Synthetic and antimicrobial studies of nitroimidazole analogues

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SYNTHETIC AND ANTIMICROBIAL STUDIES OF
NITROIMIDAZOLE ANALOGUES

by

ADELAIDE TITILAYO OLURONKE MORENIKE ADEBAYO
B.Sc., M.Sc.

A Doctoral Thesis

Submitted in partial fulfilment of the requirements
for the award of
Doctor of Philosophy of the
Loughborough University of Technology

March 1988

Supervisors: W.R. Bowman, B.Sc., Ph.D.
W.G. Salt, B.Sc., Ph.D.
Department of Chemistry

by Adelaide Titilayo Oluronke Morenike Adebayo, 1988
DEDICATION

For my dear grandmothers
ACKNOWLEDGEMENTS

It is a pleasure to thank the academic and technical staff of the Chemistry Department of Loughborough University for their help and guidance throughout the course of this work. I also thank Professor M.C.R. Symons of Leicester University for results obtained by e.s.r. spectroscopy; and May and Baker Co.Ltd., Dagenham, England for the gift of metronidazole.

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I am very grateful to Mr A. Oyelowo for proof reading the manuscript.

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Finally, I am very grateful to my parents, my sister, brother, Mr A. Oyelowo and Mrs F.B. Adetoro for moral support.
SUMMARY

New 2-, 4- and 5-nitroimidazole analogues were synthesised using the following methods:

i) $S_{RN1}$ free radical reaction between nitroimidazole anion and halonitro substrates

ii) oxidative addition of the anion of 2-nitropropane to nitroimidazole anions. N-1-Alkyl derivatives of imidazole, benzimidazole, 5-nitrobenzimidazole, 5- and 6-nitroindazole were similarly prepared. These compounds showed antimicrobial activity; anaerobes were more sensitive than aerobes.

In vitro mode of action studies of 1-(1-methyl-1-nitroethyl)-4-nitroimidazole and 1-(methyl-1-nitroethyl)-2-methyl-4-nitroimidazole showed that the radical anions lose nitroimidazol-1-yl anion rather than nitrite anion.

A range of $\alpha$-substituted 1,2-dimethyl-5-nitroimidazoles \([N1-CH_2-X\text{ with }X = H, Br, Cl, OH, \text{Me}_3, \text{Me}_2\text{N(CH}_2)_2\text{CH}_3, \text{SPh, (pyrimid-2-yl)-S, 4(5)-nitroimidazol-1-yl]}\) and $\alpha$-substituted 1-methyl-2-isopropyl-5-nitroimidazoles \([N1-C(\text{Me}_2)X\text{ with }X = H, Br}\) were synthesised.

In vitro studies on N1-CH2-X showed:

i) Non chain redox reaction with thiolates and nitronates for X = (pyrid-2-yl)-S, (pyrimid-2-yl)-S, SPh, OCONH2, H.

ii) $S_{RN1}$ reactions with the anion of 2-nitropropane and 4(5)-nitroimidazole.

iii) $S_{N2}$ reaction with thiolates, for X = Br, Cl.

iv) Anaerobic bacteria were more sensitive than the aerobes tested, and the order of activity was $X = OH \geq HBr > Cl > \text{Me}_2\text{N(CH}_2)_2\text{Me} > \text{Me}_3$.

Electron spin resonance spectroscopy (carried out in collaboration with Prof. Symons, Leicester University) was used to determine the structure of the radical anion of the nitroimidazoles, N1-CH2-X with (X = Cl, Br), 1-(1-methyl-1-nitroethyl)-4-nitroimidazole, and 1-(p-nitrobenzyl)-4-nitroimidazole, and their pattern of dissociation to
radicals and anions.

Polarographic reductions of 1-(p-nitrobenzyl)-2-nitroimidazole and 1-methyl 2-(2-nitroimidazol-1-yl-methyl)-5-nitroimidazole in the presence and absence of DNA were carried out and the polymer viscosity assessed.
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**PART A**

**CHAPTER 1**

**INTRODUCTION**

The interest in nitroimidazoles as chemotherapeutic agents began in 1955 with the elucidation of the structure of azomycin by Nakamura\(^1\) and the subsequent discovery of the in vivo activity of 1-(hydroxy-ethyl)-2-methyl-5-nitro-imidazole (metronidazole) against *Trichomonas vaginalis* in 1961.\(^2\) Since then, an ever growing number of 2-, and 5-nitroimidazoles have been synthesised. The market leaders and some of those introduced into clinical evaluation include (5-nitro-): metronidazole, dimetridazole, ronidazole, ipronidazole, tinidazole, *and* (2-nitro): benznidazole and misonidazole (structures [1-7]). Current clinical use of these drugs are listed in Table 1.1.

![Chemical structures of nitroimidazoles](image)

The activity of the nitroimidazoles is largely limited to anaerobic organisms though *Giardnerella vaginalis* is killed by a metabolite of metronidazole.\(^3\) In general, both
Table 1.1 Clinical uses of Nitroimidazole Drugs in Humans

<table>
<thead>
<tr>
<th>Drug-type</th>
<th>Disease</th>
<th>Causative Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Nitroimidazole</td>
<td>Trichomoniasis, vaginitis</td>
<td><em>Trichomonas vaginalis</em> (protozoan)</td>
</tr>
<tr>
<td>derivatives</td>
<td>Vincent's angina</td>
<td>various, <em>e.g.</em> <em>Bacteroides</em> (bacteria)</td>
</tr>
<tr>
<td></td>
<td>Amoebiasis</td>
<td><em>Entamoeba histolytica</em> (protozoan)</td>
</tr>
<tr>
<td></td>
<td>Post-operative sepsis</td>
<td><em>Clostridia, Bacteroides</em> (bacteria)</td>
</tr>
<tr>
<td></td>
<td>Gangarene</td>
<td><em>Clostridia</em> (bacteria)</td>
</tr>
<tr>
<td></td>
<td>Turkey, Poultry blackheads</td>
<td><em>Histomonas melagreadis</em> (protozoan)</td>
</tr>
<tr>
<td></td>
<td>Swine dysentry</td>
<td></td>
</tr>
<tr>
<td>2-Nitroimidazole</td>
<td>Chaga's disease</td>
<td><em>Trypanosoma cruzi</em> (protozoan)</td>
</tr>
<tr>
<td>derivatives</td>
<td>Hypoxic cell tumours</td>
<td></td>
</tr>
</tbody>
</table>

Gram positive and negative anaerobes are sensitive. Anaerobes are organisms capable of existence in either the total exclusion of oxygen (obligate anaerobes) for example *Clostridium tetani*, or occasional exclusion (facultative anaerobes) for example, *Clostridium histolyticum*. Although anaerobiosis is widespread in prokaryotic bacteria, it is by no means confined to them. A number of eukaryotic anaerobes (all symbionts in the digestive system of animals) are known among the protozoans, *e.g.* *T. vaginalis* and *Entamoeba histolytica*.

Anaerobic organisms cause a variety of disease processes in humans, *e.g.* trichomoniasis caused by the protozoan parasite, *T. vaginalis* (Table 1.1). Prior to 1961, vaginal trichomoniasis was widespread and the disease was commonly treated with vinegar or lactic acid douches or pessaries or methyl violet impregnated tampons. Such treatment was ineffective because the parasite often sheltered within mucous membranes or the confines of Skene's or Bartholin's glands, creating reservoirs which become inaccessible to these topical agents. Re-infection of the vagina was thus common. Metronidazole [1] was an important...
chemotherapeutic advance because it could be used systemically and was clinically effective.

Histomoniasis is a poultry disease caused by the protozoan parasite *Histomonas meleagridis*. It is a serious economic problem to the turkey-raising industry. A variety of 5-nitroimidazoles such as dimetridazole [2], ipronidazole [4] and ronidazole [3] have proved very effective as chemotherapeutic agents.

A number of 5-nitroimidazoles have also found widespread use in the treatment of post-operative sepsis caused by Bacteroides and clostridium species. Benznidazole [7] is one of the most effective drugs for the treatment of *Trypanosoma cruzi* infection (Chaga's disease).

Although several nitro compounds, such as p-nitroacetophenone and various nitrofurans are known radiosensitizers, it was the discovery of metronidazole [1] as a radiosensitizer which boosted research in this field of radiobiology. Its ability to selectively radiosensitize hypoxic cells in vitro led to a radiotherapy trial, though 2-nitroimidazoles seem better radiosensitizers of hypoxic cells in cancer therapy.

Structure Activity Relationships in Nitroimidazoles

Despite dissimilar side chains, the activity observed in nitroimidazoles suggests a common nucleus for activity. The simplest structural unit common to all biologically active nitroimidazoles is the 1-alkyl-5-nitroimidazole nucleus (Compound [8] with $R^1 = R^2 = H$, $R^3 = CH_3$). This implies that biological inactive 5-nitroimidazole deriv-
reduction potentials, and subtle differences in structure and conformation may be of particular importance in predicting biological activity.\textsuperscript{14,15}

Butler \textit{et al}\textsuperscript{13} reported that lipophilicity in the alkyl groups of 5-nitroimidazoles, [8], affected transport phenomenon, and/or fit to receptor, and metabolism, but they found steric effects an overriding factor for activity. Electronegative groups present in R\textsuperscript{3} augment potency\textsuperscript{13,14,16-18} especially when separated by more than one carbon atom from the imidazole ring (for example, metronidazole). Conversely when R\textsuperscript{3} \neq H, an R\textsuperscript{2} group larger than methyl results in loss of activity,\textsuperscript{13} perhaps due to over-crowding around the nitro group which is essential for activity.\textsuperscript{13}

Substitution in the 2-position with a methylene group and an electronegative atom ([8] with R\textsuperscript{1} = -CH\textsubscript{2}X) is reported\textsuperscript{17} to present ideal conditions for activation and metabolism. Likewise, when R\textsuperscript{1} is a branched chain, an increase in biological activity\textsuperscript{19} is observed (for example, ipronidazole). However, when the electronegative atom or group is an ester, (R\textsuperscript{1} = CH\textsubscript{2}X, with X = CO\textsubscript{2}R) the bond is too readily metabolised for any activity.\textsuperscript{17} Similarly, nitroimidazoles with heteroatom substitution at C-2, e.g. R = OH have no significant \textit{in vivo} activity.\textsuperscript{17c}

Satranidazole [9] is a recent and novel 5-nitroimidazole possessing a methylsulphonylimidazolidinone group at the C-2 position with a C-N bond at C-2. This may be relevant to its activity which is reported to be superior to metronidazole and other 5-nitroimidazoles for amoebiasis.\textsuperscript{5,20}

Another approach to the observed SAR of nitroimidazoles is the \textit{in vitro} antimicrobial testing against a range of anaerobic and aerobic organisms. The results from the antimicrobial testing are reported as the minimal inhibitory concentration (MIC) of the drug required to inhibit or kill the particular strain of micro-organisms under investigation. The lower the MIC value, the more potent the drug for that strain. Table 1.2 represents the sensitivities of some anaerobic and aerobic organisms to metronidazole.
Table 1.2 The Sensitivities of Anaerobic and Aerobic Organisms in vitro to metronidazole

<table>
<thead>
<tr>
<th>Anaerobic organism</th>
<th>MIC(µg/ml)</th>
<th>Aerobic Organisms</th>
<th>MIC(µg/ml)</th>
</tr>
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<tr>
<td>Clostridium tetani</td>
<td>0.5</td>
<td>Staphylococcus aureus (Gram +ve)</td>
<td>32</td>
</tr>
<tr>
<td>Cl. septicum</td>
<td>0.5-1.0</td>
<td>Bacillus cereus (Gram +ve)</td>
<td>32</td>
</tr>
<tr>
<td>Cl. sporogenes</td>
<td>0.06</td>
<td>Escherichia coli (Gram -ve)</td>
<td>250</td>
</tr>
<tr>
<td>Cl. histolyticum</td>
<td>1.0-2.0</td>
<td>Salmonella typhimurium (Gram +ve)</td>
<td>32</td>
</tr>
<tr>
<td>Bacteroides species</td>
<td>2.0</td>
<td>Pseudomonas aeruginosa</td>
<td>1000</td>
</tr>
<tr>
<td>Fusobacterium fusiforme</td>
<td>0.12-0.25</td>
<td>Trypanosoma brucei (protozoa)</td>
<td>500</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. foetus (protozoa)</td>
<td></td>
<td></td>
<td></td>
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Mode of Action of Biologically Active Nitroimidazoles

The spectrum of activity of nitroimidazoles is unique in that it encompasses both Gram positive and Gram negative bacteria, protozoa, a few nematodes and hypoxic tumour cells. However, these nitroimidazoles are only selectively toxic to those organisms which are anaerobic or capable of anaerobic metabolism. Anaerobes are different from aerobes in their biochemistry of respiration in several important respects. Evolution of hydrogen gas is the result of electron transport mechanisms in the absence of oxygen as the final electron acceptor, and is characteristic of both anaerobic protozoa and bacteria. Consequently, redox mechanisms in anaerobes occur at a much lower (more negative) potential than in aerobes. Edwards et al. found
that metronidazole [1] specifically inhibited hydrogen output though not carbon dioxide in *T. vaginalis* ²², ²³ and in bacteria, ²⁴ and concluded that the mechanism of hydrogen output in *T. vaginalis* is a modified pyruvate dehydrogenase reaction, the clostridial-type pyruvate phosphoroclastic reaction (scheme 1.1). The inhibition point is now accepted to be the reduced electron transfer point ferridoxin in bacteria or its equivalent in protozoa which has a redox potential of −470mV, far lower than that in aerobic cells. The inhibition is directly related to reduction of the nitro group of nitroimidazole; ²⁴ the latter acting as an electron sink accepting electrons from reduced ferridoxin by the nitro group. ²² The inhibition was found to be transient in clostridia ²⁵ and in protozoa, ²⁶ and once all the drug was reduced, the phosphoroclastic reaction recovered. Therefore, it was assumed that the compound responsible for cell death was a reduction product of the drug, but which exerts effect at some other site in the cell. ²⁷

The nature of the biologically active reduced species is still unknown, but reaction appears essential for antibacterial damage to DNA and in radiosensitization of hypoxic cells. ²⁸ It creates a favourable concentration gradient for the passive uptake of the drug from the environment into the cell. ²⁷

Electrochemical reduction of nitrocompounds is known to produce a one-electron reduction product, the nitro radical-anion. ²⁹ However, by analogy to nitrobenzenes, and based on the evidence obtained from a variety of chemical, radiolytic, electrolytic and enzymic reduction systems, reduction of nitroimidazoles proceeds stepwise and involves formation of reactive radical-anion [10], nitroso, hydroxylamine and amine derivatives via one, two, four and six electron reductions respectively. ²⁹, ³⁰ This is generalized in scheme 1.2. Whether all these intermediates occur in vivo has yet to be established. Recent studies ³¹, ³² have demonstrated the presence of the radical-anion intermediate in vivo. These include studies using electron spin resonance (e.s.r.) spectroscopy on nitroimidazoles in the cattle parasite, *T. foetus*, ³¹ and the measurement of the steady state
The pyruvate phosphoroclastic reaction.27

Scheme 1.1

(E-TPP is a membrane bound enzyme-thiamine pyrophosphate complex. ETP is a low redox electron transfer protein known to be ferridoxin in clostridia. The redox potentials in mV are given for ferridoxin and the hydrogenase reaction).

Scheme 1.2: Radical reduction of nitroimidazoles to aminimidazoles

intracellular levels of the radical-anions of metronidazole32 generated metabolically in T. vaginalis demonstrating radical-anion quenching by oxygen. The mammalian reductases have also been shown to be inhibited by oxygen, the effect of oxygen was shown to result from its reaction with the free radical-anion and not related to enzyme inhibition.30,33-34

The rate of appearance of the radical-anions of other nitrocompounds as diverse as ethyl p-nitrobenzoate, nitrofurazone
and niridazole is inhibited by oxygen.\(^{30,34}\) Inhibition in the presence of oxygen regenerates the parent compound and superoxide radical-anions are formed; the presence of the latter was confirmed by spin-trapping.\(^{35,36}\) The futile cycle of the radical-anion was also reported when metronidazole, reduced by an iron-cysteine system, was exposed to air.\(^{37}\) (Scheme 1.3)

\[
\begin{align*}
NADP^+ & \quad \text{F} & \quad \text{RNO}_2 & \quad \text{O}_2^2 \\
NADPH & \quad \text{F} & \quad \text{RNO}_2 & \quad \text{O}_2
\end{align*}
\]

Scheme 1.3: The futile cycle of oxygen reduction, NADPH oxidation, and superoxide formation catalysed by nitroaromatics.\(^{36}\)

Sulphhydryl compounds such as glutathione, cysteine and cysteamine are capable of protecting biological systems from the damaging effects of radiation.\(^{38}\) Whether sulphhydryl compounds act as radical scavengers, hydrogen donors or inhibit oxidative activation is not clear. However, prolonged exposure of mammalian cells to nitroimidazoles under hypoxic conditions leads to marked reduction of sulphhydryl levels.\(^{39,40}\) There has been considerable interest in the possibility that such loss plays a major role in the radiosensitizing and chemotherapeutic properties of the nitroheterocycles. A postulate is that the sulphhydryls react with the radical-anion, as presented in equation 1.1, thus protecting the cell from its presence.

\[
\text{R-NO}_2^\cdot + \text{R}^1\text{-SH} \rightarrow \text{RNO} + \text{R}^1\text{S}^- + \cdot\text{OH} \quad (1.1)
\]

**Effect of Reduced Nitroimidazoles on DNA**

The subsequent reaction site of the unquenched radical-anion (or other reduction species responsible for activity) is less clear but a hypothetical scheme involving binding of highly reactive intermediate(s) to macromolecules has been proposed.\(^{24,41}\) Evidence that metronidazole inhibited uptake of [\(^{14}\)C-labelled] thymine in *T. vaginalis* has been used to suggest that DNA was the site of action of the
It was also shown that radiation induced binding of the drug to DNA, but only under hypoxic or anoxic conditions. In vitro studies also confirmed this observation. One of these studies used an electrolytic technique by which the drug was reduced in the presence of DNA at a constant voltage related to drug redox potential. The drug's redox potential was assessed polarographically as $E_{1/2}$, the half-way potential, that is, the point at which the drug is 50% reduced. The voltage chosen for electrolytic reduction of the drug in the presence of DNA was lower (more negative) than the $E_{1/2}$. The degree of damage was measured as a marked decrease in the viscosity of DNA taken as indicating strand breakage or strand separation. If damage was by intercalation of the drug into DNA, or by helix-bending of the latter, then an increase in viscosity would have been obtained. Also intercalators cause an increase in the temperature of the mid-point helix of DNA (Tm) but a decrease was reported by Edwards et al. In a separate study, these authors showed thymine as the target of DNA damage supporting the work of Ings et al.

**Effect of Reduced Nitroimidazoles in Radiosensitization**

In addition to an ability to kill sensitive anaerobic organisms, metronidazole and other nitroimidazoles radiosensitize hypoxic cells of tumours and cancers.

Most anti-cancer drugs are designed to exploit the more rapid proliferation of tumour cells than normal cells, and are cytotoxic in certain phases of the cell cycle. Nitroimidazoles as tumour radiosensitizers are quite different since they have an equal sensitizing action on normal cells at any stage in their cell cycle. Their tumour-specific action relates to the presence of a large fraction of hypoxic cells in tumours, and not in normal tissues. Hypoxic cells develop in tumours because of the unbalanced growth of the tumour cells and of the vascular components needed to provide adequate blood supply and oxygen requirements. Such cells occur around the area of tumour necrosis and relatively resistant to radiation. These hypoxic cells may be sensitized to the killing effects of radiation by various chemicals, the largest and most important group being the electron-affinic nitro compounds. The sensitizing effic-
iciency is directly related to their electron-affinity or redox-potentials. The choice of compounds for in vivo use was constrained because the compound must be non-toxic. Nitrofurans were rejected on this basis. Metronidazole [1], and more recently misonidazole [6], are adjuncts to radiotherapy because they are small (for diffusion) and lack clinical toxicity. Misonidazole has superior radiosensitizing properties because it is more electron-affinic than metronidazole but the former is more toxic because it is more stable. Misonidazole and metronidazole are cytotoxic to hypoxic mammalian cells in the absence of radiation, indicating that the overall effect of the drugs as radiosensitizers includes a cytotoxic effect. More recent studies showed that the cytotoxic effect in hypoxic cells is identical to that in anaerobes.

The main function of an electron-affinic nitroimidazole radiosensitizer is to enhance or potentiate the damage fixation step and is reduced in the process to the one-electron radical anion $[\text{RNO}_2^-]$ which is responsible (or its further reduced equivalent) for cytotoxicity.

**Pharmacology and Metabolism of Nitroimidazoles**

Comparative studies have shown that almost all the nitroimidazoles can be administered orally (tablets), rectally (suppositories) and in urgent cases parenterally (intravenous injection). The two most investigated drugs metronidazole [1] and misonidazole [6], 5- and 2-nitro derivatives respectively, become distributed widely in the body, and the apparent volume of distribution is about 80% of the body weight for the metronidazole and 90% for misonidazole. The drugs penetrate well into the body tissues and fluid compartments with very little drug bound to plasma protein (1-11%). The elimination half-life of 8-9 hours for [1], misonidazole of 10-14 hours and for tinidazole, a half-life of 9-12 hours has been reported.

Metabolic studies have concentrated mostly on 5-nitroimidazoles and particularly on metronidazole. Metronidazole in humans and mice and in rats was shown to follow the pathway shown in scheme 1.4. The reports showed that the major metabolic pathway involved oxidation of the
hydroxy side chain in the N-1 position of metronidazole [1] to give carboxymethyl derivative [11]. A conjugate with glucuronic acid was also formed, possibly the adduct [15]. No evidence was reported of the reduction of the nitro group. Most of the drug (about 80%) is ultimately excreted in urine with only a small fraction (15%) being excreted as unchanged drug.

Metabolic studies of 2-nitroimidazoles are still very
scarce. Schwartz et al.\textsuperscript{56} reported that the preferred route of excretion was in the urine and that metabolites which had retained the nitroimidazole structure accounted for more than half of the radioactivity when C-2 labelled mis­
onidazole was used in their study.

