, 1-methyl-5-nitroimidazol-2-yl(trimethyl)ammonium chloride, 1-(methyl-1-nitroethyl)-4-nitroimidazole, and 1-(*p*-nitrobenzyl)-4-nitroimidazole were prepared by literature procedures.[7] 1-Methyl-2-nitroimidazole was prepared by methylation of 2-nitroimidazole using diazomethane.[23] 2-Nitroimidazole was prepared by the literature procedure.[24]

Acknowledgements

We thank Lorraine Harris for measuring the e.s.r. spectra, Adelaide Adebayo and David Bowen for the preparation of the compounds, and Dr. W. G. Salt for discussions.

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Received 10*th August* 1987; *Paper* 7/1473