**Toxicity Studies of Nitroimidazoles**

At the dosage levels used for the treatment of *T. vaginalis* infection, metronidazole has been well tolerated. The toxic effects induced by metronidazole [1] can be taken as a guide to the study of the toxicology of other 5-nitro­
imidazoles.\textsuperscript{57} The most common side effects involve the gastrointestinal tract, nausea, dryness of mouth, anorexia, diarrhoea, metallic taste and vomiting. As regards the central nervous system, dizziness, vertigo, (and, very rarely, ataxia and paresthesias) have been reported.\textsuperscript{58} In some individuals, the consumption of alcoholic beverages with metronidazole may produce a disulfiram-like effect. However, it was not found clinically useful in the treat­
ment of alcoholism, since co-administration with disulfiram can produce psychosis,\textsuperscript{59} the basis of which is unknown.\textsuperscript{59}

Metronizadole has been shown to be carcinogenic in mice and rats, and mutagenic in bacteria,\textsuperscript{60} but no tumours were observed in hampsters.\textsuperscript{61} Concern regarding possible genetic effects in humans increased when it was found that salmonella typhinurium showed a marked increase in mutation rate when exposed to urine from patients receiving metro­
nidazole in therapeutic dosage for the treatment of tricho­
oniasis.\textsuperscript{62} This observation taken together with other studies\textsuperscript{63} showed that the reduction products of metro­
nidazole are more likely responsible for mutagenic and carcinogenic effects observed in mice and bacteria. From these reports, it would seem that the use of metronidazole is accompanied by a theoretical risk to humans. There is, however, no direct evidence of carcinogenic or mutagenic effects in humans.

**Chemistry and Preparation of Some Therapeutically used Nitro­
imidazoles** — Part B

The chemistry of imidazoles is discussed first, fol­
lowed by the preparation of therapeutically useful nitro­
imidazoles.
Chemistry of imidazoles

Five-membered heterocycles containing two nitrogen atoms in positions one and three are known as 1,3-diazoles or imidazoles [16].

The aromaticity of imidazoles is due to contribution of one \( \pi \)-electron from each carbon and the 'pyridine' nitrogen and two from the pyrrole nitrogen to make up an aromatic sextet.

Diazoles are not as reactive as benzene or pyrrole. Although they undergo typically the same types of reactions, such as electrophilic aromatic substitution (halogenation, nitration, sulphonation, alkylation etc.), more vigorous reaction conditions are required. This reduced activity is due to the electronegative pyridine-type nitrogen atom (N-3).

Some of the chemical reactions of imidazole, and its derivatives (e.g. nitroimidazole) relevant to the course of study are discussed.

Tautomerism

When the N-H (amino-nitrogen) of imidazole is unsubstituted, the positions 4 and 5, are equivalent due to rapid tautomeric shift of hydrogen from one nitrogen to the other (equation 1.2). As a result of tautomerism, it is not possible to separate isomers of imidazole in which the 4, or 5- positions are substituted but not the amino nitrogen, (equation 1.3) although they are reported to enter into chemical reactions in one of the tautomeric forms.\(^6\)\(^4\)

The cations and anions of both isomers are also equivalent.

Various pieces of evidence support tautomerism in imidazoles. The proton \(^1\)H n.m.r. spectrum of imidazole shows a single peak for the 4- and 5- hydrogen atoms, indicating that they must be magnetically equivalent.
In neutral organic solvents, the base B in equation 1.2 could be another imidazole molecule such that an intermolecular process involving two or more imidazole molecules is set up. This large association of imidazole molecules (scheme 1.5) is hydrogen-bonded in such a way that fast proton exchange is possible. It is this rapid exchange which is responsible for the absence of spin-spin splitting with the NH proton in the $^1$H n.m.r. spectrum, and for the observation that this proton gives rise to a broad, distinct signal only in concentrated solutions in such solvents as benzene, chloroform and acetone. In protic solvents such as water, the solvent itself is involved as the base. The NH proton of imidazole can be shown to exchange with deuterium very rapidly in D$_2$O. The rate equation for tautomerism of imidazole has been shown to contain kinetic terms which correspond to catalysis by H$^+$, OH$^-$ (H$_2$O) and imidazolium cations. Thus, this prototropy makes the 4- and 5- positions magnetically and chemically equivalent.

Another way to study tautomerism is through pK$_a$ measurements. A comparison of the basic pK$_a$ values for 4(5)-
-nitroimidazole with those of 1-methyl-4- and 1-methyl-5-
nitroimidazole, [19] and [20] respectively, leads to the conclusion that the 4-nitro tautomer [17] predominates (scheme 1.6).

![Scheme 1.6](image)

The 4-nitro:5-nitro ratio has been reported as about 400:1. The influence of the methyl group on the tautomeric ratio is small by comparison of the pKₐ values for imidazole (ca.7.0) and 1-methylimidazole (ca.7.1), and hence 1-methyl-4-nitroimidazole resembles the major isomer.

In 2-nitroimidazole, only one compound is obtained because both 4- and 5- positions are equivalent.

The presence of prototropic tautomerism is also shown when an attempt is made to synthesize the two individual tautomers of a pair. Both synthetic pathways lead to the same compound (scheme 1.7), or to a mixture of products which behaves as if it was a single compound.

Other substituents favour one tautomeric form at the expense of the other. For example, 4 (or 5)-bromoimidazole exists mainly as the 4-bromo derivative [21] (Scheme 1.8).

![Scheme 1.7](image)
Evidence for this comes from examination of $^1$H n.m.r. spectra of deuterated derivatives in concentrated acidic medium. Such a medium converts imidazole into the cation which is able to undergo proton exchange only very slowly; coupling with the NH proton is observed in consequence. When 2-deuterio-4(5)-bromoimidazole [23] was treated with concentrated $D_2SO_4$, the product had a coupling constant of 2.7 Hz. 1,2-Deuterio-4(5)-bromoimidazole [24] in concentrated $H_2SO_4$ showed a smaller coupling constant of 1.9 Hz. On the basis of the assumption that a larger coupling constant is due to adjacent protons, and the smaller one is due to distance protons (cross-ring coupling), it was concluded that the compound exists as 4-bromoimidazole.

The observation that the coupling constants for 4(5)-fluoroimidazole correspond closely with those of 4-fluoro-1-methylimidazole suggests that in solvents of low polarity the 4-fluoro derivative is the dominant tautomer.

Although the prototropy between N-1 and N-3 of imidazole is normally very rapid, as it is with benzimidazole, it is possible to prepare benzimidazoles (of general struc-
ture [25]) in which the rate of prototropy has decreased to the extent that the two forms can be demonstrated. The

![Diagram of a molecule](image)

retarding effect described in this instance is ascribed to intramolecular hydrogen-bonding between NH and the carbonyl oxygen.\(^1\)

The related heterocyclic systems, pyrazole [26], indazole [27] (1,2-diazoles) and benzimidazole [28] also undergo tautomeric shift of hydrogen as represented in equations 1.4 - 1.6.

\[(\text{pyrazole [26]}) \leftrightarrow (\text{Indazole [27]}) \leftrightarrow (\text{Isoindazole})\]

\[(\text{Benzimidazole [28]}) \leftrightarrow (\text{Isoindazole})\]

When the NH group is substituted, e.g., 1-alkylimidazoles, then prototropic tautomerism is no longer possible. Thus methylation of 4-methylimidazole gives a mixture of two quite distinct compounds, 1,4- and 1,5-dimethylimidazole in about 50:50 ratio.

**Halogenation of Imidazole**

The effect of two nitrogen atoms in the five-membered
imidazole ring should result in more ready halogenation compared with benzene.\textsuperscript{69} Imidazoles are readily halogenated (e.g. \( \text{Br}_2\cdot\text{CHCl}_3 \) gives 2,4,5-tribromoimidazole\textsuperscript{72} in the absence of catalysts required for the halogenation of benzene). It is difficult to prevent bromination of all the vacant ring positions,\textsuperscript{73} and substituted imidazoles are similarly brominated by N-bromosuccinimide (NBS).

Bromocyanogen has been used to give a monobrominated imidazole \textsuperscript{[29]} (equation 1.7).

\[
\begin{align*}
\text{BrCN} & \quad \text{imidazole} \\
\text{imidazole} & \quad \text{BrCN}
\end{align*}
\]

(1.7)

However, various attempts to monobrominate in the 4-position have failed, e.g. 2,3,5,6-tetrabromocyclohexa-2,5-dienone\textsuperscript{72} gave a mixture of products, (equation 1.8).

\[
\begin{align*}
\text{imidazole} + \text{BrCN} & \quad \text{imidazole-BrCN-BrCN}
\end{align*}
\]

(1.8)

Iodine also readily reacts with imidazole in aqueous alkali solution to give 2,4,5-tri-iodoimidazole and 4,5-di-iodoimidazole (equation 1.8). The latter product has been wrongly assigned as 2,4-di-iodoimidazole.\textsuperscript{75} Mono-iodination at C-2 is obtained by lithiation using the 1-trityl analogue of imidazole\textsuperscript{68} (equation 1.9) in low yields.

\[
\begin{align*}
\text{imidazole-CPh}_3 & \quad \text{imidazole-CPh}_3-Li \\
\text{imidazole-CPh}_3-Li & \quad \text{imidazole-I}
\end{align*}
\]

(1.9)

**Nitration of Imidazoles**

Nitration is a prerequisite reaction to the synthesis of therapeutically useful 5-nitroimidazoles and their analogues.
2-Nitroimidazole [30] (azomycin) is the only naturally occurring nitroimidazole. Its discovery as an antibiotic in 1955 set off the search for synthetic analogues which has led to the presently used nitroimidazole antimicrobial drugs.

Nitration of imidazole does not give nitration in the 2-position, and therefore, azomycin and its derivatives are synthesised from 2-aminoimidazole.

The latter is prepared, from 2,2-diethoxyethylamine and 5-methylisothiourea in the presence of an acid, as shown in scheme 1.9. The 2-aminoimidazole is diazotized and all the resultant diazo-intermediates react by a Sandmeyer reaction to generate 2-nitroimidazole (equation 1.10).

\[
\text{Scheme 1.9}
\]

Ever since the earliest work of Pyman, it has been known that nitration of imidazoles by conventional methods leads to the introduction of a nitro group into the equivalent C-4 and C-5 positions, if the amine nitrogen is not substituted (equation 1.11). It is not possible to separate these isomers of imidazole, although as mentioned above, they are reported to enter into chemical reactions in one or the other of the tautomeric forms.
Variations in reaction conditions such as heating imidazole nitrate with sulphuric acid or addition of sodium or potassium nitrates to imidazole also give the 4-nitro isomer as the major product. Exhaustive nitrilation in 'mixed acids' gives successively 4-nitro- and 4,5-dinitroimidazole, but not 2,4,5-trinitroimidazole. The latter compound can, however, be prepared by nitrilation of 2,4-dinitroimidazole. 78

Nitric acid in acetic anhydride (nitroimium acetate) appears to form only the nitrate salt in many instances. With nitric-sulphuric acid mixtures, 1-methyl- and 1,2-dimethylimidazole gives mixtures of the 4- and 5-nitroisomers in the approximate ratios of 5:2 and 2:1 respectively. 79

The kinetics of nitrilation of imidazole has been studied in sulphuric acid medium. The yields of 4-nitroimidazole are dependent on the acidity with ring opening becoming a side reaction. The kinetic results which could be separated indicated that the species being nitrilated is the imidazole cation, but side reactions complicate matters. 79

The mechanism of nitrilation is similar to that of benzene, although more vigorous conditions than for the benzene ring are required. 80 (scheme 1.10).

Ridd et al. 64, 67 showed that the 4-nitro-isomer predominates in a 400:1 (4:5NO2) ratio, consequently, the nitrated product is conventionally referred to as 4-nitroimidazole.
Nitration of imidazole substituted in the 4-position automatically directs to give only the 5-nitro compound. No nitration is observed at C-2 position but that of 1-substituted imidazoles gives mainly the 4-nitro isomer as in the unsubstituted imidazole. 73

1-Alkylimidazoles with substituents present at the C-2 position also affect the orientation of nitration. With a +I (electron donating) group at C-2, nitration occurs mainly at the C-5 position (equation 1.12). R.C. Tweit et al. 81 reported that nitration of 1-alkylimidazole-2-yl-sulphides proceeds to give the 5-nitro-isomer as the major product (equation 1.13). The +I groups stabilize the intermediate σ-complex leading to nitration in the 5-position (scheme 1.11).
4-substitution

\[
\begin{array}{c}
\text{major} \\
\text{minor}
\end{array}
\]

5-substitution

Scheme 1.11

ipso-Nitration of iodoimidazole has been reported\textsuperscript{74b,82} but recent studies\textsuperscript{74} have demonstrated that some compounds, reported to be 2-iodo derivatives are in fact 4-iodoimidazoles. Accordingly, when the results reported earlier are amended in the light of these finds, iodo groups in the 4- and 5- positions of imidazoles may be replaced by the nitro group (scheme 1.12).
N-Alkylation of Imidazoles

The mechanism and orientation of N-substitution in imidazole derivatives were first discussed by Pyman\textsuperscript{83} as part of the general problems of N-substitution in amidines. Ridd et al.\textsuperscript{64a,67} later showed that the detailed kinetics of mechanism of reaction invoked prototropic equilibrium and is dependent on the acidity of the medium.

Thus, electrophilic attack on imidazole at a ring nitrogen can involve the neutral species, the conjugate base, or the conjugate acid. This would lead to four possible transition states [32] - [35] for the reaction (scheme 1.13).

Although the two nitrogens in the neutral molecule are equally susceptible to attack by the electrophile, reaction

\[
\text{Scheme 1.13}
\]

is normally confined to the nitrogen which has an unshared electron pair orthogonal to the ring. Reaction with the NH nitrogen [33] would require the use of two electrons from
the 6π system to form a bond, disrupting the aromaticity of the ring, (designated S_{E2} mechanism substituted electrophilic bimolecular on amino nitrogen\textsuperscript{64a}) For this reason the transition state [32] would be energetically more favourable than [33] and, in consequence, reactions with the neutral imidazole molecule follow the sequence shown in scheme 1.14. This mechanism was designated S_{E2} \textsuperscript{1} (substitution, electrophilic, bimolecular, on the pyridine nitrogen) by Ridd et al.\textsuperscript{64a} When the imidazole already has a group other than at N-1 a quaternized product results. Such a reaction sequence is typical of the classical method of alkylation of imidazoles. For example, either the HI produced or excess MeI reacted with imidazole or 1-methylimidazole (scheme 1.14). The

\begin{align*}
\text{Scheme 1.14 : Mechanism of neutral alkylation of imidazole}
\end{align*}

salts formed are impurities and are also resistant to methylation. In spite of these limitations, the reaction has found considerable utility in the synthesis of 1-substituted imidazole, particularly the methylation of unsymmetrical imidazoles (discussed later).

The attack at either nitrogen of the imidazole anion [34] will be a highly favoured reaction. Such a reaction sequence applies when imidazoles are alkylated in basic
medium, conditions which lead usually to good yields of 1-alkylimidazole with none of the problems of quaternization which can accompany alkylation of neutral molecules. This reaction is also of importance in the methylation of unsymmetrical imidazoles (see later discussion). The reaction mechanism is designated $S_{E2}^{cB}$ (Substitution electrophilic bimolecular involving the conjugate base of imidazole) by Ridd et al. [64a,67]

Any reaction involving a transition state of type [35] is not favoured and such electrophilic substitutions of imidazolium cation appear to be absent.

Consequently, imidazoles are readily alkylated in neutral or basic medium following either an $S_{E2}^{1}$ or an $S_{E2}^{cB}$ mechanism. The method of choice now commonly involves alkylation of the heterocycle in basic medium (equation 1.14) since these conditions do not suffer from side reactions

\[
\begin{align*}
\text{imidazole} + X^{-} & \rightarrow \text{imidazolium species} \\
\text{R} & \rightarrow \text{RX} + X^{-}
\end{align*}
\]

which produce the imidazolium species. Thus, the reaction of an imidazole with an alkyl halide (or related compound) is carried out in the presence of the hydroxide of an alkali metal or alkaline earth metal, sodium ethoxide, or sodamide, in solvents such as ethanol, dioxane, acetone or liquid ammonia.

Related reactions which utilize the imidazole anion include alkylation of the silver salt or an imidazolyl Grignard reagent. The former has been employed specifically for the introduction of trityl and carbohydrate residues. An example of the latter is the preparation of 1-glucopyranosyl-5-methylimidazole from the silver salt of 4-methylimidazole and $\alpha$-acetobromoglucose [84] (equation 1.15).

Many alkylations involving unsaturated compounds, probably proceed via the imidazole anion. 1-Allylimidazole is obtained by reaction of the heterocycle with allyl chloride in the presence of sodium hydroxide in acetonitrile (equation 1.16).
Alkaline medium, however, is not always suitable since branched-chain alkyl halides are subject to elimination, and there are striking changes in orientation of substitution in unsymmetrically substituted imidazole.

**Effects of Substituents on N-Alkylation of Imidazoles**

The mechanism of N-alkylation in unsymmetrical imidazole, as in imidazoles has been shown to invoke prototropic equilibrium and is dependent on the acidity of the medium. The same mechanism (scheme 1.13) of substitution has been proposed by Ridd et al. and similarly, the SE$_2$ and SE$_2$cB are the two feasible mechanisms.

When imidazole is substituted in the 4- or 5- position, there is a directional effect imposed on electrophilic attack on the nitrogen atoms. This orientation may be related to the tautomeric nature of the substrate. The usual substitution effects as observed for benzene derivative apply, i.e. electron-withdrawing groups decrease the rate of substitution and vice versa for +I groups. Steric and inductive effects are likely to determine direction of substitution.

In basic medium, the mechanism involves the anion, and the two possible products [36] and [37] (equation 1.17) are obtained in a ratio which reflects the electronic and steric effects of the substituent group R and those of reagent which is much less than the tautomer ratio.
A large R-group tends to direct substitution to the more remote nitrogen. An electron-withdrawing R-group reduces the basic nature of the adjacent nitrogen more than that of the remote nitrogen and gives the same orientation \[36\]; conversely, an electron-releasing group leads mainly to \[37\]. The alkaline benzylation of 2,4-dialkylated imidazoles gives the least sterically hindered product (equation 1.18). Also in basic medium, the methylation of 4-nitroimidazole gives a 9:1 ratio of 1-methyl-4-nitro:1-methyl-5-nitro product (electronic effects) (scheme 1.15).

Scheme 1.15: Methylation of 4(5)-nitroimidazole in basic conditions.
In neutral conditions, it is the tautomeric isomer present in higher concentration that reacts to give the major N-methylated product. Although the electron-withdrawing group is adjacent to the 'pyridine' nitrogen (N3), it is more basic (nucleophilic) than the amino N-1 nitrogen (since reaction at this centre would destroy the aromatic sextet). Although the N-3 nitrogen in the minor tautomer is more basic and may react faster, this isomer is only present in negligible amounts. The reaction was shown to be kinetically and not thermodynamically controlled. The ratio of isomers (1-methyl-5-nitro:1-methyl-4-nitroimidazole) was found to be 350:1.

There are however a few instances when it is the minor tautomeric isomer that reacts. In an $S^1_E$ process, 2-methyl-4-phenylimidazole is methylated to give a mixture of the 4-phenyl and 5-phenyl isomers in the ratio 5.7:1. The phenyl group exerts some steric hindrance and results in a preference for the formation of the 1,4-product in spite of the fact that this involves methylation of the minor tautomomer (scheme 1.16).

![Scheme 1.16 Methylation of 2-methyl-4(5)-phenylimidazole](image)

tautomer major ratio

Ridd et al. showed that methylation of unsymmetrical imidazoles by both $S^1_E$ and $S^1_E$ mechanisms are kinetically controlled, i.e. the more basic (nucleophilic) nitrogen of the major tautomer under acid or base conditions reacts to give the observed product. The rate coefficients for reaction at the two nitrogen centres in conjugate base and neutral conditions are shown below.

The rate coefficients showed that under basic conditions ($S^1_E$), the two nitrogen atoms react in an 8:1 ratio of 4-nitro to 5-nitroimidazole. In the reaction by
the \( S^1 \) mechanism, the relationship between rates and equilibria indicates that the difference between rate coefficients of the two tautomers should be much less than the difference between their concentrations, \(^{67}\) the predominant tautomer should therefore react via the \( S^1 \) mechanism to give the predominant isomer (1-methyl-5-nitroimidazole), and the isomer ratio should more nearly approach the tautomer ratio (1-methyl-5-nitro-:1-methyl-4-nitro-imidazole [350:1]). \(^{67}\) Imidazole derivatives, containing +I groups give isomer ratios in reverse order.

For 4(5)-nitroimidazole, the rate coefficient for the methylation of the conjugate base is greater than that for the methylation of the neutral molecule by a factor of a thousand. In other substituted imidazoles, this factor is probably similar. The transition from the \( S^2cB \) to the \( S^1 \) mechanisms should therefore occur about 3pH units below the acidic \( pK_a \) of the neutral molecule. Consequently, this change of mechanism should always change the main product of substitution. This occurs because the most basic nitrogen atom in the conjugate base is necessarily blocked by a proton in the predominant tautomer of the neutral imidazole, the predominant tautomer determines the product. \(^{67}\)

Under neutral conditions, 2-methyl-4-nitroimidazole can be fused with alkyl tosylates to give mainly the 1-alkyl-2-methyl-5-nitroimidazole isomer. \(^{67}\)

As for imidazoles, the product of methylation of nitroimidazoles via \( S^1 \) mechanism with methyl iodide or dimethyl sulphate can undergo further methylation at the
tertiary nitrogen to give an undesired quaternized by-product. In spite of this limitation, methylation in neutral or acid conditions has found considerable utility in the synthesis of 1-alkyl-5-nitroimidazoles, some of which are the current drug of choice in the treatment of anaerobic diseases.²¹,⁴¹

Methylation with diazomethane yields mainly 1-methyl-5-nitro-derivatives (equation 1.19). The direction of alkylation can therefore be controlled by the method of alkylation. However, large scale quantities of diazomethane should not be used in the laboratory due to the explosive nature of the reagent.

N-Alkylation of 2-nitroimidazoles always gives only one product because the 4- and 5- positions are equivalent, except when those positions are substituted, then the above consideration for imidazoles substituted in the 4- and 5-position should apply.

Preparation of Therapeutically Used Nitroimidazoles

The above discussions are particularly important to the synthesis of therapeutically used nitroimidazoles, using different alkylating agents. In order to obtain 5-nitroimidazoles, neutral conditions must be used, e.g. for the synthesis of dimetridazole [2] (1,2-dimethyl-5-nitroimidazole), (equation 1.20).

A number of clinically important nitroimidazoles have been synthesised using an appropriate alkylating agent as represented in schemes 1.18 and 1.19.

Synthesis via introduction of functional groups at the C-2 position of imidazole is represented in scheme 1.19.
Scheme 1.18: Synthetic pathway to some clinically used nitroimidazoles

Scheme 1.19: Synthetic pathway to some clinically used nitroimidazoles
Other Clinical Uses of Imidazoles

There are far fewer effective antifungal compounds than antibacterials. Griseofulvin and polyenes\textsuperscript{21} (for example nystatin) are important antifungal agents which cannot be readily synthesised. Imidazole derivatives are providing useful antifungal agents, particularly as topical agents, e.g. ketoconazole. Miconazole [40] and econazole [41] are synthesised as outlined in scheme 1.20. Imidazoles are also finding increasing use as antiviral agents.

\[\text{Scheme 1.20}\]

Part C
Synthesis Involving Radical and Radical-Anion Intermediates

The mode of action of nitroimidazole drugs initially proceeds via reduction to the corresponding radical-anions which are relatively stable. Edwards\textsuperscript{21} and Ings\textsuperscript{41} showed that reduction of the drug in the cell is the driving force for the entry of neutral nitroimidazoles into the cell from the surrounding environment. Various methods have been used to detect the one-electron reduction product of nitroimidazoles (see discussion, Part A).
This ability of nitroimidazoles to accept an electron indicates that synthesis proceeding via their radical-anion may provide useful routes for the preparation of new analogues. One of the aims of the project was to exploit these potential synthetic methods. This section of the introduction therefore discusses the general background to the chemistry of radicals, radical-anions and useful synthetic reactions proceeding via radical anions, applicable to the course of study.

The study and use of reactions which proceed via transient radical-anion formation are now established as an important part of organic chemistry. Radical-anions are molecules which, in addition to having one or more unpaired electrons, have a net negative charge. The formation of radical-anions may be schematically represented as:

\[ M + e^- \rightarrow M^- \]
\[ M^+ + A^- \rightarrow MA^- \]

The electron enters the lowest unoccupied molecular orbital (LUMO), commonly a \( \pi^* \)-orbital. A simple example of this is the formation of benzene radical-anion \([42]\). Radical-anions are classified according to the orbital in which the unpaired electrons reside. These orbitals may be \( \sigma, \pi \) or p-type and bonding, antibonding or nonbonding. By far the most common are the \( \pi \)-types.

The first radical-anion was probably observed as early as 1836\(^9\) when potassium hydroxide solution was added to benzil producing a deep blue colour which is now attributed to the formation of compound \([43]\).

\[
\begin{align*}
\text{[42]} \\
&\quad \quad \quad \\
\end{align*}
\]

\[
\begin{align*}
[43]
\end{align*}
\]
Further work\textsuperscript{85b} showed the reaction was given by many diketones. More recently, it has been well demonstrated that radical-anions may be generated in many compounds containing π-bonds,\textsuperscript{85c} notably in ketones, azo and nitro compounds, simple and conjugated olefins, and aromatics. In general, the more extended the π-system, the easier is the formation of the radical-anion, due to the resonance stabilization obtained.

The formation of radical-anions for reaction and study has been achieved by several diverse means.\textsuperscript{86} Electrolytic reduction has found wide application and has been used to produce the radical-anions of aromatic systems (nitroimidazole, benzene, furan), π-diketones, simple aliphatic ketones, nitriles and nitroalkanes.

Reduction using alkali metals has also been used, and some stable radical-anions may be produced using standard reducing agents such as sodium dithionite, glucose and sodium borohydride. Ashby et al.\textsuperscript{86} and several other researchers demonstrated the reaction of aromatic substrates via a single electron transfer (s.e.t.)\textsuperscript{87} (scheme 1.21).

\[
\text{Ph}_3\text{C}-\text{Br} + \text{MH} \xrightarrow{\text{s.e.t.}} [(\text{Ph}_3\text{CBr})^z + \text{MH}^+] \xrightarrow{\text{s.e.t.}} [\text{Ph}_3\text{C}^+ + \text{Br}^- + \text{M-H}^+] \text{Solvent Cage}
\]

\[\text{[Ph}_3\text{C}^- + \text{Br}^- + \text{M-H}^+] \rightarrow \text{MBr} + \text{Ph}_3\text{CH} \]

Scheme 1.21 Solvent Cage

The generation of radical-anions by photostimulated or thermally stimulated single electron transfer (s.e.t.) from carbanions, nitronates, radical-anions, amines and sulphur anions to unsaturated systems, notably nitro compounds, aryl substrates and olefins has found most use synthetically.\textsuperscript{33} The process may be represented as shown below.\textsuperscript{89} (equations 1.22 and 1.23).

\[\text{R}^- + \text{n} \rightarrow \text{R}^+ + \text{n}^z \quad (1.22)\]

\[2\text{R}^+ \rightarrow \text{R-R} \quad (1.23)\]

The radical-anion, \(n^z\) so formed may react further via several different pathways.
Radical-anions are not very stable, although in exceptional cases, e.g. the naphthalene radical-anion, they may be isolated and stored. Atmospheric oxygen readily destroys radical-anions by removing an electron (equation 1.24) by s.e.t. In consequence, all reactions involving radical-anions are best conducted under an inert atmosphere.

$$\text{R}^+ + \text{O}_2 \xrightarrow{\text{s.e.t.}} \text{R} + \text{O}_2^\cdot$$

(1.24)

Radical-anions have also been observed to dimerise, and ketyls (radical-anions derived from ketones) existed predominantly in the form of pinacolates in suitable solvents. In contrast, aromatic radical-anions show little tendency to dimerise.

If the radical-anion has a suitable leaving group, it is particularly unstable and the group X may be rapidly eliminated to give a radical and stable anion (equation 1.25).

$$[\text{R-X}]^+ \rightarrow \text{R}^+ + \text{X}^-$$

(1.25)

The fate of R depends on its structure and the nature of the other chemical species present, but it may, in suitable circumstances, react with another anion, Y\textsuperscript{-}, to generate a new radical-anion.

The frontier molecular orbital (MO) approach to formation of radical-anions demonstrates that the strongest interaction between two reacting centres occurs with the frontier orbitals of similar energies. Consequently, for example, the singly occupied MO (SOMO) of an aryl radical will interact with the highest occupied MO (HOMO) of the nucleophile.

This three-electron interaction will generate one two-electron bonding orbital (a 6 bond) and an antibonding orbital (scheme 1.22), which does not necessarily bear the extra electron. The strongest interaction occurs when the two species approach each other along the axes of the new bond. (equation 1.26).
Scheme 1.22: Changes in energy during the coupling of a phenyl radical and a methyl anion and in the product toluene radical-anion

\[
\begin{align*}
\text{Ph}^+ + \text{CH}_3^- & \rightarrow [\text{PhCH}_3]^2^- \\
\text{Ph}^+ & \rightarrow \text{Ar}^+ \rightarrow \text{ArCH}_3^2^- \\
\sigma^* & \rightarrow \sigma & \pi^* & \rightarrow \pi^*
\end{align*}
\]

In an hypothetical reaction of an aryl radical with a methyl anion (equation 1.27), toluene radical-anion is obtained, in which the odd electron occupies the \( \pi^* \) MO, because the energy of this MO is lower than the C-C \( \sigma^* \) MO. 92

\[
\begin{align*}
\text{Ar}^+ + \text{Nu}^- & \rightarrow [\text{Ar}^+ \text{Nu}]^2^- \\
\text{Ar}^+ + \text{CH}_3^- & \rightarrow [\text{ArCH}_3]^2^- \\
\text{Ar}^+ & \rightarrow \text{Ar}^+ \rightarrow \text{ArCH}_3^2^- \\
\text{Nu}^- & \rightarrow \text{Nu}^- \rightarrow \text{Nu}^2^- \\
1\text{e}^- & \rightarrow 2\text{e}^- & 2\text{e}^- & \rightarrow 3\text{e}^-
\end{align*}
\]  

However, when the carbanion is of the type \(-\text{CH}_2 Z\) (where \(Z\) is an unsaturated moiety, e.g. nitro group), the odd electron of the radical-anion is located in the lowest unoccupied MO (LUMO) of either the aryl or \(Z\) groups, (equation 1.28). The radical-anion predominantly formed will

\[
\begin{align*}
\text{Ar}^+ \text{CH}_2 Z & \rightarrow \text{Ar}^+ \text{CH}_2 Z \\
\text{Ar}^+ \text{CH}_2 Z & \rightarrow \text{Ar} \text{CH}_2 Z
\end{align*}
\]

be the one where the electron is located in the LUMO of lowest energy, which will also be formed when the neutral molecule accepts an electron.

**Radical-anions as Intermediates in Nucleophilic Substitution**

The most useful of the synthetic reactions proceeding via radical-anions are chain nucleophilic substitutions involving s.e.t. A wide range of substrates undergo chain nucleophilic substitution reactions and some of the more important examples are discussed.
One of the most important developments involving radical-anion intermediates has been the elucidation of the $S_{RN1}$ mechanism. Bunnett suggested the terminology $S_{RN1}$ (substitution, radical-nucleophilic, unimolecular) to describe the reactions which has been shown to proceed via intermediate radical-anions and radicals rather than Meisenheimer complexes. The reactions were carried out in liquid ammonia or dipolar aprotic solvents and are stimulated by solvated electrons, light or heat. A simple ionic substitution mechanism via addition and elimination steps hardly accounts for this stimulation and the following mechanism has been proposed (scheme 1.23).

$$\text{ArX} + \text{electron donor} \xrightarrow{\text{s.e.t.}} [\text{ArX}]^- + \text{residue} \quad (1.29)$$

$$[\text{ArX}]^- \rightarrow \text{Ar}^+ + X^- \quad (1.30)$$

$$\text{Ar}^+ + Y^- \rightarrow [\text{ArY}]^- \quad (1.31)$$

$$[\text{ArY}]^- + \text{ArX} \xrightarrow{\text{s.e.t.}} \text{ArY} + [\text{ArX}]^- \quad (1.32)$$

$X = \text{Cl, Br, I, SPh, F, OPh}$

$Y = \text{NH}_2, \text{RS, (RO)}_2\text{PO, RCOCH}_2$ etc.

Scheme 1.23

Initiation of the chain reaction is achieved by transfer of a single electron from a suitable donor to the substrate (equation 1.29). The resulting radical-anion breaks down rapidly to give an aryl radical and a stable nucleofuge ($X^-$) (equation 1.30). The aryl radical adds to a new nucleophile ($Y^-$) to produce a new radical-anion, (equation 1.31) which then transfers one electron to a molecule of the substrate (equation 1.32), perpetuating the chain sequence. The nature of the termination steps is, as yet, poorly understood.

The first example of radical-anion serving as intermediates in aliphatic substitution was discovered during an investigation of the reactions of $p$-substituted benzyl halides with the sodium salt of 2-nitropropane. (Scheme
The salt is an ambident anion, i.e., it may react via the C- or O-center with a benzyl halide to give alkylation on carbon or oxygen respectively.

The former route (to C-alkylation) was found to predominate only when $Z = \text{NO}_2$. In all other cases, O-alkylation occurs giving the corresponding aldehyde, via the nitronic ester (e.g., [45]). When p-nitrobenzyl chloride was reacted with the sodium salt of 2-nitropropane an 83-95% yield of C-alkylated product [46] was obtained (equation 1.33). m-Nitrobenzyl chloride gave 46% yield of the C-alkylate in the same reaction but m-nitrobenzyl chloride reacted to give mainly O-alkylation.

It was found that the ratio of C- to O-alkylation occurring in the reaction of the salt of 2-nitropropane with a series of $\omega$-substituted p-nitrotoluenes depended on the nature of the $\omega$-substituent. The poorer the leaving group, the more C-alkylated product formed. The rate of reaction when C-alkylation predominated was at least 100 times that when O-alkylation predominated.
the results of various studies, it was suggested that $O$-alkylation proceeded via an $S_N^2$ mechanism whereas $C$-alkylation proceeded by an $S_{RN}^1$ mechanism.

Electron spin resonance (e.s.r.) spectroscopy had shown that the salt of 2-nitropropane can transfer an electron to nitroaromatics to form the corresponding radical-anion, and initially it proved impossible to detect the radical-anion of p-nitrobenzyl chloride due to its short lifetime at room temperature.

More support for radical-anion chain mechanism was provided by the reaction of $\alpha$-substituted p-nitrocumyl compounds [47]. Since in this system, substitution must occur

![Diagram](image)

at a tertiary carbon atom centre, $S_N^2$ type attack is not possible. Kornblum et al. [96,97] have studied the system intensively and found that $p$-nitrocumyl chloride reacted relatively rapidly with a variety of nucleophiles at room temperature in dipolar aprotic solvents to give high yields of the substituted products.

The mechanism of the reaction between $p$-nitrocumyl compounds and anions has been studied extensively. The reactions are inhibited by molecular oxygen. For example, the reaction of sodiomalonic ester with $p$-nitrocumyl chloride produces under nitrogen a 90% yield of the $C$-alkylated product but when the reaction was conducted under oxygen, only the $O$-alkylation was observed. (equation 1.34).

Oxygen scavenges the intermediate $p$-nitrocumyl radical giving the peroxy radical. The latter is converted to the hydroperoxide by hydrogen abstraction from the solvent and finally to the corresponding alcohol (equation 1.35).

Trace amounts of oxygen also inhibit the rate of reactions of $p$-nitrocumyls. The reaction of $p$-nitrocumyl chloride with sodium benzene sulphinate is normally complete in two hours under nitrogen giving a 95% yield of the sul-
phone, but in the presence of only 1 mol% of oxygen, the reaction proceeded only to 1% completion in the same time. These results suggest that the reactions are chain processes, with a small amount of oxygen being able to intercept a chain carrying radical, presumably the p-nitrocumyl radical.

The inhibition of the reactions of substituted p-nitrocumyl compounds with anions by p-dinitrobenzene (p-DNB) in low concentration has also been observed. The p-DNB readily accepts an electron acceptor and can participate in electron transfer reactions with the intermediates of the substitution chain process. Kornblum suggested that p-DNB causes the inhibition by accepting an electron from the intermediate radical-anion and thus interrupting the chain process (equation 1.36).

Di-(tert-butyl)-nitroxide, a well known radical scavenger also inhibits these SRN1 reactions as shown in equation 1.37.

Further support for a chain process was made available when it was discovered that some of the reactions of p-
\[ \text{Me-C-Cl} + \text{Me-C-Cl} \rightarrow \text{Me-C-Cl} + \text{Me-C-Cl} \quad (1.36) \]

\[ \text{Me-C-Cl} + \text{Me-C-Cl} \rightarrow \text{Me-C-Cl} + \text{Me-C-Cl} \quad (1.37) \]

\(-\text{nitrocumyl chloride could be accelerated by daylight or laboratory lights.}^{96,98,99}\)

Attempts to detect the radical-anion of \(p\)-nitrocumyl chloride in \(S_{\text{RN}}1\) reactions by e.s.r. spectroscopy have failed.\(^{100}\) This failure is attributed to the rapid expulsion of the chloride anion before the radical-anion can reach a high enough concentration to give an e.s.r. signal, i.e. the intermediate radical-anions are unstable and have short lifetimes.

In view of these facts, the sequence of scheme 1.25 was suggested as the mechanism for the reaction between \(\alpha\)-substituted \(p\)-nitrocumyl compounds and anions.

The sequence of reactions in scheme 1.25 closely resembles that proposed by Bunnett\(^{93}\) for \(S_{\text{RN}}1\) aromatic substitution. The same mechanism was also rationalised by Kornblum\(^{85b}\) and Russell\(^{85c}\) for substitution in aliphatic \(\alpha\)-substituted nitro compounds.

The presence of the \(p\)-nitro groups stabilises the intermediate radical-anion and facilitates the elimination (equation 1.39) but it is not essential for the substitution reaction to proceed. It was shown recently that the benzene ring need not be substituted at all for this type of sub-
stitution reaction to occur if hexamethylphosphoric triamide (HMPA) is used as solvent and long reaction times are used. The sodium salt of nitroethane and α-nitrocumene react in HMPA at 25° in 45 hours to give 74% of the C-alkylation product (equation 1.42).

A range of nucleophiles undergo $S_{RN}^1$ substitution at the 'benzylic' position of $p$-nitrobenzyl and $p$-nitrocumyl compounds. These nucleophiles include nitronates, enolates,
thiolates, sulphonates, nitrite and cyanide anions. Primary, secondary and tertiary amines react with difficulty and for significant reaction to occur an entraining substrate (i.e., using a stronger one electron transfer nucleophile to initiate radical-anion chain reaction) needs to be used. For example, when p-nitrocumyl chloride is treated with quinuclidine, no reaction takes place, but in the presence of catalytic amounts of the lithium salt of 2-nitropropane, the quaternary ammonium chloride is isolated in 63% yield (equation 1.43) (scheme 1.26). The nitro-

Substitution in Heterocyclic Systems

Initially, little was known about the ability of heterocyclic systems to enter into radical-anion substitution at a saturated carbon attached to the heterocyclic nucleus. The first heterocycles to have been studied were substituted pyridines (equation 1.48). The reaction was shown to proceed via the S_N1 mechanism.

The ease with which p-nitrocumyl derivatives undergo S_N1 reactions prompted Norris et al. to investigate the analogous derivatives of 5-nitro thiophenes. They showed that these heterocyclic substrates underwent substitution via the S_N1 mechanism with various anions (scheme 1.27).
Scheme 1.26

\[
\begin{align*}
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 \\
\text{Me-C-Cl} & \quad + \quad \text{Me_2CNO}_2 (1.44)
\end{align*}
\]

\[
\begin{align*}
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 \\
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 (1.45)
\end{align*}
\]

\[
\begin{align*}
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 \\
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 (1.46)
\end{align*}
\]

\[
\begin{align*}
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 \\
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 (1.47)
\end{align*}
\]

\[\text{Scheme 1.26}\]

\[
\begin{align*}
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 \\
\text{Me-C-Cl} & \quad + \quad \text{Me}_2\text{CNO}_2 (1.48)
\end{align*}
\]

89%
The analogous 5-nitrofurfuryl systems were also shown to participate in $S_{RN1}$ reactions, especially (equation 1.49).

\[
\begin{align*}
\text{O}_2\text{N}-\text{C}=\text{CH}_2 + \text{Me}_2\text{CNO}_2 & \rightarrow \text{O}_2\text{N}-\text{C}=\text{CH}_2-\text{C}-\text{NO}_2 + \text{X}^- \\
\text{X} &= \text{Br}, \text{Cl}, \text{I}
\end{align*}
\]

\(S_{RN1}\) Substitution in Aliphatic Systems

The participation of purely aliphatic systems in $S_{RN1}$ reactions has also been observed. The reaction between nitronate anions and $\alpha$-halo-nitroalkanes was described as early as 1940 (equation 1.50).

\[
\begin{align*}
\text{Me}_2\text{C} = \text{NO}_2^+ + \text{Me}_2\text{C(X)NO}_2 & \rightarrow \text{Me}-\text{C}-\text{C}-\text{Me} \\
\text{X} &= \text{Br}, \text{Cl}, \text{I}
\end{align*}
\]

In particular, the reaction between the sodium salt of 2-nitropropane and 2-chloro-2-nitropropane, 2-bromo-2-nitropropane and 2-iodo-2-nitropropane in refluxing ethanol was found to give 9, 29 and 43% yields respectively of 2,3-dimethyl-2,3-dinitrobutane.

Van Tamelen and Van Zyl have described the displacement of the halogen atom of 2-halo-2-nitropropane by diethyl ethylmalonate.
Kornblum and co-workers\textsuperscript{108} reported similar reactions using dipolar aprotic solvents. They found that the transformations took place rapidly under mild conditions \textit{e.g.} equations 1.51 and 1.52.

More recent investigations\textsuperscript{109} have confirmed and extended these studies. Catalysis by light and inhibition by catalytic amounts of DNB, di-(tert-butyl)-nitroxide (DTBN), and molecular oxygen has been observed in reactions using a variety of anions and \(\alpha\)-substituted nitro-aliphatic compounds.\textsuperscript{85b}

The proposed mechanism is given in Scheme 1.28.

\begin{align*}
R_2C(X)NO_2 + A^- &\overset{\text{s.e.t.}}{\rightarrow}\ [R_2C(X)NO_2]^+ + A^- \quad (1.53) \\
[R_2C(X)NO_2]^+ &\rightarrow R_2CNO_2 + X^- \quad (1.54) \\
R_2CNO_2 + A^- &\rightarrow [R_2C(A)NO_2]^+ \quad (1.55) \\
[R_2C(A)NO_2]^+ + R_2C(X)NO_2 &\rightarrow R_2C(A)NO_2 + [R_2C(X)NO_2]^+ \quad (1.56)
\end{align*}

\(X = \text{SO}_2\text{Ph, Cl, Br, I, NO}_2, \text{SCN},\)

\(R = \text{alkyl; A}^- = \text{SO}_2\text{Ar, nitronates, RC}^-(\text{CO}_2\text{Et})_2,\)

\(\text{RS}^-, \text{N}_2^-, \text{enolates}\)

\textbf{Scheme 1.28}

Other \(\alpha\)-substituted nitroaliphatics react with anions to yield a product which is formally the result of direct displacement of the nitro group.\textsuperscript{99} (equation 1.57)
These reactions are again subject to catalysis by light and to inhibition by catalytic amounts of radical scavengers and electron acceptors. A mechanism similar to scheme 1.28 has been proposed, see scheme 1.29.

\[
\begin{align*}
R_2C(X)NO_2 + A^- & \xrightarrow{s.e.t.} [R_2C(X)NO_2]^+ + A^- \quad (1.58) \\
[R_2C(X)NO_2]^+ & \longrightarrow R_2CX + NO_2^- \quad (1.59) \\
R_2CX + A^- & \longrightarrow [R_2C(X)A]^+ \quad (1.60) \\
[R_2C(X)A]^+ + R_2C(X)NO_2 & \longrightarrow R_2C(X)A+[R_2C(X)NO_2]^+ \quad (1.61)
\end{align*}
\]

where \( X = -\text{CO}_2\text{R}, -\text{COR}, -\text{NO}_2, -\text{CN}, \) and \( R \)

\( R = \text{alkyl} \)

\( A^- = \text{Me}_2\text{CNO}_2', \text{PhSO}_2^-, \text{EtC(CO}_2\text{Et)}_2 \)

Scheme 1.29

Schemes 1.27 and 1.28 differ in the way in which the intermediate radical-anion [48] cleaves (equation 1.62).

\[
\begin{align*}
\text{[48]} & \quad \text{a} \quad \text{b} \\
\begin{array}{c}
\begin{array}{c}
\begin{array}{c}
\begin{array}{c}
R^1 \\
R^2
\end{array}
\end{array}
\end{array}
\end{array}
& \quad \text{R}^2
\end{align*}
\]

Kornblum\textsuperscript{109} suggests that a reaction involving radical-anion is most likely to be observed when minimal energy is needed for the formation of the intermediate radical-anions. It is known that one electron reduction of a nitro group is brought about relatively easily and they are relatively stable. It is therefore not surprising that the chain sequences of scheme 1.27 which invoke nitro radical-anion formation, are feasible processes.

The energy required for the formation of \([R_2C(X)A]^+\) is
relatively higher. Generally, pathway b (and scheme 1.28) only prevails when X is a very poor leaving group.

**Substitution Reactions of Thiolates**

The substitution reaction of thiolates with various substrate (aromatic, \(^{110}\) benzylic, \(^{95}\) and aliphatic systems \(^{111}\)) is of particular interest because of their participation in many biological reactions, but has in general been poorly investigated.

Zeldin and Schechter \(^{112}\) have reported that the reaction between n-butane thiolate and 1,1,1-trinitroethane in hot ethanol leads to the formation of the corresponding disulphide (71%) and the anion of 1,1-dinitroethane (42%) (equation 1.63).

\[
\text{MeC(NO}_2\text{)}_3 + \text{BuS}^\Theta \rightarrow \text{BuSSBu} + \text{Me-C(NO}_2\text{)}_2 \quad (1.63)
\]

The authors suggested an ionic mechanism (scheme 1.30).

\[
\text{MeC(NO}_2\text{)}_3 + K^+\text{SBu} \rightarrow \text{Me}_2\text{C(NO}_2\text{)}_2K^+ + \text{BuSNO}_2 \quad (1.64)
\]

\[
\text{BuSNO}_2 + K^+\text{SBu} \rightarrow \text{BuSSBu} + KNO_2 \quad (1.65)
\]

**Scheme 1.30**

Russell and Danen \(^{100}\) offered an alternative mechanism, which invokes the intermediacy of 1,1,1-trinitroethane radical-anion (scheme 1.31).

\[
\text{MeC(NO}_2\text{)}_3 + \text{BuS}^- \xrightarrow{\text{S,ext}} \text{BuS}^- + [\text{MeC(NO}_2\text{)}_3]^2 \quad (1.66)
\]

\[
[\text{MeC(NO}_2\text{)}_3]^2 \rightarrow \text{MeC(NO}_2\text{)}_2 + \text{NO}_2^- \quad (1.67)
\]

or

\[
\rightarrow \text{MeC(NO}_2\text{)}_2 + \text{NO}_2^- \quad (1.68)
\]

\[
\text{MeC(NO}_2\text{)}_2 + \text{BuS}^- \rightarrow \text{MeC(NO}_2\text{)}_2 + \text{BuS}^- \quad (1.69)
\]

\[
2\text{BuS}^- \rightarrow \text{BuSSBu} \quad (1.70)
\]

**Scheme 1.31**
Sokolovsky et al. \cite{113} reported that the oxidation of protein thiol and peptides such as glutathione with tetranitromethane resulted primarily in disulphide formation (scheme 1.32).

\[
\text{R-SH} + \text{C(NO}_2)^4 \rightarrow \text{RSNO}_2 + \text{C(NO}_2)^3 + \text{H}^+ \quad (1.71)
\]

\[
\text{R-SH} + \text{RSNO}_2 \rightarrow \text{RSSR} + \text{NO}_2^- + \text{H}^+ \quad (1.72)
\]

For glutathione $R = \text{OCH}_2\text{CH-C-NHCH}_2\text{CO}_2\text{H}$

\[
\text{CH}_2\text{CH}_2\text{CH-COOH}
\]

\[
\text{NH}_2
\]

Scheme 1.32

Substitution of $\alpha$-substituents in various nitro-compounds\cite{93a,95,97} by thiolates have been reported by Kornblum et al.\cite{96,97} For example, the reaction of phenylthiolate with $p$-nitrocumyl chloride gave the substitution product in high yield (equation 1.73). They have also shown that these reactions proceed via an $\text{SRN}_1$ mechanism (scheme 1.33).

\[
\begin{align*}
\text{Me} \\
\text{Me-C-X} \\
\text{NO}_2
\end{align*} + \begin{array}{c}
\text{Me} \\
\text{Me-C-S} \\
\text{S} \quad \text{S}
\end{array}
\rightarrow
\begin{align*}
\text{Me} \\
\text{Me-C-S} \\
\text{NO}_2
\end{align*} + X^-
\quad (1.73)
\]

Recently, Bowman and Richardson\cite{111,114,115} studied the reactions of aliphatic $\alpha$-substituted nitro compounds with various thiolates. They found that thiolates derived from the more acidic thiols reacted to give $\alpha$-nitrosulphides by an $\text{SRN}_1$ mechanism, but thiolates derived from the less acidic thiols (more nucleophilic thiolates) were oxidized to disulphides.

Russell\cite{100,116} had initially proposed a non-chain radical oxidative dimerisation mechanism for the oxidation of thiolates by $\alpha$-substituted nitro-alkanes (scheme 1.34).
Thus, the difference in schemes 1.33 and 1.34 is that the intermediate 2-nitropropyl radical either adds to thiolate (scheme 1.33) to yield α-nitrosulphide or undergoes electron transfer (scheme 1.34) with thiolates to yield disulphide (equation 1.82).

Bowman and Richardson demonstrated that for thiolates of intermediate reactivity e.g. 4-chlorophenyl and 2-nitrophenyl thiolate, the nature of the α-substituent does not determine the route of reaction (a or b in equation 1.82). However, they failed to trap the thiyl radical (pathway b) intermediate, and demonstrated an ionic mechanism for the oxidation of thiolates (scheme 1.35). Thus, the distribution of product depends on competition between $S_N^2$ and $S_{RN}^1$ mechanisms. Therefore, the more nucleophilic the thiolate, the easier the abstraction of the 2-substituent.
RS⁻ + X → C(NO₂)Me₂ → R-S-X + Me₂C(NO₂)

RS⁻ + RSX → RSSR + X⁻

Scheme 1.35

(1.35) 51

(1.83)

(1.84)

(I > Br > Cl > NO₂ > SO₂Ph), the more disulphide formation is favoured over S⁻ product and vice versa. Summary of the overall mechanism proposed by Bowman and Richardson is shown in scheme 1.36.

MeC(X)NO₂ + RS⁻ → Me₂C(SR)NO₂ + X⁻

Me₂C(X)NO₂ + RS⁻ → RSX + Me₂C(NO₂)

RSX + RS⁻ → RSSR + X⁻

Me₂C(NO₂) + Me₂C(X)NO₂ → MeMe

Scheme 1.36

Aims

Our approach to the synthesis of potentially useful nitroimidazoles stemmed from observations that:

1. Synthesis using S⁻ reactions which have intermediate radicals, and radical-anions is now a growing area in synthetic organic chemistry. The initiation step in the mode of action of 2-, and 5-nitroimidazole drugs is the formation of radical-anions as outlined in the introduction. As a result, the current study was directed towards investigating the applicability of utilizing S⁻ reactions as a method for the preparation of a wide variety of novel nitroimidazoles with potential biological activity.

2. Electron accepting groups on C-2 and N-1 side chains improve anti-microbial activity, and increased substitution, especially branched chains at C-2, increased antitrichomonal activity. The nitro groups (aliphatic or aryl) are excellent electron accepting groups, and therefore their incorporation into the nitroimidazole nucleus may increase biological activity.
Consequently, synthesis centred on compounds of the type:

![Chemical structure](image)

\[ R = \text{H or Me, } X = \text{NO}_2, \text{aryl-NO}_2, \text{heteroaryl-NO}_2 \]

A further aim was to screen for inherent antimicrobial activity in compounds, and to determine any correlation of structure activity relationship. This involved both minimum inhibitory concentration (MIC) determinations and the reaction of some of these products with thiolate anions.

The use of e.s.r. spectroscopy to predict the behaviour of radical-anions of a representative nitroimidazoles prepared in solution reactions at room temperature was also envisaged.
CHAPTER 2

N-ALKYLATION OF DIAZOLEs

N-Alkylation of Nitroimidazole N-Anions via Radical and Radical-Anion Intermediates - PART A

The most useful synthetic reactions proceeding via radical-anions are chain nucleophilic substitution reactions involving single electron transfer (s.e.t.). The $S_{RN1}$ mechanism is now well established and several different types of substrates (RX) have been shown to react by this mechanism.

These substrates include substituted -arenes (ArX)\(^{92,117}\) and -heterocycles,\(^{118}\) o- and p-nitrobenzyl derivatives (\(\text{O}_,\text{p-NO}_2\text{C}_6\text{H}_4\text{-CH}_2\text{-X}\)) and \(\omega\)-substituted nitroalkanes [\(\text{R}_2\text{C(X)NO}_2\)]\(^{109,119}\) The range of nucleofuges (X\(^{-}\)) which are suitable for $S_{RN1}$ reactions are similar for all three groups of substrates and include Br\(^{-}\), I\(^{-}\), Cl\(^{-}\), SCN, N\(_3\)^{-}, NO\(_2\)^{-}, S\(^{-}\), S(OR), S\(_2\)O\(_2\)R, NR\(_3\), and OP(O)(OR)\(_2\).

The nature of the nucleophiles (Y\(^{-}\)) which participate in $S_{RN1}$ reactions differs between the aromatic substrates, and the aliphatic nitro- and p-nitrobenzyl substrates. Some nucleophiles undergo reactions\(^{92,109,119}\) in both groups (e.g. RS\(^{-}\), (RO)\(_2\)PO\(^{-}\)) but most nucleophiles will undergo reaction with ArX, or with nitro-halo substrates, e.g. nitro-nate anions [\(\text{R}_2\text{CNO}_2\)^{\text{\theta}}\] readily react with nitro-halo substrates (equation 2.1) but do not react with ArX substrates.\(^{109,119}\)

\[
\begin{align*}
\text{CH}_2\text{Cl} & + \text{R}_2\text{CNO}_2^{\text{\theta}} \\
\text{NO}_2 & \quad \rightarrow \\
\text{CH}_2\text{CR}_2 & \quad \text{NO}_2
\end{align*}
\]

On the other hand, some nucleophiles will react with both substrates under biased conditions, e.g. malonate anions undergo reaction with nitro-halo substrates but will only react with aryl substrates when strong electron-withdrawing groups are present on ArX.\(^{92,109,117,119}\)

Two groups of nucleophiles are largely absent from reactions with both aromatic and nitro-halo derivatives; these are the N- and O-centred anions. A few examples of
SRN₁ reactions of N-centred anions are known and include the reactions between amide (NH₂) and ArX (equation 2.2), \(^{92,117}\) p-nitrocumyl chloride and nitrite anions and quinuclidine, \(^{109,119}\) and azide and \(\alpha\)-substituted nitroalkanes. \(^{120}\) An explanation has been proposed\(^{121}\) for the lack of reactivity of O-anions; i.e. the reaction between O-anions and the intermediate radicals yield radical-anions which contain the unpaired electrons in C-O \(\delta^*\) SOMO's, which are of very high energy, thus acting as a barrier to reaction. A similar argument can be applied to the reactions of N-centred anions which would have intermediate radical-anions containing high energy C-N \(\delta^*\) SOMO's.

Edwards\(^2\) and Ings\(^4\) reported that reduction of 5-nitroimidazoles by electrons donated by pyruvate/NaDH via the hydrogenosomal enzyme, pyruvate ferridoxin oxidoreductase is a prerequisite to the mode of action of these nitroimidazoles. The radical-anions of nitroimidazoles thus formed in protozoa and anaerobic bacterial cells treated with nitroimidazoles have been detected by e.s.r. spectroscopy. \(^{36}\) Consequently, it was considered that synthesis via radical-anions would provide an ideal opportunity for synthesis of some novel nitroimidazoles.

This section describes the application of the \(S_{RN1}\) mechanism, and the related oxidative addition reactions of nitronates, to the N-alkylation of nitroimidazoles. The central step in these reactions is the addition of the N-anions, obtained by deprotonation of the nitroimidazoles, to radical intermediates. The studies were based on the prediction that these N-anions, because of their aromatic nature, may yield intermediate radical-anions which were of sufficiently low energy to allow reaction to proceed, unlike previous observations of the lack of reaction of N-centred anions in \(S_{RN1}\) reactions.

The investigation had the added advantage that most of
the starting materials could be readily synthesised (see experimental section) and are shown below.

Nitroimidazoles for $S_{RN1}$ and oxidative addition reactions:

Substrates (RX) for $S_{RN1}$ reactions:

\[
\begin{align*}
&\begin{array}{c}
\text{Me} \\
\text{NO}_2
\end{array} & X = \text{Cl} [54], \text{Br} [55], \text{NO}_2 [56]
\end{align*}
\]

Anion of 2-nitropropane for oxidative addition reaction $\text{Me}_2\text{C=NO}_2^-$ [61]

2.1 Synthesis using $S_{RN1}$ reactions

2.1.1 N-Alkylation of 4(5)-nitro- and 2-methyl-4(5)-nitroimidazoles

The 2-substituted-2-nitropropanes ([54], [55], [56]) reacted slowly with both the imidazole anions [51], [52] to yield the corresponding 1-(1-methyl-1-nitroethyl)-4-nitroimidazoles [62] (equation 2.3) and 1-(1-methyl-1-nitroethyl)-2-methyl-4-nitroimidazoles [63] (equation 2.4).
The reactions were carried out under conditions (nitrogen, light catalysis) conducive to $S_{RN1}$ reactions. The results are presented in Table 2.1.

The rates of reaction were slower than normally observed for $S_{RN1}$ reactions of $\alpha$-substituted nitroalkanes. The slow rates may be due to steric hindrance of the addition to the imidazole anions, $[50]$ and $[51]$, to the intermediate radical (Me$_2$CNO$_2$) (see later).

The related bromo-nitro analogue, 5-bromo-5-nitro-1,3-dioxane $[57]$, which is the commercially used antimicrobial agent, bronidox, also reacted with the anion of 4(5)-nitroimidazole $[51]$ to give the corresponding N-1-alkylated product $[64]$ in 81% yield (equation 2.5).

Reactions between the anions ($[51]$ and $[52]$) and $p$-nitrobenzyl chloride $[58]$ (equation 2.6) and (2-Chloromethyl)-1-methyl-5-nitroimidazole $[60]$ (equation 2.7) gave the corresponding 1-alkyl-4-nitroimidazoles.
Table 2.1  $\text{S}_{\text{RN}}^1$ reactions between 4(5)-nitroimidazole anions [51] and [52] and substrates RX

<table>
<thead>
<tr>
<th>Nitroimidazole anion (1)</th>
<th>RX</th>
<th>Conditions$^a$</th>
<th>1-alkyl-4-nitroimidazole [$72$]</th>
<th>unaltered RX Nitroimidazole$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Me}_2\text{C(\text{Br})NO}_2$</td>
<td>44 h; 8 h; 4 h</td>
<td>92(59); 68; 48(31) 0; 0; 29 0; 0; 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 h; 6 h, O$_2$</td>
<td>41; 0; 0</td>
<td>25; 3; 28 0; 40; 27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 h, p-dinitrobenzene (5 mol. %)</td>
<td>52, 49</td>
<td>20, 8 3, 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 h, di-t-butylnitroxide, nitrooxide (10 mol. %)</td>
<td>3</td>
<td>3</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>6 h, dark</td>
<td>28, 28</td>
<td>58, 23 0, 0</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}_2\text{C(\text{Cl})NO}_2$</td>
<td>30 h</td>
<td>11(6) 50</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$\text{P-NO}_2\text{C}_6\text{H}_4\text{-CH}_2$</td>
<td>8 h; 5h; 2h</td>
<td>100(75); 60; 55 0; 0; 0 0; 0; 0</td>
<td>1: 30 0; 16 48; 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 h, O$_2$; 2 h, dark</td>
<td>1: 30</td>
<td>0; 16 48; 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 h, p-dinitrobenzene (10 mol. %)</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 h, di-t-butylnitroxide (10, 25 mol. %)</td>
<td>38; 3</td>
<td>0; 11 4; 57</td>
<td></td>
</tr>
<tr>
<td>$\text{[55]}$ (X=Br)</td>
<td>27 h</td>
<td>81(42)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>0</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>$\text{[52]}$</td>
<td>72 h; 8 h</td>
<td>41(37); 31(20) 0; 0; 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Me}_2\text{C(\text{Cl})NO}_2$</td>
<td>96 h; 25 h; 4 h</td>
<td>12(5); 9; 6 0; 0; 0 6; 14; 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>80(37)</td>
<td>0; 0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22 h</td>
<td>100(73)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>$\text{P-NO}_2\text{C}_6\text{H}_4\text{-CH}_2\text{-Cl}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{[59]}$ (X=Br)</td>
<td>24 h$^e$; 16 h$^e$</td>
<td>50(35); 41 (27) 0; 0</td>
<td>0; 0; 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28 h$^e$</td>
<td>28(19)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>36 h</td>
<td>0</td>
<td>0</td>
<td>45</td>
</tr>
</tbody>
</table>

$^a$ All reactions were carried out in DMSO under nitrogen with fluorescent lamps (2 x 150W) with nitroimidazole (1 equiv.), t-BuOK (1.5 equiv.), and RX (1.5 equiv.) unless otherwise stated.

$^b$ % Yields are based on nitroimidazole and were calculated by n.m.r. spectroscopy using an internal standard; yields of pure isolated material are in parenthesis.

$^c$ % Nitroimidazole recovered by filtration after pouring the reaction into water (a larger amount remains in solution).

$^d$ Also carried out using light at 250 nm with similar results.

$^e$ NaH used in place of t-BuOK.
The results are shown in Table 2.1.

An attempt at aromatic $\text{SRN}_1$ substitution using iodo benzene failed (equation 2.8). The reaction between imidazole and iodo benzene has also been reported to be unsuccessful.\(^{92}\) Similarly, reaction with bromonitromethane failed, possibly due to immediate deprotonation\(^{122}\) of $\text{BrCH}_2\text{NO}_2$ by the nitroimidazole anion. The bromonitromethane is quite acidic (two electron-withdrawing groups) (equation 2.9) and therefore the anion [51] may remove a proton rather than react (equation 2.10).

\[ \text{O}_2\text{N} + \text{BrCH}_2\text{NO}_2 \rightleftharpoons \text{O}_2\text{N} + \text{Br}^+\text{CHNO}_2 \]  

\[ \text{O}_2\text{N} + \text{I}^- \rightarrow \text{O}_2\text{N} + \text{I}^- \]
Evidence for the $S_{\text{RN}1}$ mechanism for these reactions was obtained by using the normal criteria for establishing the mechanism for two representative reactions (see Table 2.1).

The use of oxygen, p-dinitrobenzene, and di-(tert-butyl)-nitroxide to investigate the reaction of nitroimidazoles with 2-substituted-2-nitropropanes and p-nitrobenzyl chloride clearly showed that the production of N-alkylated imidazoles proceeded largely via a pathway involving radical and radical-anion intermediates. The results are summarised in Table 2.1.

It can be seen from the data in the table that the reactions are completely inhibited by passing a stream of oxygen through the reaction mixtures. Oxygen is a stable diradical and an efficient scavenger of carbon free-radicals, including the 2-nitropropyl and p-nitrobenzyl radicals. The radicals add to oxygen to form peroxy radicals which ultimately break down to acetone and nitrite and p-nitrobenzyl alcohol, respectively. Di-(tert-butyl)-nitroxide is also a stable free radical which is known to trap carbon radicals. The addition of only 10 mol % di-(tert-butyl)-nitroxide (DTBN) to the reaction between 2-bromo-2-nitropropane and the nitroimidazole anion [51] significantly depresses the synthesis of 1-alkynitroimidazole; while inhibition is only partial in the reaction with p-nitrobenzyl chloride. However, addition of 25 mol % DTBN significantly inhibited the latter reaction.

p-Dinitrobenzene [p-DNB] forms a relatively stable radical-anion and will accept an electron from other radical-anions and electron donors readily. It has been used as a diagnostic method for the $S_{\text{RN}1}$ mechanism because it is able to intercept intermediate radical-anions in the free radical, radical-anion chain sequence and thus inhibit the reaction. However, addition of 5 mol % to the reaction...
between the nitroimidazole anion and 2-bromo-2-nitropropane and 10% to that between the anion and p-nitrobenzyl chloride gave little inhibition. It has been pointed out\textsuperscript{123} that if one of the intermediate radical-anions has a similar reduction potential to that of p-DNB, then inhibition will be poor, i.e. the radical-anion which is an intermediate in the chain reaction may not readily undergo single electron transfer (s.e.t.) to p-DNB. In view of this, the poor inhibition does not preclude the reaction proceeding via radical-anion intermediates. Oxygen is also thought to undergo electron transfer with radical-anions\textsuperscript{96} (equation 2.11) and may well be intercepting the radical-anion intermediate as well as acting as a radical trap.

\[
O_2 + [RA]^- \rightarrow RA + [O_2]^2^{-} \tag{2.11}
\]

Wrapping the reaction flask in aluminium foil to exclude all light results in a reduction in the yields of 1-alkynitroimidazoles indicating a level of light catalysis. Strong red colours were observed in all the reactions which faded on completion. These colours may be caused by charge-transfer complexes between nitroimidazole anions and substrates prior to light catalysed s.e.t.

These results of the inhibition studies are similar to those observed for other reactions known to proceed by the \( S_{RN1} \) mechanism\textsuperscript{92,109,117,119} and thus are good evidence for assigning the \( S_{RN1} \) mechanism to these reactions as fully illustrated by scheme 2.1.

The mechanism is a chain process with initiation, propagation, and termination steps. The initiation occurs by an electron transfer from the nitroimidazole anion \([51]\) and \([52]\) to 2-substituted-2-nitropropane (and p-nitrobenzyl chloride) to give the radical-anion of the latter (equation 2.12). In the absence of inhibitors, the radical-anions break down to form 2-nitropropyl and p-nitrobenzyl radicals, and bromide and chloride anions respectively (equation 2.13). An electron transfer between the newly formed radical-anions and molecules of the starting material complete the propagating cycle. (equation 2.15). Radical-anions are known to be able to transfer electrons readily\textsuperscript{92}.
The termination steps of the sequence are not well understood. Dimerisation of the intermediate 2-nitropropyl and p-nitrobenzyl radicals before coupling with anions may be one route to termination. Alternatively, the same radical could abstract an hydrogen atom from the solvent to form 2-nitropropane or p-nitrotoluene respectively, which cannot re-enter the propagation cycle.

**N-Alkylation of 2-nitroimidazole**

The anion of 2-nitroimidazole, [53], also underwent $S_{RN1}$ reactions with the 'benzylic' substrates [58], and ([59] and [60]) to give the expected products, [69] and [70] (equations 2.16 and 2.17), but failed to react with Me$_2$C(X)NO$_2$ even under forcing conditions. The results are presented in Table 2.1. In the reactions of Me$_2$C(X)NO$_2$ (X=Br and NO$_2$), 2,3-dimethyl-2,3-dinitrobutane was also isolated, resulting from abstraction of the $\alpha$-substituent to yield the anion of 2-nitropropane (Me$_2$C(NO$_2$)$_3$) (see later) and subsequent reaction$^{109,119}$ of this anion with Me$_2$C(X)NO$_2$ by the $S_{RN1}$ mechanism.
Proof of Structure

The generally known synthetic procedures for 1-substituted-4- and 5-nitroimidazoles involve either the introduction of a nitro group by electrophilic substitution of imidazoles or alkylation (arylation) of the N-1 nitrogen atom. The ultimate orientation of the nitro group, however, depends on a number of factors, the most important of which appear to be the nature of the alkylating agent used and the reaction conditions employed. (This was discussed fully in the introduction).

The 5-nitro derivatives, however, generally possess significantly greater biological activity than the 4-nitroisomers and hence it is important that an unambiguous method of structure assignment be available. Previous attempts to provide such a method have included the application of i.r., u.v., $^1$H n.m.r. and $^{13}$C n.m.r. spectroscopy.\textsuperscript{124}

The use of $^1$H n.m.r. spectroscopy chemical-shift data
for structural assignment is limited because the $b$-values are dependent on the solvent used for analysis and concentration of individual compound in solution, and more importantly, the difference in chemical shift values ($^1$H n.m.r. spectroscopic data for some representative 1-substituted-4- and 5-nitroimidazoles are presented in Table 2.2) is too small for accurate structural assignment.

$^{13}$C n.m.r. spectroscopic chemical shifts of a variety of substituted imidazoles have been reported$^{124}$ and data for some 4(5)-nitroimidazoles and 1-substituted-4- and 5-nitro isomers of established structure are summarised in Table 2.3. These show that the values for the ring carbon atoms are definitive for the groups of compounds and can thus be used to assign the position of the nitro group.

The identity of products obtained from radical N-alkylation was determined by comparison of the $^{13}$C n.m.r. spectroscopic chemical shift values with those of the known nitroimidazoles (Table 2.3). $^{13}$C n.m.r. spectroscopy clearly identifies the position of the nitro group, i.e. the signal for $C_4$ in the $^{13}$C n.m.r. spectrum in the 5-nitroisomer ranges from 131-134 p.p.m., whereas the signal for $C_5$ in the 4-nitro isomer is 119-123 p.p.m. It is assumed that the nature of the 1-alkyl group would not significantly alter the range. The structure and $^{13}$C n.m.r. spectroscopic chemical shift data for two representatives of radical N-alkylated nitroimidazoles [62] and [65] are presented below:

![Chemical structures](attachment:image.png)

The chemical shift data for the rest of the products are presented in the experimental section.

These analyses showed that the products of radical N-alkylation are all the 4-nitro isomers.
Table 2.2 Representative $^1$H n.m.r. data for N-1 alkynitroimidazole derivatives

<table>
<thead>
<tr>
<th>Chemical Shift ($\delta$ - ppm)</th>
<th>Proton No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C$_2$-Me</td>
</tr>
<tr>
<td>O$_2$N-</td>
<td>2.48</td>
</tr>
<tr>
<td><img src="image1" alt="Chemical Structure 1" /></td>
<td></td>
</tr>
<tr>
<td>O$_2$N-</td>
<td>2.43</td>
</tr>
<tr>
<td><img src="image2" alt="Chemical Structure 2" /></td>
<td></td>
</tr>
<tr>
<td>O$_2$N-</td>
<td>2.45(s)</td>
</tr>
<tr>
<td><img src="image3" alt="Chemical Structure 3" /></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Chemical Shifts</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td>C(_2)-Me</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>150.64(s)</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>151.14</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>144.17</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>145.66</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>145.23(s)</td>
</tr>
</tbody>
</table>

a) ref 124
2.2 Synthesis using oxidative addition reactions

The first oxidative addition was reported in 1961 for the preparation of geminal dinitroalkanes\textsuperscript{(125)} (equation 2.18).

\[
\begin{align*}
R^2 & \quad \text{AgNO}_2, \text{NaNO}_2 \quad R^2 \\
\text{NO}_2 & \quad \text{NO}_2 \\
\end{align*}
\]

The mechanism, elucidated 3 years later invoked the initial oxidation of the nitro-anion \textsuperscript{(71)} by an oxidizing agent, \textsuperscript{(126)} \text{Ag}^+ to give a nitro-radical (equation 2.19) which reacted with the nitrite to yield a dinitro radical-anion (equation 2.20). The radical-anion then yielded the dinitroalkane product after transfer of an electron from the product to the oxidizing agent (scheme 2.2).

\[
\begin{align*}
R_2^\text{C} = \text{NO}_2^- + \text{Ag}^+ & \quad \text{s.e.t.} \quad R_2^\text{CN}O_2^- + \text{Ag}^0 \\
R_2^\text{CN}O_2^- + \text{NO}_2^- & \quad \text{s.e.t.} \quad [R_2^\text{C(NO}_2^-)_2]^+ \\
[R_2^\text{C(NO}_2^-)_2]^+ + \text{Ag}^+ & \quad \text{s.e.t.} \quad R_2^\text{C(NO}_2^-)_2 + \text{Ag}^0 \\
\end{align*}
\]

Scheme 2.2 Oxidative addition reaction mechanism

Matacz et al.\textsuperscript{(127)} showed in 1979 that aqueous potassium ferricyanide can replace expensive silver salts. This useful reagent was used\textsuperscript{(127)} to prepare various secondary nitro compounds, for example, equation 2.22.

\[
\begin{align*}
\text{Me}_2^\text{C} = \text{NO}_2^- + \text{Fe(CN)}_6^{3-} & \quad \text{s.e.t.} \quad \text{Me}_2^\text{C(A)NO}_2 \\
\end{align*}
\]

Scheme 2.2 Oxidative addition reaction mechanism

Bowman and Rakshit\textsuperscript{(118)} used this simple, cheap reagent to prepare a series of heterocyclic compounds (equations 2.23 and 2.24).

The nitroimidazole anions \textsuperscript{(51)} and \textsuperscript{(52)}, were successfully reacted with anion of 2-nitropropane \textsuperscript{(61)} (equation 2.25).
in water and CH₂Cl₂ using 2 equivalents of potassium ferri-

cyanide to yield the corresponding 4-nitroimidazolyl deriv-

ative.

Recent improvements,¹²⁰ using only a catalytic amount

des, and sodium persulphate to re-
generate the ferricyanide from ferrocyanide has been reported

equation 2.26).

(2.26)

The oxidation addition reaction using the catalytic

eck step in the oxidation sequence is the addition of
<table>
<thead>
<tr>
<th>Nitroimidazole</th>
<th>Conditions(^b)</th>
<th>K(_3)Fe(CN)(_6) (Equiv.)</th>
<th>1-[Me(_2)C(NO(_2))]2,3-dimethyl-nitroimidazole</th>
<th>% Yield(^a) 2,3-dimethyl-2,3-dinitrobutane</th>
<th>Unaltered Nitroimidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>4(5)-Nitroimidazole</td>
<td>60 min</td>
<td>1.5</td>
<td>21([72], R(^1)=H)</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>1.5</td>
<td>8</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>0.2(^c)</td>
<td>34</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>2-methyl-4(5)-</td>
<td>5 min</td>
<td>1.5</td>
<td>(14)([72], R(^1)=Me)</td>
<td>(13)</td>
<td>-</td>
</tr>
<tr>
<td>nitroimidazole</td>
<td>30 min</td>
<td>1.5</td>
<td>(13)</td>
<td>(15)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>1.5</td>
<td>(12)</td>
<td>(20)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>1.75</td>
<td>16, 17</td>
<td>14, 14</td>
<td>19, 9</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>2.0</td>
<td>9, 12</td>
<td>2, -</td>
<td>24, -</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>0.2(^c)</td>
<td>31</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>2-nitroimidazole</td>
<td>75 min</td>
<td>0.2(^c)</td>
<td>0</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\) % Yield base on starting nitroimidazole; yields analysed by \(^1\)H n.m.r. are in parenthesis.
\(^b\) All reactions were carried out in CH\(_2\)Cl\(_2\)-H\(_2\)O under nitrogen with Me\(_2\)CNO\(_2\)\(^-\) (1.5 equiv.) and NaOH (2.5 equiv.).
\(^c\) Plus Na\(_2\)S\(_2\)O\(_8\) (2 equiv.)
Scheme 2.3: Oxidative addition of 4(5)-nitroimidazole anions to \( \text{Me}_2\text{CNO}_2^- \)

Experimentally, the best yields of 1-alkyl-4-nitroimidazoles [72] were obtained when one molar equivalent of sodium persulphate (2 redox equivalents) and only a catalytic amounts of \( \text{K}_3\text{Fe(CN)}_6 \) were used. The oxidation using only potassium ferricyanide \(^{118,127}\) in the initial studies gave poor yields of 1-alkylated products and longer reaction times only resulted in the production of more of the 2,3-dimethyl-2,3-dinitrobutane by-product. This by-product results from the addition of the \( \text{Me}_2\text{CNO}_2^- \) [61] to the intermediate \( \text{Me}_2\text{CNO}_2^- \) radical in competition with the nitroimidazole anion (equation 2.30). Traces of 2,2-dinitropropane...
were also obtained in all the reactions. This by-product arises from the addition of nitrite anion to the Me₂CNO₂ radicals. The origin of the nitrite anion is not known.

**Reasons for the Regio-selectivity of the N-Alkylations**

The addition of nucleophiles to radicals is a key step in all SRN₁ reactions. Further evidence is provided by the oxidative addition of anions to Me₂CNO₂ radicals. The second step (equation 2.28) is the same as the third step of the SRN₁ mechanism (equation 2.14, scheme 2.1). The following anions add to Me₂CNO₂ radicals: NO₂⁻, CN⁻, PhSO₂⁻, SCN⁻, azide, and heterocyclic thiolates.

When two anions compete for the same radical, the most nucleophilic anion will react faster to form the corresponding radical-anion. For instance, enolate anions react preferentially over nitronate anions with α-keto-radicals (RCHCOR). The proposal has been made that the addition of anions to radicals is kinetically controlled, therefore the strongest base (nucleophile) will add preferentially, even though the product may be less stable.

The proposal has been applied to the addition of ambident anions to Me₂CNO₂ radicals, which are generated by oxidation of Me₂CNO₂⁻ in oxidative addition reactions, or as intermediates in SRN₁ reactions. All the ambident anions reported in the literature react selectively at the more nucleophilic centre; aryl thiolates (S not C), enolates (C not O), nitronates (C not O), thiocyanates (S not N), sulphinates (S not O) and dialkyl phosphites (P not O).

The addition of the anion of 2-pyridinethiol is of interest. The N-centred anion is ca. 10⁴ times more basic than the S-centred anion but only addition via the sulphur is observed. The S-anion is, however, more nucleophilic towards carbon than the N-anion, which suggests that nucleophilicity rather than basicity is the dominating factor in the kinetic control (equations 2.32 and 2.33).

The reason for the kinetic control is because the stability of the new radical-anion is not reached until well after the transition state. In the above examples the transition state will be the radical-anion with the unpaired electron in the σ* MO of the C-Nu bond, which then relaxes via reorganisation of the MO's to yield the nitro
These results, in which the less stable product is formed, suggest that the earlier proposal of the thermodynamic control in the $S_{RN1}$ reactions of $p$-nitrobenzyl substrates is unlikely.

4(5)-Nitroimidazole anions are ambident and are able to react via either of the N-centres to yield 4- or 5-nitroimidazole products, which is one of the major problems of nitroimidazole chemistry. In these $S_{RN1}$ reactions, the anions [51] and [52] can react with the intermediate radicals (R$^1$) (equation 2.14) to yield either 1-alkyl-4-nitroimidazole radical-anions [68] or the analogous 5-nitroimidazole radical-anions. In most of the reactions the 4-isomer is exclusively formed. Detailed searching for traces of any 5-nitro isomer in two reactions showed an absence of the 5-nitro isomer in the reaction between $Me_2C(Br)NO_2$ and the anion, [51], and traces of the 5-isomer were observed by $^{1}H$ and $^{13}C$ n.m.r. spectroscopy in the reaction between the anion, [51], and $p$-nitrobenzyl chloride; the 5-isomer was not isolated. The position of the nitro group in all these reactions was determined particularly by the $^{13}C$ n.m.r. spectroscopy which clearly distinguishes between the 4- and 5-nitro isomers (see previous section).

The regio-selectivity observed in the $S_{RN1}$ reactions is the same as observed in the reaction between the anion of 4(5)-nitroimidazole [51], and dimethyl sulphate $^{64a,67}$ (equation 2.34) (see introduction). In this reaction the regio-selectivity (the ratio of the 4-nitro to the 5-nitro products was $ca. 8:1$) was explained by the greater nucleophilicity of the nitrogen atom further away from the nitro group (i.e. higher electron density on this nitrogen as
opposed to the nitrogen atom adjacent to the nitro group).\textsuperscript{64a,67}
The kinetic control of the attack of the ambident 4(5)-nitroimidazole anions by the electrophile, Me\textsubscript{2}SO\textsubscript{4}, can be readily compared to attack by the strongly electrophilic radical, MeCN0\textsubscript{2}·.

It is therefore proposed that the same logic applies to radical reactions, i.e. kinetic control of the nucleophilic attack by the nitroimidazole anions [51] and [52] (equation 2.14, scheme 2.1) to yield 4-nitroimidazole radical-anions [68]. In S\textsubscript{RN}· reactions involving ambident anion, exclusive formation of products via the most nucleophilic centre has, so far, always been observed.\textsuperscript{118,133}

These results provide further confirmatory evidence that the addition of ambident anions to radicals is under kinetic control.\textsuperscript{131}

**Steric Effects in the S\textsubscript{RN}· and Oxidative Addition Reactions of Nitroimidazole Anions**

Several of the above reactions appear to be affected by steric hindrance even though S\textsubscript{RN}· reactions are not easily influenced by steric factors.\textsuperscript{131c,134a}

Norris and co-workers\textsuperscript{134} have, however, shown that there is a level of steric hindrance at which addition of the nucleophile to the intermediate radical is blocked. They\textsuperscript{134} have tested the steric limits of the S\textsubscript{RN}· reactions of 2-nitrobenzyl derivatives. At a certain steric bulk of the anion and the intermediate radical, addition is hindered, and other reactions predominate, e.g. reduction. An example of the steric limit is shown below in the reaction of malononitrile anions.

In the reactions of nitroimidazoles, steric hindrance is indicated by; 1) the preference for 4-nitro-over 5-nitro-products, 2) the faster rates of reaction between the less
hindered anion of 4(5)-nitroimidazole [51], relative to the more hindered anion of 2-methyl-4(5)-nitroimidazole, [52], and the sterically crowded nitropropanes, MeC(X)NO₂, 3) the lack of reactivity of 2-nitroimidazole anions with Me₂C(X)NO₂ and Me₂CNO₂-, and 4) the faster rates of reaction for all imidazole anions with p-nitrobenzyl chloride as compared with Me₂C(X)NO₂. It can be suggested, therefore, that substituents in the α-positions of the imidazole anions (2-NO₂, 5-NO₂, and 2-Me) cause steric hindrance in the addition of these anions to radicals, especially to more bulky radicals (i.e., Me₂CNO₂ as compared to p-NO₂-C₆H₄-CH₂). Therefore the preferential formation of 4-nitroisomers relative to 5-nitroisomers is caused by electronic and steric factors.

In order to confirm these proposals, competitive reactions were carried out. Evidence suggests¹³⁵ that the slowest step in the S₉N₁ chain is the dissociation of the first intermediate radical-anion (equation 2.13 scheme 2.1). Therefore, reactions proceeding by radical-anions which dissociate faster would be predicted to react faster. Studies of the dissociation of radical-anions at low temperature using e.s.r. spectroscopy have shown that the radical-
For e.g.

steric bulk in 5-nitro anion

relief of steric bulk in 4-nitro anion

major product

anions of \( \text{Me}_2\text{C(X)NO}_2 \) dissociate considerably faster than the radical anion of \( p\)-nitrobenzyl chloride\(^\text{136}\) (the order observed is \( X = I > Br > Cl \gg p\)-\( \text{NO}_2\)-\( \text{C}_6\text{H}_4\text{CH}_2\text{-Cl} \)) but the reverse order of reactivity is apparently observed with nitroimidazole reactions. Therefore, both imidazole anions \([51]\) and \([52]\) were reacted with a mixture of \( \text{Me}_2\text{C(Br)NO}_2 \)
and \( p\)-\( \text{NO}_2\)-\( \text{C}_6\text{H}_4\text{-CH}_2\text{Cl} \) under similar reaction conditions (scheme 2.4).

Products from \( p\)-\( \text{NO}_2\)-\( \text{C}_6\text{H}_4\text{-CH}_2\text{Cl} \) were preferentially formed over products from \( \text{Me}_2\text{C(Br)NO}_2 \) with both anions, and lower yields (lower rates) were observed for the more hindered anion, \([52]\), providing confirmatory evidence for steric hindrance. Caution must however be practised in chain reactions, in which the initiating reactions (equation 2.12, scheme 2.1) may influence observed results.

Mechanism of the Reaction between Nitroimidazole Anions and Radicals

In the introduction, it was proposed that the lack of reaction between N-centred anions or amines and radicals in \( S_{RN-1} \) reaction is because of the high energy of the C-N \( \sigma^* \) SUMO in which the unpaired electron of the resultant radical-anions reside. However, certain reactions have been reported, the most notable being the \( S_{RN-1} \) reactions between \( p\)-nitrocumyl chloride and amines\(^\text{137}\) (see introduction).

Nitrite anion has only been reported in one \( S_{RN-1} \) reaction, i.e. with \( p\)-nitrocumyl chloride (equation 2.36), yielding \( p\)-dinitrocumene in 94% yield.

However, the reaction does not proceed unless an entrainment technique is used. The lack of reaction between
Scheme 2.4: Competitive reactions between [55] and [58]

Nitrite and various 2-substituted-2-nitropropanes, Me₂C(X)NO₂ with X = Cl, Br and I, under conditions favouring the $S_{RN}^{-1}$ mechanism is unusual. Even using the entrainment technique with Me₂CNO₂⁻ gives no reaction. However, the lack of reaction also appears to be caused by problems with the initiation step because nitrite anion adds easily to the radical intermediate, Me₂CNO₂⁻, and the intermediate radical-anion
is a common intermediate. The oxidative addition of nitrite anions to various nitronate anions proceeds in high yields. It should be noted that the step (equation 2.38, scheme 2.3) is the same as the third step of the potential $S_{RN1}$ mechanism for nitrite anion and $Me_2C(X)NO_2^-$.

Azide anions participate in $S_{RN1}$ reactions with several substrates (see introduction section).

In order to explain why nitroimidazole anions are able to react, and $sp^3$ hybridized $N$-nucleophiles do not react, the following are proposed: the electrons in the imidazole anion are symmetrically delocalised in the π-molecular orbitals of the ring, and therefore when the anion reacts with a radical the initially formed radical-anion has the unpaired electrons in a π* MO of relatively low energy and not in a C-N σ* MO, thereby allowing the reaction to take place. The nitro group will further lower the energy of the SOMO by conjugation. The initially formed radical-anion almost certainly undergoes smooth re-organisation of molecular orbitals to form a radical-anion (scheme 2.5) of lower energy consisting of the unpaired electron in a NO$_2$/aromatic, π* SOMO and with a C-N σ* molecular orbital. Thus, the lone pair of electrons of the anion [51] which were in a $sp^2$ orbital undergo smooth reorganisation into the p-orbital (and part of the aromatic system) of the product radical-anion [76]. A determination of the structure of these radical-anions using electron spin resonance spectroscopy at low temperature has been carried out (see later).

Reactions between Anions and 2-(Halomethyl)-1-methyl-5-nitroimidazoles

The reaction between the anion of 2-nitropropane and
2-(chloromethyl)-1-methyl-5-nitroimidazole was carried out to give 1-(1-methyl-5-nitroimidazol-2-yl)-2-methylprop-1-ene (equation 2.37). At the same time as our initial investigation, Crozet and Surzur\textsuperscript{140} reported the same reaction. Therefore, further investigations were not carried out. A corresponding reaction using the anion of 1-nitroethane in place of Me\textsubscript{2}CNO\textsubscript{2} did not yield the corresponding product.

The present work provides further examples of SRN\textsubscript{1} reactions between these 2-halomethyl-nitroimidazoles and anions [51] and [52].

Although inhibition studies of these reactions were not carried out, it is unlikely that the mechanism would differ from that observed for the reaction between the same anions and p-nitrobenzyl chloride. 2-(Halomethyl)-5-nitroimidazoles therefore appear to show similar SRN\textsubscript{1} reactivity to that of the analogous p-nitrobenzyl- and p-nitrocumyl-halides, 2-(halomethyl)-5-nitrofurans,\textsuperscript{105} and \(\alpha\)-substituted 2-(2-propyl)-5-nitrothiophenes.\textsuperscript{104}

**SRN\textsubscript{1} Reactivity of 1-(1-Methyl-1-nitroethyl)-4-nitroimidazoles**

2-Substituted-2-nitropropanes, Me\textsubscript{2}C(X)NO\textsubscript{2}, react with nucleophiles by the SRN\textsubscript{1} mechanism with loss of either of the \(\alpha\)-substituent (X) or nitrite anion i.e. the intermediate radical-anion, Me\textsubscript{2}C(X)NO\textsubscript{2} is able to dissociate to either Me\textsubscript{2}CNO\textsubscript{2} and X\textsuperscript{−}, or Me\textsubscript{2}CX and NO\textsubscript{2} (see introduction).\textsuperscript{109,119-120} The former route is observed for X=halogen, SCN, SR, S(O)R, SO\textsubscript{2}R, and NO\textsubscript{2} and the latter route for X=COR, CO\textsubscript{2}R, R, N\textsubscript{3}, NO\textsubscript{2}, and p-NO\textsubscript{2}-C\textsubscript{6}H\textsubscript{4}N\textsubscript{3}i.e. the rate and direction of dissociation is largely determined by relative bond
strength and nucleofugicity, but the structure of the radical-anion (i.e. the location of the unpaired electron) has only small influence. It appears that when the relative nucleofugicities are similar that the structure is important, e.g. $N_3^-$ is a better nucleofuge than $NO_2^-$ in polar $S_{N2}$ reactions but vice versa for $S_{RN1}$ reactions.

This dissociation behaviour therefore poses a question regarding the $S_{RN1}$ reactions between 2-halo-2-nitropropanes and 4(5)-nitroimidazole anions: i.e. why does the product of the reaction, 1-(1-methyl-1-nitroethyl)-4-nitroimidazole and its 2-methyl analogue, not react further as shown in scheme 2.6 by Route A to yield a disubstituted product (e.g. [78], with $Nu=4$-nitroimidazole). The question can be answered by proposing that the nucleofugicity of 4(5)-nitroimidazole anions is superior to that of nitrite anions in radical-anions, that is, further $S_{RN1}$ reactions between the $N$-alkylated products [72] and the 4(5)-nitroimidazole anion [51] does not yield new products because the nucleofuge equals nucleophile as illustrated by Route B in Scheme 2.6.

In order to test this hypothesis, and to test the $S_{RN1}$
reactivity of 1-(1-methyl-1-nitroethyl)-4-nitroimidazoles, several reactions were carried out. The results are shown in Table 2.5. As expected, reaction between the nitroimidazole [72], with R=H [62], and the anion of 4(5)-nitroimidazole [51], gave unaltered starting materials. However, reaction between both of the nitroimidazoles, [72] with R=H [62], and R=Me [63], and Me₂CNO⁻ gave the S<sub>RN</sub>₁ product 2,3-dimethyl-2,3-dinitrobutane in reasonable yields (Route B, Scheme 2.6, Nu = Me₂CNO⁻). The formation of the product was inhibited in both cases by addition of radical traps (di-t-butyl nitroxide and O₂) and strong electron acceptors (p-dinitrobenzene and O₂) thereby indicating S<sub>RN</sub>₁ mechanisms. The results are shown in Table 2.5.

The nitroimidazole, [72] with R = Me [63], was reacted with the anion of 4(5)-nitroimidazole on the basis that the 2-methyl-4(5)-nitroimidazole [52] would act as a better nucleofuge than 4(5)-nitroimidazole [51] in this potentially reversible reaction (scheme 2.6 with Nu = [51]) due to steric hindrance (see earlier section). This predicted steric effect was observed with 47% of the S<sub>RN</sub>₁ product [62] being formed (equation 2.38 Table 2.5) and only 26% of the unaltered starting material [63].

As the final confirmation of nucleofugicity proposal, the nitroimidazole, [72] with R = Me [63], was reacted with sodium benzene sulphinate. No reaction would be expected because the loss of phenylsulphinate from the intermediate radical-anion [Me₂C(SO₂Ph)NO₂]⁻ would be much faster than loss of the nitroimidazole anion [52], from the intermediate radical-anion, [68] with R = Me. Indeed, no reaction was observed and only starting materials were recovered. These results show that nitroimidazole anions are able to act as nucleofuges as well as nucleophiles in the S<sub>RN</sub>₁ reactions of 2-substituted-2-nitropropanes and that nitroimidazoles are better nucleofuges (Route B, scheme 2.6) than nitrite.
### Table 2.5 Reaction between 1-(1-methyl-1-nitroethyl)-4-nitroimidazoles [72] and nucleophiles

<table>
<thead>
<tr>
<th>1-(-methyl-1-nitroethyl)-4-nitroimidazole [72]</th>
<th>Nucleophile</th>
<th>Conditions</th>
<th>Product [81]</th>
<th>% Yield$^a$</th>
<th>Unaltered$^b$</th>
<th>Unreacted Nucleophile$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me$_2$CNO$_2$</td>
<td>DMSO, 24 h</td>
<td>-</td>
<td>[81] $^\text{Nu}$=Me$_2$C(NO$_2$)</td>
<td>(30), (35) (28, (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMF, 31 h</td>
<td>-</td>
<td></td>
<td>6, 81</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DTBN$_d$ (50 mol. %)</td>
<td>16</td>
<td>40; 40, 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMF, 31 h</td>
<td>-</td>
<td></td>
<td>16; 40, 32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^\text{p-DNB}$ (40 mol. %)</td>
<td>4; 10</td>
<td>64; 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMF, 4.5 h; 11 h</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[72] R=Me</td>
<td>[51]</td>
<td>DMSO, 14 h</td>
<td>[72], 47 R=H</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me$_2$CNO$_2$</td>
<td>DMSO, 14 h</td>
<td>-</td>
<td>[81] $^\text{Nu}$=Me$_2$C(NO$_2$)</td>
<td>(54); (51) 0; (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMF, 4.5 h; 5 h</td>
<td>-</td>
<td>12; 7; 12; 49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMF, DTBN$_d$ (50 mol. %)</td>
<td>13</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5 h; 5 h</td>
<td>-</td>
<td></td>
<td>13; 49, 89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMF, 4.5 h, O$_2$, dark</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^\text{p-DNB}$ (40 mol. %)</td>
<td>16</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhSO$_2$</td>
<td>DMSO, 24 h</td>
<td>0</td>
<td>(86)</td>
<td>(88)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

$^a$ % Yields were calculated by $^1$H n.m.r. spectroscopy using an internal standard. Isolated yields are shown in parenthesis. $^b$ Isolated as the conjugate acid. $^c$ 3 Equiv. $^d$ DTBN = di-t-butyl-nitroxide. $^e$ p-DNB = p-di-nitrobenzene.
anions (Route A, scheme 2.6) from intermediate radical-anions, [68].

The Use of E.s.r. Spectroscopy to show the Presence of Radical and Radical-anions and to predict the direction of $S_{RN1}$ Reactions

Mechanistic studies have suggested that in the $S_{RN1}$ mechanism, electron-capture by substrates, $RC(\cdot)N0_2$, yields intermediate radical-anion with finite lifetimes (equation 2.39). These substrates usually have a low energy LUMO into which the transferred electron is localised (e.g., nitro group) and form a 'stable' radical-anion. These radical-anions then dissociate rapidly in the second step of the $S_{RN1}$ mechanism (equation 2.39) to radicals and anions.

$\text{R}_2C(\cdot)N0_2 + e^- \rightarrow \text{R}_2\cdot + NO_2^- \quad (2.39)$

Dissociative electron-capture

In studies of $\alpha$-substituted nitro-compounds proceeding by the $S_{RN1}$ mechanism, loss of the $\alpha$-substituent ($X$) from the intermediate radical-anion has been observed for $X = I, Br, Cl, SO_2R, SR, S(O)R, \text{and SCN};$ while loss of nitrite has been observed for $X = \text{COR, CO}_2R, \text{CN, NO}_2, \text{P}_2\text{NO}_2\text{C}_6\text{H}_4\text{N}_2,$ and Me.

The use of e.s.r. spectroscopy to detect and identify the radical-anion, and radical species postulated as intermediates in $S_{RN1}$ mechanisms has proved to be of some importance in providing evidence for these species. E.s.r. spectroscopy at low temperature has been used to provide evidence for the $S_{RN1}$ reactions of 2-substituted-2-nitropropanes [Me$_2C(\cdot)N0_2$], $\alpha$-substituted-2-methyl-5-nitrofurans, and $p$-nitrobenzyl- and $p$-nitrocumyl derivatives.

There are two different radical-anion intermediates in $S_{RN1}$ reactions (e.g., equations (2.13) and (2.14), scheme 2.1), the latter is more stable and a number have been observed using e.s.r. spectroscopy, e.g. $[\text{R}_2C(\cdot)0_2]\text{C(\cdot)NO}_2\text{R}_2^\cdot$ from the addition of $\text{R}_2\text{CNO}_2^-$ anions to $\text{RCNO}_2$ radicals, and
p-nitrobenzyl derivatives. E.s.r. spectroscopy at low temperature has been successfully used in providing evidence for unstable species.

1-(1-Methyl-1-nitroethyl)-4-nitroimidazole [62] and 1-(p-nitrobenzyl)-4-nitroimidazole derivatives are structurally analogous to \( \text{Me}_2C(X)\text{NO}_2 \) and \( p-\text{NO}_2-C_6H_4-\text{CH}_2-X \) respectively. Consequently, the interest to use e.s.r. spectroscopy to confirm that these compounds do also accept electrons to form radical-anions at low temperature as their structural analogues, and thus confirm solution \( SRN_1 \) reactions. Secondly, whether the pattern of dissociation of the initial radical-anion formed can predict the direction of reaction observed in solution reaction at room temperature.

Three representative compounds synthesised in these studies, [62], [65] and [69], were submitted to Prof. Symons of Leicester University who carried out the e.s.r. spectroscopy studies. A brief experimental procedure and relevant results and discussion from Prof. Symons' studies are presented.

Degassed samples were irradiated as dilute solutions (ca. 1% v.v.) in \( \text{CD}_3\text{OD} \) or MeTHF. They were frozen as small beads in liquid nitrogen and irradiated at 77K in a Vickrad \( ^6\text{Co} \) \( \gamma \)-ray source to doses of up to 1 Mrad. E.s.r. spectra were measured on a Varian E109 spectrometer. Samples were annealed to selected temperatures or until changes occurred in the e.s.r. spectra, and re-cooled to 77K for study. For the \( \text{CD}_3\text{OD} \) systems solute radicals were detectable at 77K, but for the MeTHF systems, it was necessary to anneal until the solvent radical features were lost before well defined solute features were observed.

The e.s.r. spectrum of the radical-anion of 1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62] closely resembles that of 1,2-dimethyl-4-nitroimidazole [79] and the 5-nitro analogue [2] (all the 4- and 5-nitroimidazoles radical-anion are similar) suggesting that the unpaired electron resides in the ring nitro group in preference to the aliphatic nitro group. However, detection of \( \text{Me}_2\text{CNO}_2 \) radicals in the spectrum suggests that electron addition to both nitro
groups occur, the latter giving only the dissociation product. No overlap of MO's between the two nitro groups was observed.

In contrast, the values obtained in the e.s.r. spectrum for the radical-anions of 1-(p-nitrobenzyl)-4-nitroimidazole [65] indicates that they have a similar structure to other \([\text{p-O}_2\text{N-C}_6\text{H}_4-\text{CH}_2-X]\)^2 radical-anions,\(^{141}\) with the electron on the aryl p-nitro group and not on the imidazole nitro group (equation 2.40).

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{R} & \quad \text{Me} \\
\text{CH}_2 & \quad \text{NO}_2
\end{align*}
\]

One of the key steps in the \(S_{RN1}\) mechanism is the dissociation of the intermediate radical-anions (scheme 2.1, equation 2.13). Therefore, any data obtained from the e.s.r. studies pertaining to this step provides useful evidence for this stage of the reaction.
The lack of dissociation observed for the radical-anions of 1-(p-nitrobenzyl)-4-nitroimidazole [65], \( ^{-}O_{2}N-C_{6}H_{4}-CH_{2}-X \) with \( X = 1-(4\text{-nitroimidazole}) \) is in keeping with the lack of dissociation observed under these conditions for \( ^{-}O_{2}N-C_{6}H_{4}-CH_{2}-X \) derivatives.

The 4-nitroimidazole analogue, the radical-anion of 1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62] does, however, exhibit dissociation to \( \text{Me}_{2}C\text{NO}_{2} \) radicals. This observation can be explained by two different radical-anions being formed on electron capture by the imidazole [62], these are summarised in equations 2.41 and 2.42. These

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{N} & \quad \text{O}_2\text{N} \\
\text{NO}_2 & \quad \text{NO}_2
\end{align*}
\]

results support the observed \( S_{RN1} \) reactions (discussed previously, e.g. equation 2.38) for the 1-(1-methyl-1-nitroethyl)-4-nitroimidazole derivatives, i.e. loss of 4-nitroimidazolyl anions rather than loss of nitrite anions from the intermediate radical anion [62]. None of the 2-, 4-, or 5-nitroimidazole radical-anions observed by e.s.r. spectroscopy dissociated to give species which suggests that loss of nitrite anions do not take place.

The biological implication of these results are discussed later.

**Non-Radical N-Alkylation of 4(5)-Nitroimidazoles**

Most of the clinically-used nitroimidazoles are synthesised by N-alkylation under neutral conditions (N-alkylation of unsymmetrical imidazoles has been discussed in the introduction). N-Alkylation of nitroimidazoles under basic conditions, which proceeds by an \( S_{B2CB} \) mechanism, generally gives rise to the 4-nitro isomers, while under neutral conditions, the reaction proceeds by an \( S_{B21} \) mechanism to give the 5-nitro isomer.
Consequently, a number of alkylation were carried out in neutral solution with the aim of synthesising new 5-nitroimidazole analogues with electron accepting groups (NO$_2$) in the N-side chain.

The attempt to react 4(5)-nitroimidazole with 2-nitropropyl tosylate (equation 2.43) under neutral conditions gave an intractable gummy material. Although, the synthesis was reported in a U.S. Patent it was not possible to obtain the expected product. Similarly, the reaction between the 4(5)-nitroimidazole and bromonitromethane failed, probably because the lone pair of electrons on the pyridine nitrogen of the nitroimidazole abstracted bromine from bromonitromethane to form a salt which does not react further. (equation 2.44). The reactions were not investigated further.

\[
\begin{align*}
\text{O}_2\text{N} & + \text{Me-} \text{SO}_2 & \text{HCNO}_2 \text{CH}_3 & \rightarrow \text{O}_2\text{N} & + \text{SO}_3 \text{Me} \\
\text{[80]} & & & & (2.43)
\end{align*}
\]

**Conclusion**

The anions of 2- and 4(5), and 2-methyl-4(5)-nitroimidazole have been shown to undergo $S_{RN1}$ reactions with a range of halo-nitroalkanes to yield the corresponding 4-nitro N-1-nitroalkyl-derivatives. The anions of 2-methyl-4(5)-nitro- and 4(5)-nitroimidazole, but not the anion of 2-nitroimidazole, underwent oxidative addition to the anion of 2-nitropropane (using potassium ferricyanide and sodium persulphate as oxidants) to yield the corresponding 4-nitro, N-1-(1-methyl-1-nitroethyl) derivatives.

These nitroimidazoles were able to react as nucleophiles in $S_{RN1}$ reactions, whereas, other sp$^3$ hybridised N-centred nucleophiles do not. This is because the initially
formed radical-anion has an unpaired electron in a $\pi^*$ MO of relatively low energy (the aromatic system), and not in a C-N $\sigma^*$ MO (as in other N-centred nucleophiles), thereby allowing the reaction to take place. The NO$_2$ group further assisted in lowering the $\pi^*$ MO orbital by conjugation.

Although $S_{RN1}$ reactions were reported to be generally free from steric effects,$^{131c,134a}$ except in a few cases,$^{134}$ steric hindrance in reactions of nitroimidazole anions with various substrates has been demonstrated by competitive reactions between the imidazole anion [51] and 2-bromo-2-nitropropane and p-nitrobenzyl chloride. 2-Nitroimidazole anion also reacted with p-nitrobenzyl chloride to give the required product but failed to react with 2-substituted-2-nitropropanes. Hence, steric hindrance is also a factor in causing the exclusive formation of 1-alkyl-4-nitroimidazoles.

The anions of 2-methyl-4-nitro- and 4-nitro-imidazole have been shown to act as nucleofuges in the $S_{RN1}$ reactions between their 1-(1-methyl-1-nitroethyl)-derivatives [Me$_2$C(NO$_2$)$_2$X] and anions (which include Me$_2$CNO$_2^-$ and PhSO$_2^-$). Loss of nitrite anion was not observed in these reactions.

The use of e.s.r. spectroscopy has confirmed the behaviour of the radicals and radical-anions proposed as intermediates in the $S_{RN1}$ and oxidative addition reactions.

The aim of this section was to produce novel nitroimidazole analogues with possible antimicrobial analogues. Although these reactions produce 4-nitro isomer, which has been shown$^{13}$ to possess less antimicrobial activity, they are novel interesting reactions, showing that N-centres of nitroimidazoles can participate both as nucleophiles and nucleofuges in $S_{RN1}$ reactions.
PART B
Radical N-Alkylation of Diazoles and Nitrodiazoles

N-1-Alkyl-4-, and 2-nitroimidazoles were prepared as described in Part A of this discussion by a reaction between the anions of 2- and 4(5)-nitroimidazoles and the appropriate alkylating agent (equation 2.45) via the $S_{RN}^1$ mechanism.

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{R}^1 & \quad \text{+ RX} \\
& \quad \text{h} \text{v} \text{DMSO} \\
\text{O}_2\text{N} & \quad \text{N} \\
\text{R}^1 & \quad \text{R}
\end{align*}
\]

\[(2.45)\]

\[R = \text{Me}_2\text{C}-\text{NO}_2, \quad X = \text{Cl, Br, NO}_2 \quad R^1 = \text{H, Me} \]

\[R = \text{-CH}_2\text{-}[\text{ phenol]} \quad X = \text{Cl} \]

\[R = \text{O} \quad \text{NO}_2 \quad X = \text{Br} \]

This section discusses the scope of extending the radical N-alkylation of nitroimidazoles to other diazoles, in order firstly, to gain an understanding of the generality of the reaction (i.e. as a useful synthetic method), and secondly, to prepare new nitrodiazole analogues for biological testing. One of the explanations for the formation of the intermediate radical-anions, arising from the reaction between the anion of 4(5)-nitroimidazoles and the 2-nitro-2-propyl radical (equation 2.14, scheme 2.1, page 61), was that the unpaired electron is in a $\pi^*$ MO of low energy, and that the energy of this species was further lowered by conjugation with the nitro group. Thus, a reason for the extension of the present work was to find out whether this conjugation (involving the nitro group) was essential for the observed reaction of nitroimidazoles. Consequently, the choice of diazoles included those with and without the nitro group. The compounds which were readily available were imidazole, benzimidazole, 5-nitrobenzimidazole and 5- and 6-nitroindazoles. The anions of these diazoles, [83]-[87], together with the $S_{RN}^1$ substrates used in this study are listed below:
Diazole anions:

Substrates:

\[
\begin{align*}
\text{Me} & \quad X \\
\text{Me} & \quad \text{NO}_2
\end{align*}
\]

\[X = \text{Br} \quad [55] \]
\[= \text{Cl} \quad [54] \]
\[= \text{NO}_2 [56] \]

\[\text{Me}_2\text{C} = \text{NO}_2 \quad \Theta \quad \text{(for oxidative addition reactions)} \]

\[\text{p-O}_2\text{N-C}_6\text{H}_4-\text{CH}_2\text{-Cl} \quad [58] \]

The anion of imidazole, [83], was reacted with 2-substituted-2-nitropropanes under conditions suitable to \( S_{\text{RN}} \) reactions (dry conditions, \( \text{N}_2 \), and light catalysis) to yield 1-(1-methyl-1-nitroethyl)-imidazole (equation 2.46) in a relatively low yield compared with the corresponding reaction between 4(5)-nitroimidazole and 2-substituted-2-nitropropanes. Similarly, the reaction between the diazole anions ([84]-[87]) gave the corresponding 1-alkyl-diazole derivatives in low yields (equations 2.47-2.50) (Table 2.6). In each of these reactions, the presence of two compounds was observed as indicated by t.l.c. analysis and \(^1\text{H} \) n.m.r. spectroscopy of the crude mixtures. The yield of each product in the mixture was calculated from the crude mixture using \(^1\text{H} \) n.m.r. spectroscopy with a known quantity of an internal standard, \( \text{p-dimethoxybenzene} \). The latter compound was chosen because it has no chemical shift values in the vicinity of those expected of the products, and is readily soluble in normal \(^1\text{H} \) n.m.r. solvents. The methods of separation and identification of these compounds...
Table 2.6 $S_{RN1}$ Reactions of the anions of Diazoles and Nitrodiazoles [83]-[87]

<table>
<thead>
<tr>
<th>Diazole and nitro-diazole anion</th>
<th>RX</th>
<th>Reaction condition</th>
<th>N-alkylated product</th>
<th>$S_{N1}$ Substituted product</th>
<th>Unreacted diazole and RX</th>
<th>Unreacted nitro-diazole</th>
<th>Dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>[83]</td>
<td>$\text{Me}_2\text{C(Cl)}\text{NO}_2$</td>
<td>DMSO, 96 h</td>
<td>2</td>
<td>-</td>
<td>25</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\text{Me}_2\text{C(Br)}\text{NO}_2$</td>
<td>DMSO, 24 h</td>
<td>3</td>
<td>-</td>
<td>22</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>$\text{Me}_2\text{C(Cl)}\text{NO}_2$</td>
<td>HMPA, 48 h</td>
<td>8</td>
<td>-</td>
<td>24</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\text{Me}_2\text{C(NO}_2\text{)}_2$</td>
<td>HMPA, 24 h</td>
<td>8</td>
<td>-</td>
<td>25</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>[84]</td>
<td>$\text{Me}_2\text{C(Cl)}\text{NO}_2$</td>
<td>DMSO, 72 h</td>
<td>5</td>
<td>1</td>
<td>28</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\text{Me}_2\text{C(Br)}\text{NO}_2$</td>
<td>DMSO, 68 h</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>$\text{Me}_2\text{C(NO}_2\text{)}_2$</td>
<td>DMSO, 48 h</td>
<td>5</td>
<td>1</td>
<td>29</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>[86]</td>
<td>$\text{Me}_2\text{C(Cl)}\text{NO}_2$</td>
<td>DMSO, 72 h</td>
<td>20</td>
<td>12</td>
<td>22</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>[87]</td>
<td>$\text{Me}_2\text{C(Cl)}\text{NO}_2$</td>
<td>DMSO, 48 h</td>
<td>12</td>
<td>7</td>
<td>18</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>[85]</td>
<td>$\text{Me}_2\text{C(Cl)}\text{NO}_2$</td>
<td>DMSO, 48 h</td>
<td>17</td>
<td>12</td>
<td>16</td>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

a All reactions were carried out in dipolar aprotic solvents under nitrogen and irradiated with fluorescent lamps (2 x 150w) with diazole (1 equiv.), sodium hydride (1.5 equiv.) and RX (1.5 equiv.) unless otherwise stated.

b % Yields are based on diazole and nitrodiazole and were calculated by $^1\text{H}$ n.m.r. spectroscopy using an internal standard.

c % Diazole and nitrodiazole recovered by filtration after pouring the reaction mixture into water (a large amount remains in solution).
are discussed later. The products are designated, for convenience as: N-alkylated product, S_N1 substituted product

and 5- and 6-nitro isomers where applicable as shown in equations (2.47-2.50). A possible explanation for the S_N1
substituted product is proposed later. The various 2-substituted-2-nitropropanes, ([82] with \( X = \text{Br} [55], \text{Cl} [54], \text{NO}_2 [56])

\[
\begin{align*}
\text{[87]} + \text{Me}_2\text{C}(X)\text{NO}_2 & \rightarrow \text{[2.50]} \\
\text{SN}_1 \text{ substituted product} [95] & \text{N-alkylated product} [96]
\end{align*}
\]

reaction, all gave similar yields (Table 2.6). The rates of reaction of these nitropropanes were slower than normally observed for \( \text{SN}_1 \) reactions of these compounds. The slow rate is probably due to steric hindrance (see later). The slow rate observed in the reactions between diazole anions and 2-substituted-2-nitropropanes prevented inhibition studies - the normal diagnostic criteria for \( \text{SN}_1 \) reactions. However, the \( \text{SN}_1 \) mechanism is most likely, because as yet, there is no known example of substitution at a tertiary carbon atom via an \( \text{SN}_2 \) mechanism in the literature. Similarly, an \( \text{SN}_1 \) mechanism is most unlikely because it would require an intermediate \( \alpha \)-nitroalkyl cation which would be of extremely high energy. The reaction between the anions of 4(5)-nitroimidazoles and 2-substituted-2-nitropropanes [82] have been shown \(^{143} \) using the normal criteria of inhibition studies to proceed via the \( \text{SN}_1 \) mechanism (see Part A of this discussion). It is unlikely that these closely related diazoles react differently, and consequently, the \( \text{SN}_1 \) mechanism is proposed for these reactions (scheme 2.7). As was observed in the 4(5)-nitroimidazole reactions, strong red colours were observed, and this we associated with a charge-transfer complex between the diazole anions, [83]-[87] and the substrates [54]-[56].

The reaction between the diazole anions [83]-[87] and the substrates [54]-[56], in HMPA was only slightly better than in DMSO, and in both solvents, a decreased yield of products was obtained with time.
Scheme 2.7: Reaction between the anion of 5-nitrobenzimidazole [85] and 2-substituted-2-nitropropanes by the $S_{RN1}$ mechanism.

Lawless and Hawley$^{144}$ reported that the stability of radical-anions is dependent on the solvent, and found that the stability decreased in the order: DMSO $>$ acetonitrile $>$ DMF. Similarly, Norris et al.$^{134b,145}$ reported that reactions are faster in HMPA than in DMSO. They showed that the reaction (equation 2.55) was completed in 1.5h in HMPA whereas it was still incomplete in DMSO after 11 days.

The low overall yields of isolated materials in the alkylation reactions (Table 2.6) were probably caused by decomposition of the starting materials due to the extended periods of irradiation.
Synthesis by the oxidative Addition Reaction

The oxidative addition method was successfully used to add the anion of 2-nitropropane [61] to the anion of 4(5)-nitroimidazoles, 143a [51] and is discussed fully in Part A (equation 2.56).

![Chemical structure diagram](image)

\[ \text{Bu}^t \text{H-C-Cl} + \text{MeCO}_2 \text{Et} \xrightarrow{1.5h \text{ HMPA}} \text{Bu}^t \text{H-C-CO}_2 \text{Et} \]

\[ \text{(2.55)} \]

Similarly, the method was used to add the diazole anions, [83]-[87], to the anion of 2-nitropropane, [61], to give the same 1-alkyl-diazole derivatives as observed in the SRN1 reactions (e.g. equation 2.57). For diazoles, [84], [85], [86] and [87], the same products were obtained.

![Chemical structure diagram](image)

\[ \text{O}_2\text{N}^5 \text{N}_2 \text{Me}_2\text{CNO}_2 \xrightarrow{\text{K}_3\text{Fe(CN)}_6, \text{Na}_2\text{S}_2\text{O}_8} \text{O}_2\text{N}^5 \text{N}_2 \text{Me}_2\text{CNO}_2 \]

\[ \text{(2.56)} \]

As before, the compounds were designated N-alkylated, S_N1-substituted product and isomers. The yields from the reactions are recorded in Table 2.7. The overall isolated yields in the oxidative addition of the anion of 2-nitropropane [61] to the diazole anions ([83]-[87]) are improved compared with similar reactions in the S_RN1 reactions. These improved yields could be due to shorter reaction time.
### Table 2.1: Oxidative addition of nitro- and non-nitrodiazole anions (83)-(87) to the anion of 2-nitropropane. (Me₂CNO₂⁻) (61)

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>![83]</td>
<td>1.0</td>
<td>1.0</td>
<td>120min,NaOH</td>
<td>(28)</td>
<td>42(28)</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.05</td>
<td>120min,NaOH</td>
<td>(30)</td>
<td>30(17)</td>
<td>11</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>![84]</td>
<td>0.85</td>
<td>1.0</td>
<td>60min,NaOH</td>
<td>(42)</td>
<td>15(5)</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>60min,NaOH</td>
<td>(19)</td>
<td>40(5)</td>
<td>23</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>60min,NaOH</td>
<td>(16)</td>
<td>43(3)</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.5</td>
<td>75min,NaOH</td>
<td>(48)</td>
<td>19(3)</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>![85]</td>
<td>1.0</td>
<td>1.5</td>
<td>30min,KOH</td>
<td>(25)</td>
<td>16(8)</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.5</td>
<td>75min,KOH</td>
<td>(42)</td>
<td>32(4)</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.5</td>
<td>75min,KOH</td>
<td>(37)</td>
<td>16(4)</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.5</td>
<td>37min,KOH</td>
<td>0</td>
<td>59(2)</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>![86]</td>
<td>1.0</td>
<td>1.5</td>
<td>75min,KOH</td>
<td>(37)</td>
<td>18(4)</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>![87]</td>
<td>1.0</td>
<td>1.5</td>
<td>75min,KOH</td>
<td>0</td>
<td>59(2)</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>![88]</td>
<td>1.0</td>
<td>1.5</td>
<td>75min,KOH</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

A = unaltered diazole  
B = 2,2-dinitropropane  
C = 2,3-dimethyl-2,3-dinitrobutane  
D = All reactions were carried out in dichloromethane distilled water (1:1) under nitrogen with diazole anion, base, the anion of 2-nitropropane, potassium ferricyanide (0.2 equiv.) and sodium persulphate (2 equiv.).  
E = & Yields are based on diazole anion and were calculated by n.m.r. spectroscopy using an internal standard. Isolated yields are in parenthesis.  
F = Dizole obtained after neutralizing the aqueous layer.  
G = KOH (2.0 equiv.).  
H = KOH (4.0 equiv.).  
I = The SN₂ substituted product was not isolated pure.  
J = no persulphate, use potassium ferricyanide (2.0 equiv.).
However, as observed for the $S_{RN1}$ reactions, the crude product consisted of a mixture of 2-products. The ratio of the percentage yields calculated for each product in the mixture, by $^1$H n.m.r. spectroscopy, closely resembled those obtained in the $S_{RN1}$ reactions.

Most of the crude mixtures from these reactions were purified by column chromatography on neutral alumina and an appropriate eluent (see experimental section). 5-And 6-nitrobenzimidazole, and 6-nitroindazole and 6-nitroisoindazole derivatives were separated in pure form using preparative t.l.c. on alumina. In the latter mixture, the $S_{N1}$ substituted product, [95], could be separated pure but not the N-alkylated 6-nitroisoindazole product, [96]; however, the crude mixture was purified by column chromatography for analysis.

The probable mechanism of the oxidative addition of the anion of 2-nitropropane to the diazole anions ([83]-[87]) is shown in scheme 2.8, using 5(6)-nitrobenzimidazole anion [84] as an example.

As mentioned in Part A for 4(5)-nitroimidazoles, the key step in the oxidative addition reaction method is the addition of the diazole anions to the 2-nitro-2-propyl radical $[\text{Me}_2\text{CNO}_2]$ (equation 2.59, scheme 2.8), which is the same as step 3 in the $S_{RN1}$ mechanism (equation 2.53, scheme 2.7). Therefore, it was not surprising that the same ratios of products were obtained. By-products, 2,3-dimethyl-2,3-dinitrobutane and 2,2-dinitropropane, were also obtained as in the 4(5)-nitroimidazole reactions (equations 2.61-2.62), although the origin of the nitrite anion to form the latter by-product is not known.

The various attempts, (using the reaction between benzimidazole anion [84] and the anion of 2-nitropropane [61] (equation 2.47)) to optimise the reaction conditions are also presented in Table 2.7.

The diazole anions ([83]-[87]) are ambident and are able to react via either of the $N$-centres to yield alkylated products at either $N$-atom as observed in the $N$-alkylation of 4(5)-nitroimidazoles.

Ridd et al. showed that the tautomers of substituted benzimidazoles are present in equal concentration (see
later), consequently, alkylation should lead to equal amounts of isomers. This was also reported to be the case in substituted indazoles.

In imidazoles and benzimidazole, both N-centres are equivalent, and thus, alkylation leads to only one compound. The reaction between the benzimidazole anion [84] and the 2-substituted-2-nitropropane, or the anion of 2-nitropropane gave two products which are not isomers (i.e. not a direct consequence of the ambident nature of benzimidazole) (equation 2.47). The second product [90] obtained (5%) arose from the displacement of the aliphatic nitro group in the N-alkylated benzimidazole, [89], (42%), and replacement by another benzimidazole anion, [84].

A similar substitution of the aliphatic nitro group by 5- and 6-nitroindazole anions ([86] and [87]) to give the corresponding products [93] and [94] was obtained.
2-Substituted-2-nitropropanes have been shown\textsuperscript{109} to undergo $S_{RN}1$ substitution with loss of either the nitro group or the $\alpha$-substituent ($X$). Kornblum\textsuperscript{109} suggested that the radical formed with the minimum energy usually predominates (equation 2.64). This is the basis of the synthesis of these $N$-alkylated diazoles (schemes 2.7 and 2.8).

If the loss of the nitro group (to give $\text{Me}_2\text{CX}$) (equation 2.65) is the mechanism by which the substituted products, [90], [93] and [94], were obtained, then the substitution should be expected in all the products [88]–[96], since the radical formed would be of low energy.\textsuperscript{109} However, as mentioned above, not all the products underwent further substitution.

Further evidence to preclude the loss of nitro group from these radical-anions of 2-substituted-2-nitropropane analogues comes from the previous work on 1-alkyl-4-nitroimidazole (see Part A).

The reactions between 1-(1-methyl-1-nitroethyl)-4-nitroimidazole and anions showed that the intermediate radical-anion dissociates with loss of the nitroimidazole group and not the nitrite. It is therefore unlikely that these intermediate radical anions should lose the nitrite.
anion rather than the diazole anion. If this were the case, the direction of dissociation (equations 2.64 and 2.65) would be determined by relative nucleofugicities. For example, the anion of 5-nitrobenzimidazole would be lost faster than the anion of benzimidazole from the respective intermediate radical-anions. Also, as loss of nitrite is observed from the benzimidazole and not from the nitrobenzimidazole analogue, the $S_{RN}$ mechanism can reasonably be excluded for this dissubstitution.

A more plausible explanation is that the substitution of the nitro group is via an $S_{N1}$ reaction. The loss of the nitro group could be initiated by the lone pair of electrons on the substituted amino nitrogen.

In the N-1-substituted indazole series, such a reaction would lead to loss of aromaticity in the pyrazole ring but retain aromaticity in the benzo-portion of the molecule. The intermediate cation is, therefore, stabilised, as shown in equation 2.69. The overall effect is that a stable carbonium centre is generated which can allow $S_{N1}$ substitution to take place.

\[ \text{(2.69)} \]

If the lone-pair on N-1-substituted isoindazoles were engaged in such a reaction, aromaticity would be lost in the whole molecule (equation 2.70), consequently, the $S_{N1}$ reaction is not favourable. The lone-pair on the N-1 nitrogen of isoindazoles is essential for maintaining the aromatic character.

\[ \text{(2.70)} \]
In benzimidazole, a situation similar to that of N-1 substituted nitroindazoles is observed. The N-1 electrons are available for $S_{N1}$ reaction although the imidazole aromatic portion is lost, the benzene aromatic system remained (equation 2.71). However, in the 5- or 6-nitrobenzimidazole analogues, no nitrite anion loss is observed. This can be explained by the $-I$ effect of the nitro group in the benzene ring withdrawing electron density from the N-1 centre, thereby destabilising the intermediate carbocation required for $S_{N1}$ substitution. Using this argument, it could be predicted that a $+I$ group would increase the rate of $S_{N1}$ reaction. It is probable that the $-I$ effect is less significant in the 5- and 6-nitroindazoles than in 5- and 6-nitrobenzimidazole due to the proximity of the second nitrogen heteroatom in the former compounds, hence the observed $S_{N1}$ reaction.

Reaction between diazole anions ([83]-[87]) and p-nitrobenzyl chloride [58]

The anion of 5(6)-nitrobenzimidazole [85] was reacted with p-nitrobenzyl chloride under conditions conducive to $S_{RN1}$ reactions ($N_2$, light catalysis) to give two isomeric products in moderate yields (equation 2.72) (Table 2.8).

Similarly, the reaction between the anion of 5-nitroindazole [86] and p-nitrobenzyl chloride gave two isomeric products,
5-nitroindazole and 5-nitroisoindazole isomers, in good yields (equation 2.73). The results are shown in Table 2.8.

![Diagram 1](image1.png)

The reaction with the anion of 6-nitroindazole [87] also gave two isomers as observed for the 5-nitroindazole anion [86] (equation 2.74). The results are shown in Table 2.8.

![Diagram 2](image2.png)

$^1$H n.m.r. spectroscopy was used to measure the ratio of isomers present in the reaction mixture. These isomers were purified by column chromatography (see experimental section for details). The recrystallised products were subjected to micro- and spectroscopic analyses for structural identification. The identity of the two isomeric products obtained for each diazole anion ([85]–[87]) were assigned as shown in equations (2.72 to 2.74) based on their $^1$H n.m.r. and $^{13}$C n.m.r. spectroscopic data (discussed later in this section).

The reaction between p-nitrobenzyl chloride and the anion of benzimidazole [84] gave intractible mixtures of
### Table 2.8: Reactions Between Diazole Anions ([85]-[87]) and p-Nitrobenzyl Chloride

Diazole anion + p-nitrobenzyl chloride $\rightarrow$ N-alkylated products + Cl$^-$

<table>
<thead>
<tr>
<th>Diazole anion</th>
<th>Reaction condition</th>
<th>N-alkylated products</th>
<th>Unreacted Diazole</th>
<th>Unreacted RX</th>
</tr>
</thead>
<tbody>
<tr>
<td>![O2N][85]</td>
<td>22h</td>
<td>5-Nitro-N-alkylated</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-Nitro-N-alkylated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>![O2N][86]</td>
<td>20h</td>
<td>5-or 6-nitro N-alkylated</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-or 6-nitro isoindazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22h, ½h</td>
<td>(33)</td>
<td>(33)</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>40 mol % DTBN</td>
<td>(45)</td>
<td>(45), 14</td>
<td>(25), 14</td>
</tr>
<tr>
<td></td>
<td>½h, Dark, Dark + O$_2$</td>
<td>25</td>
<td>25, 21</td>
<td>25, 21</td>
</tr>
<tr>
<td>![O2N][87]</td>
<td>22h</td>
<td>5-or 6-nitro N-alkylated</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-or 6-nitro isoindazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-or 6-nitro N-alkylated</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-or 6-nitro isoindazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-or 6-nitro N-alkylated</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-or 6-nitro isoindazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>![O2N][84]</td>
<td>22h</td>
<td>-</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>![O2N][83]</td>
<td>5h</td>
<td>-</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
</table>

- Diazo recovered by filtration after pouring the reaction into water.
- All reactions were carried out in DMSO under nitrogen and irradiated with fluorescent lamps (2 x 150 watts), with diazoles anion ([83]-[87]) (1 equiv.), sodium hydride (1.5 equiv.) and p-nitrobenzyl chloride (1 equiv.).
- Yields based on nitroimidazole and were calculated by n.m.r. spectroscopy using an internal standard, isolated yields are in parenthesis.
- Standard reaction for inhibition studies.
unidentifiable oily products. Likewise, it was not possible to assign structure to the product of the reaction between p-nitrobenzyl chloride and the anion of imidazole [83].

In order to assign a mechanism to the reaction between diazole anions ([85]-[87]), and p-nitrobenzyl chloride, inhibition and light catalysis studies, the normal criteria, diagnostic of $S_{RN1}$ reactions, were carried out for the reaction between p-nitrobenzyl chloride and the anion of 6-nitroindazole, [87]. The results (presented in Table 2.8) showed that the reaction (equation 2.74) is only slightly inhibited, and therefore, the reactions do not conclusively proceed via the $S_{RN1}$ mechanism. Although the results suggest an $S_{N2}$ type mechanism, an $S_{RN1}$ mechanism cannot be precluded.

p-Nitrobenzyl chloride is well known to participate in $S_{RN1}$ reactions (see introduction) with various anions and was observed to undergo $S_{RN1}$ substitution with the anion of 4(5)-nitroimidazole (equation 2.6, Part A) and with imidazole. It is therefore unlikely that the anions of 4(5)-nitroimidazole and imidazole undergo $S_{RN1}$ substitution and 6-nitroindazole does not. Further studies are required to fully elucidate this problem.

Comparison of Electrophilic and Radical N-Alkylation Reactions

The diazole anions ([83]-[87]) are ambident and are able to react via the $N$-centres to yield $N$-alkylated products. In both $S_{RN1}$ and oxidative addition reactions, the diazole anions ([83]-[87]) can react with the intermediate 2-nitro-2-propyl radical $[\text{Me}_2\text{CNO}_2]$ (equations 2.63 and 2.69) to give two $N$-alkylated products (equations 2.47 to 2.50).

The effect of the ambident nature of the diazole anion on the orientation of substitution is not so marked in unsubstituted imidazole and benzimidazole. In these cases, both $N$-centres are equivalent, therefore, substitution at either $N$-centre would lead to the same product (equations 2.46 and 2.47). The second product obtained for benzimidazole is not its $N$-alkylated isomer but the further "substituted $S_{N1}$ product" discussed above.

In substituted diazoles, radical $N$-alkylation gave the two possible $N$-alkylated isomers. For the nitroindazoles,
the "substituted $S_{N-1}$ products" [93] and [95] were obtained as an indication of one of the respective $N$-alkylated nitroindazoles.

The other nitroisoindazole isomers are represented by structures [94] and [96]. The ratio of isomers for each diazole anion ([83]-[87]) is presented in Table 2.9. The ratios (approximately 1:1) obtained, showed lack of regioselectivity in radical $N$-alkylation of these diazole anions unlike $N$-alkylation of 4(5)-nitroimidazoles.\textsuperscript{143}\textsuperscript{a}

It is interesting to compare these $S_{N-1}$ alkylation reactions with those of Ridd and Smith.\textsuperscript{64\textsuperscript{a}, 67} They have reported that the anion of 5(6)-nitrobenzimidazole is alkylated by dimethyl sulphate to give a mixture of isomers ($6-\text{NO}_2 : 5-\text{NO}_2 = 40-50\% : 50-60\%$) (Table 2.9) indicating that the position of the nitro group in the ambident anion is not significant in determining the direction of alkylation and that nucleophilicity at each $N$-centre is similar.

Likewise, the lack of regioselectivity observed in the radical $N$-alkylation ($S_{R\text{N}-1}$ and oxidative reactions) of 5- and 6-nitroindazole anions ([86] and [87]) has also been reported for the reactions between the diazole anions ([86] and [87]) and dimethyl sulphate.\textsuperscript{146} The isomeric ratio reported\textsuperscript{146} for each isomer is given in Table 2.9.

These results are in contrast with the strong selectivity shown\textsuperscript{64\textsuperscript{a}, 67, 143\textsuperscript{a}} in the equivalent alkylation of 4(5)-nitroimidazole in which the ratio of 4:5 nitroisomers is 9:1.

The radical alkylation of the anions 4(5)-nitroimidazole,\textsuperscript{143} 5(6)-nitrobenzimidazole [85] and 5- and 6-nitroindazole ([86] and [87]) give similar ratios of isomers to that observed in the polar alkylation (see Table 2.9). These results indicate that the electronic control in these ambident anions in adding to electrophiles is similar to that for adding to free-radicals.

These radicals are electron deficient and are 'electrophile like' in reactivity. The 2-nitro-2-propyl radical is a reactive electrophilic radical because of the strong electron-withdrawing (−I) effect of the nitro group. Therefore, it is not surprising that in a kinetically controlled

$$\text{Me}_2C^\cdot \rightarrow \text{NO}_2$$
Table 2.9: Comparison of Isomeric Ratios from Electrophilic and Radical N-Alkylation of Diazoles

<table>
<thead>
<tr>
<th>R^1 + Y</th>
<th>N-alkydialane</th>
<th>Reacting species</th>
<th>Ratio of isomers in crude mixture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-NO_2 Isomer</td>
</tr>
<tr>
<td>Me_2SO_4</td>
<td>Me^+</td>
<td>53-61^b</td>
<td>39-47</td>
</tr>
<tr>
<td>Me_2C(X)NO_2</td>
<td>[Me_2CNO_2]</td>
<td>58</td>
<td>42</td>
</tr>
<tr>
<td>Me_2CNO^-</td>
<td>[Me_2CNO_2]</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>p-NO_2BzCl</td>
<td>radical and/or electrophile</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>5-Nitroindazole</td>
<td>5-Nitroindazole</td>
<td></td>
</tr>
<tr>
<td>Me_2SO_4</td>
<td>Me^+</td>
<td>47^c</td>
<td>53</td>
</tr>
<tr>
<td>Me_2C(X)NO_2</td>
<td>[Me_2CNO_2]</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Me_2CNO^-</td>
<td>[Me_2CNO_2]</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>p-NO_2BzCl</td>
<td>radical and/or electrophile</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>6-Nitroindazole</td>
<td>6-Nitroindazole</td>
<td></td>
</tr>
<tr>
<td>Me_2SO_4</td>
<td>Me^+</td>
<td>50^c</td>
<td>50</td>
</tr>
<tr>
<td>Me_2C(X)NO_2</td>
<td>[Me_2CNO_2]</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>Me_2CNO^-</td>
<td>[Me_2CNO_2]</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>p-NO_2BzCl</td>
<td>radical and/or electrophile</td>
<td>46</td>
<td>54</td>
</tr>
</tbody>
</table>

a) Yields are based on nitrodiazole and were calculated by n.m.r. spectroscopy using an internal standard.
b) ref 67,c) ref 146
reaction, the ambident anion behaves similarly towards electrophiles and radicals.

Structure Identification of N-Alkylated Diazoles

N-Alkylation of diazole anions ([83]-[87]) gave rise to interesting compounds - the isomeric N-alkyl-derivatives and $S_{N1}$ substituted products [88]-[96]. The formation of mixtures of these isomers and $S_{N1}$ products in synthetic procedures require methods for distinguishing between them.

The low solubility of these compounds prevented their spectral analyses in the same solvent for easy comparison, consequently, analyses of their spectroscopic data were carried out with caution, and particularly when comparing these data with the literature values.

For clarity, the spectroscopic analyses of N-alkylated diazoles are discussed under two general headings: i) N-alkylbenzimidazole and ii) N-alkynitroindazole and isoindazole derivatives.

i) N-Alkylbenzimidazole derivatives

The spectroscopic analyses of 1-(1-methyl-1-nitroethyl) derivatives of imidazole [88], benzimidazole [89] and 5- and 6-nitrobenzimidazole [91], [92], 2,2-(dibenzimidazol-1-yl) propane [90], together with 1-(p-nitrobenzyl)-5- and 6-nitrobenzimidazole [99] and [100] are discussed.

The $^1$H n.m.r. spectrum of 1-(1-methyl-1-nitroethyl) imidazole [88] was compared with the spectrum for 1-methylimidazole [105]. The $^1$H n.m.r. spectroscopic data are shown in the structures below:

\[
\delta_H = 7.1
\]

\[
\delta_H = 7.73
\tag{88}
\]

\[
\delta_H = 2.26
\tag{88}
\]

\[
\delta_H = 7.08
\]

\[
\delta_H = 7.47
\tag{105}
\]

\[
\delta_H = 6.88
\tag{105}
\]

\[
\delta_H = 3.70
\tag{105}
\]

The $^1$H n.m.r. spectra data of compounds [89], [90], [91], [92], [99] and [100] together with their structure and numbering systems are presented in Table 2.10. (Page 106).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Proton</th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Compound 1" /></td>
<td>a</td>
<td>-</td>
<td>7.65 (s)</td>
<td>7.4-7.0</td>
<td>7.0</td>
<td></td>
<td>C2=He 2.37</td>
</tr>
<tr>
<td><img src="image2" alt="Compound 2" /></td>
<td>b</td>
<td>8.12</td>
<td>8.74</td>
<td>-</td>
<td>7.56</td>
<td>Me, 3.93</td>
<td>J4,6=2.5Hz J6,7=8.0</td>
</tr>
<tr>
<td><img src="image3" alt="Compound 3" /></td>
<td>c</td>
<td>8.01</td>
<td>7.65-7.12</td>
<td>7.75</td>
<td>Me2, 2.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image4" alt="Compound 4" /></td>
<td>d</td>
<td>8.5</td>
<td>7.96</td>
<td>7.12</td>
<td>7.35</td>
<td>6.76</td>
<td>Me2, 2.48</td>
</tr>
<tr>
<td><img src="image5" alt="Compound 5" /></td>
<td>e</td>
<td>8.56 (brs)</td>
<td>7.78</td>
<td>8.12</td>
<td>-</td>
<td>8.10</td>
<td>Me2, 2.44</td>
</tr>
<tr>
<td><img src="image6" alt="Compound 6" /></td>
<td>f</td>
<td>8.74 (brs)</td>
<td>8.52</td>
<td>-</td>
<td>8.11</td>
<td>7.59</td>
<td>Me2, 2.42</td>
</tr>
<tr>
<td><img src="image7" alt="Compound 7" /></td>
<td>g</td>
<td>8.43</td>
<td>8.51</td>
<td>-</td>
<td>8.04</td>
<td>7.52</td>
<td></td>
</tr>
<tr>
<td><img src="image8" alt="Compound 8" /></td>
<td>h</td>
<td>8.49</td>
<td>7.74</td>
<td>8.04</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) δ H in DMSO-d6 b) δ H in CDCl3 c) δ H in CDCl3/DMSO-d6 d) δ H in acetone-d6/CDCl3 e) δ H in acetone-d6/CDCl3
The only precedent in the literature\textsuperscript{147} is the $^1$H n.m.r.
spectroscopic study of 1-methyl-5- and 6-nitrobenzimidazole. $^1$H N.m.r. and $^{13}$C n.m.r. spectra of the 5(6)-nitrobenzimidazole
could not be obtained due to lack of solubility.

The interpretation of the $^1$H n.m.r. spectroscopic data
for each isomer of the diazoles was complicated due to over-
lapping of the proton signals. Ellis et al.\textsuperscript{147} simplified
the spectra using a lanthanide shift reagent for the N-methyl-
series. They also presented data for both isomers without
the shift reagent.

In both our work, and that of Ellis,\textsuperscript{147} the spectrum
of the 5-nitro isomer showed the least overlapping. Like-
wise, a simplification (less overlapping) was observed in
the spectrum of the $S_N1$ substituted product [90].

The distinguishing feature is the signal for H-4 in
the 5-nitro-isomer which is at a lower field than the cor-
responding H-7 in the 6-nitro-isomer (Table 2.10).

This effect is probably due to the greater downfield
effect of the $\varphi$-imino group (5-nitro-isomer) as opposed to
the $\varphi$-amino group in the 6-nitro isomer.

The same argument can be used to explain the signal
for H-7 in the 5-nitro-isomer which is upfield [for example,
$S_H = 7.52$ ppm in 1-(p-nitrobenzyl) derivative] from the H-4
in the 6-nitro-isomer ($S_H = 7.74$ ppm).

Our data is slightly different from that in the liter-
ature\textsuperscript{147} (Table 2.10) due to the use of different solvents
because of solubility problems. However, the isomers
showed the same relative values supporting the earlier
assignment.

In the 6-nitro-isomer, the signal for H-2 could be
predicted to be downfield from that for H-2 in the 5-nitro-
isomer as is observed in the $^1$H n.m.r. spectrum. There is a
close agreement between the coupling constants of our com-
ounds and those of Ellis\textsuperscript{147} (Table 2.10).

The $^1$H n.m.r. spectroscopic data for the 1-(1-methyl-
-1-nitroethyl)- and 1-(p-nitrobenzyl)-analogues (the struc-
tures are shown below), showed close agreement, except for
the H-2 signal shifting downfield in the former alkyl der-
ivative (e.g. [91]) due to deshielding by the side chain
nitro group.
The $^1$H n.m.r. spectrum of benzimidazole $S_N1$ product [90] is simplified compared with that of the 1-(1-methyl-1-nitroethyl) derivative. The structures and spectroscopic data are presented in Table 2.10. The second benzimidazole nucleus was indicated by twice the expected integration of signals for the benzimidazole nucleus. The prediction of the structure was supported by the micro-analysis, accurate mass measurement (mass spectrometry) and the i.r. spectrum which showed the absence of C-NO$_2$ absorption at 1550 and 1350 cm$^{-1}$.

The chemical shift values of the diazole [89] are in close agreement with those of diazole derivative [90] and those reported in the literature, although it was not possible to assign chemical shift values to individual protons, H-4, H-5 and H-6 due to overlapping in benzimidazole derivative [89] and the N-lmethylbenzimidazole. An interpretation of the A$_2$B$_2$ pattern of the spectrum of benzimidazole has been reported, but there has been no detailed, specific analysis of the chemical shifts of protons in unsymmetrical substituted derivatives.

In the benzimidazole $S_N1$ substituted product [90], the shielding of the signal for H-7 caused by the shielding ring current of the second benzimidazole ring was very pronounced causing an upfield shift of approximately 1.0 p.p.m. from the chemical shift value observed for the same proton in the benzimidazole N-alkylated product, [89]. As a result, the chemical shifts of the other protons, H-4 and H-5, which appeared as a multiplet in compound [90] could be more easily distinguished. (Table 2.10).
The chemical shift differences between different protons are, however, too small for total structural identification, particularly bearing in mind solvent and concentration effects. Consequently, $^{13}$C n.m.r. spectroscopy was used to support identification of the different products.

The off-resonance proton-decoupling distinguished between quaternary and methine carbons as singlets and doublets respectively. Of value is the large chemical shift differences for the different carbons in the $^{13}$C n.m.r. spectra.

The $^{13}$C n.m.r. spectra for the benzimidazole [99] compared very well with the data for 1-methyl- and 1-acetyl-benzimidazoles (Table 2.11).

It was not possible to compare chemical shift values for the N-alkyl 5- and 6-nitro benzimidazole derivatives with literature values because most studies on the $^{13}$C n.m.r. spectra of benzimidazole and its nitro analogues have concerned 1-unsubstituted compounds or N-alkyl or aryl benzimidazoles.148,150

The N-1 chain methyl and benzyl carbons of the different analogues are readily distinguished.

The assignment of the chemical shift values to the methine carbons was achieved by comparison with similar compounds, for example, 1-methyl-5-nitroindazole151 5-nitro-benzimidazole,148 1-methyl-, and 1-acetylbenzimidazoles.150 This comparison showed the signal for C-7 upfield from the rest of the carbon signals because it is the homocyclic carbon atom. By similar comparison, the signal for C-8 would be expected upfield of that for C-9. The signal for C-8 is adjacent to an amino group as opposed to imino group in C-9. (structures [92] and [99]).

ii) N-Alkyl-5- and 6-Nitroindazole and Isoindazole Derivatives

The spectroscopic analyses and structures of 1-(p-nitro-benzyl)-5- and 6-nitroindazole and isoindazoles derivatives [101], [102], [103] and [104], 1-(1-methyl-1-nitroethyl)-5- and 6-nitroisoindazole [94], [96] and the S$_N$1 substituted indazole derivatives [93] and [95] are presented in Tables 2.12, 2.13, 2.14 and 2.15 respectively. The spectra were
Table 2.11: $^{13}$C n.m.r. Chemical Shifts (δppm) of 1-alkyl-benzimidazoles and 1-alkyl-5- and 6-nitrobenzimidazoles

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td><img src="image1" alt="Structure of Compound a" /></td>
<td>-132.7</td>
</tr>
<tr>
<td><img src="image2" alt="Structure of Compound b" /></td>
<td>147.15</td>
</tr>
<tr>
<td><img src="image3" alt="Structure of Compound c" /></td>
<td>143.1</td>
</tr>
<tr>
<td><img src="image4" alt="Structure of Compound d" /></td>
<td>141.2</td>
</tr>
<tr>
<td><img src="image5" alt="Structure of Compound e" /></td>
<td>140.6</td>
</tr>
<tr>
<td><img src="image6" alt="Structure of Compound f" /></td>
<td>147.98</td>
</tr>
<tr>
<td><img src="image7" alt="Structure of Compound g" /></td>
<td>144.17</td>
</tr>
</tbody>
</table>

a) ref.146  b) ref.148  c) ref.150  d) DMSO-d$_6$/CDCl$_3$
### Table 2.12: $^1$H n.m.r. Chemical Shifts (δ ppm) for 5-nitro-indoles and isoindazoles

<table>
<thead>
<tr>
<th>Compound</th>
<th>Proton</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Structure 1]</td>
<td></td>
<td>8.37</td>
<td>8.81</td>
<td>-</td>
<td>8.26</td>
<td>7.77</td>
<td>-</td>
</tr>
<tr>
<td>![Structure 106]</td>
<td>a</td>
<td>8.15</td>
<td>8.66</td>
<td>-</td>
<td>8.23</td>
<td>7.44</td>
<td>Me, 4.15</td>
</tr>
<tr>
<td>![Structure 106]</td>
<td>b</td>
<td>8.36</td>
<td>8.77</td>
<td>-</td>
<td>8.21</td>
<td>7.80</td>
<td>Me, 4.12</td>
</tr>
<tr>
<td>![Structure 107]</td>
<td>a</td>
<td>8.21</td>
<td>8.66</td>
<td>-</td>
<td>8.07</td>
<td>7.72</td>
<td>Me, 4.26</td>
</tr>
<tr>
<td>![Structure 107]</td>
<td>b</td>
<td>8.73</td>
<td>8.82</td>
<td>-</td>
<td>8.01</td>
<td>7.72</td>
<td>Me, 4.27</td>
</tr>
<tr>
<td>![Structure 93]</td>
<td>c,r</td>
<td>8.34</td>
<td>8.62</td>
<td>-</td>
<td>7.81</td>
<td>6.52</td>
<td>Me$_2$, 2.47</td>
</tr>
<tr>
<td>![Structure 93]</td>
<td>d,o</td>
<td>8.33</td>
<td>8.66</td>
<td>-</td>
<td>7.87</td>
<td>6.50</td>
<td>Me$_2$, 2.50</td>
</tr>
<tr>
<td>![Structure 94]</td>
<td>d,o</td>
<td>8.63</td>
<td>8.75</td>
<td>-</td>
<td>8.10</td>
<td>7.75</td>
<td>Me$_2$, 2.45</td>
</tr>
<tr>
<td>![Structure 94]</td>
<td>d,r</td>
<td>8.62</td>
<td>8.75</td>
<td>-</td>
<td>8.10</td>
<td>7.75</td>
<td>Me$_2$, 2.42</td>
</tr>
<tr>
<td>![Structure 101]</td>
<td>c,r</td>
<td>8.35</td>
<td>8.69</td>
<td>-</td>
<td>8.13</td>
<td>7.83</td>
<td></td>
</tr>
<tr>
<td>![Structure 101]</td>
<td>c,c</td>
<td>8.58</td>
<td>8.40</td>
<td>-</td>
<td>8.17</td>
<td>7.75</td>
<td></td>
</tr>
<tr>
<td>![Structure 102]</td>
<td>c,c</td>
<td>8.58</td>
<td>8.40</td>
<td>-</td>
<td>8.17</td>
<td>7.75</td>
<td></td>
</tr>
</tbody>
</table>

a) $^6_H$ in CDCl$_3$ from ref. 146;  b) $^6_H$ in DMSO-d$_6$ from ref. 152;  
c,r) recrystallised product, $^6_H$ in CDCl$_3$;  
d,o) product off-prep. plate, $^6_H$ in CDCl$_3$;  
d,r) recrystallized product, $^6_H$ in CDCl$_3$
Table 2.12B: Coupling constant (Hz) of substituted-5-nitroindazoles and isoindazoles

<table>
<thead>
<tr>
<th>Compound</th>
<th>$J_{3,7}$</th>
<th>$J_{4,5}$</th>
<th>$J_{4,6}$</th>
<th>$J_{4,7}$</th>
<th>$J_{5,6}$</th>
<th>$J_{5,7}$</th>
<th>$J_{6,7}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a[106]</td>
<td>0.84</td>
<td></td>
<td>2.15</td>
<td>0.68</td>
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<td></td>
<td>9.17</td>
</tr>
<tr>
<td>a[107]</td>
<td>0.84</td>
<td></td>
<td>2.15</td>
<td>0.75</td>
<td></td>
<td></td>
<td>9.65</td>
</tr>
<tr>
<td>[93]</td>
<td>0.9</td>
<td></td>
<td>2.0</td>
<td>0.6</td>
<td></td>
<td></td>
<td>8.3</td>
</tr>
<tr>
<td>[94]</td>
<td>0.9</td>
<td></td>
<td>2.0</td>
<td>0.7</td>
<td></td>
<td></td>
<td>9.2</td>
</tr>
</tbody>
</table>
Table 2.13: $^1$H n.m.r. spectroscopic chemical shifts ($\delta$) of $H$-substituted 6-nitroindazoles and isooindazoles

<table>
<thead>
<tr>
<th>Compound</th>
<th>Proton</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>a</td>
<td>8.35</td>
<td>8.02</td>
<td>7.92</td>
<td>-</td>
<td>8.46</td>
<td>-</td>
</tr>
<tr>
<td><img src="image2.png" alt="Image" /></td>
<td>c</td>
<td>8.05</td>
<td>7.7</td>
<td>7.64</td>
<td>-</td>
<td>8.35</td>
<td>-</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td>a</td>
<td>8.26</td>
<td>7.90</td>
<td>7.90</td>
<td>-</td>
<td>8.46</td>
<td>1-Me, 4.18</td>
</tr>
<tr>
<td><img src="image4.png" alt="Image" /></td>
<td>b</td>
<td>8.09</td>
<td>7.81</td>
<td>7.97</td>
<td>-</td>
<td>8.35</td>
<td>1-Me, 4.18</td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td>a</td>
<td>8.56</td>
<td>7.95</td>
<td>7.80</td>
<td>-</td>
<td>8.56</td>
<td>2-Me, 4.18</td>
</tr>
<tr>
<td><img src="image6.png" alt="Image" /></td>
<td>b</td>
<td>8.03</td>
<td>7.73</td>
<td>7.85</td>
<td>-</td>
<td>8.63</td>
<td>2-Me, 4.30</td>
</tr>
<tr>
<td><img src="image7.png" alt="Image" /></td>
<td>d</td>
<td>8.64</td>
<td>7.65</td>
<td>7.9</td>
<td>-</td>
<td>8.44</td>
<td>2-Me, 2.43</td>
</tr>
<tr>
<td><img src="image8.png" alt="Image" /></td>
<td>d</td>
<td>8.21</td>
<td>7.75</td>
<td>7.88</td>
<td>-</td>
<td>7.37</td>
<td>1-Me, 2.50</td>
</tr>
<tr>
<td><img src="image9.png" alt="Image" /></td>
<td>d</td>
<td>8.34</td>
<td>7.65</td>
<td>7.9</td>
<td>7.30</td>
<td>1-Me, 2.50</td>
<td></td>
</tr>
<tr>
<td><img src="image10.png" alt="Image" /></td>
<td>e</td>
<td>8.21</td>
<td>7.91</td>
<td>7.91</td>
<td>-</td>
<td>8.52</td>
<td></td>
</tr>
<tr>
<td><img src="image11.png" alt="Image" /></td>
<td>f</td>
<td>8.73</td>
<td>7.94</td>
<td>7.75</td>
<td>-</td>
<td>8.53</td>
<td></td>
</tr>
</tbody>
</table>

a) $\delta_H$ in DMSO-d$_6$ ref. 146; b) $\delta_H$ in CDCl$_3$ ref. 152; c) sample re-run in CDCl$_3$; d) $\delta_H$ in CDCl$_3$.

dm) $\delta_H$ of purified mixture in CDCl$_3$/DMSO-d$_6$. Data obtained by elimination of the spectrum of the 6-nitroisoindazole isomer.
e) $\delta_H$ in acetone-d$_6$/CDCl$_3$; f) $\delta_H$ in acetone-d$_6$/DMSO-d$_6$/CDCl$_3$.
Table 2.14: $^{13}$C n.m.r. Chemical shift (δ ppm) for 5-nitroindazoles and isoindazoles

<table>
<thead>
<tr>
<th>Compound</th>
<th>Carbon</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>136.7 110.7 141.5 120.6 110.9 141.8 122.1</td>
<td>-</td>
</tr>
</tbody>
</table>

| ![Structure 2](image2.png) | 127.1 110.7 141.7 120.6 110.3 141.3 122.6 | Me, 35.0 |

| ![Structure 3](image3.png) | 128.7 119.3 141.8 120.1 117.6 149.3 120.0 | Me, 40.6 |

| ![Structure 4](image4.png) | 128.9 110.5 142.1 119.8 117.9 149.3 120.1 | - |

| ![Structure 5](image5.png) | 136.8 110.9 141.2 121.1 110.3 141.3 122.9 | 123.5 128.3 |

| ![Structure 6](image6.png) | 136.8 118.8 140.18 120.78 110.3 143.02 121.59 | C-Me$_2$ 24.91; C-Me$_2$ 79.33 |

| ![Structure 7](image7.png) | 129.8 119.05 - 120.04 118.23 149.8 120.64 | C-Me$_2$ 27.09; C-Me$_2$ 80.37 |

---

a) $^6$C in DMSO-d$_6$; b) assignments may have to be reversed; c) $^6$C in DMSO-d$_6$/CDCl$_3$
Table 2.15: \[^{13}C\] n.m.r. Chemical shifts (\(\delta\) ppm) of 6-nitroindazoles and isoindazoles

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Compound 1" /></td>
<td>(\delta) 134.0 121.6 114.6 145.6 106.8 136.4 125.9 -</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><img src="image2" alt="Compound 2" /></td>
<td>(\delta) 133.64 121.17 111.99 145.72 106.74 138.50 125.62 -</td>
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</tr>
<tr>
<td><img src="image3" alt="Compound 3" /></td>
<td>(\delta) 132.7 121.6 114.6 145.4 106.4 127.9 126.9 Na, 35.7</td>
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<tr>
<td><img src="image4" alt="Compound 4" /></td>
<td>(\delta) 124.1 122.2 114.3 146.6 114.6 147.8 124.0 Na, 40.8</td>
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<tr>
<td><img src="image5" alt="Compound 5" /></td>
<td>(\delta) 126.20 123.30 115.32 145.94 115.65 147.2 -C=NO(_2), 100.34 Na, 27.19</td>
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</tr>
<tr>
<td><img src="image6" alt="Compound 6" /></td>
<td>(\delta) 134.06 122.58 114.34 146.52 108.56 136.95 128.97 -C=NO(_2), 80.31 Na, 27.78</td>
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</tr>
<tr>
<td><img src="image7" alt="Compound 7" /></td>
<td>(\delta) 134.19 123.30 115.58 146.48 105.92 138.82 126.61 -C=NO(_2), 79.99 Na, 25.01</td>
<td></td>
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</tr>
<tr>
<td><img src="image8" alt="Compound 8" /></td>
<td>(\delta) 134.77 122.52 115.4 146.58 106.83 130.59 127.00</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><img src="image9" alt="Compound 9" /></td>
<td>(\delta) 126.35 122.66 115.03 143.51 114.92 146.68 123.77</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

a) ref.152; b) \(\delta\) \(^13\)C in CDCl\(_3)/DMSO-d\(_6\); c) assignment can be reversed\(^{152}\); d) \(\delta\) \(^13\)C of isolated product in CDCl\(_3)/acetone-d\(_6\); e) \(\delta\) \(^13\)C deducted from \(\delta\) \(^{13}\)C n.m.r. spectrum of 'column-pure mixture' in DMSO-d\(_6)/CDCl\(_3\); f) \(\delta\) \(^13\)C in DMSO-d\(_6)/CDCl\(_3\)
assigned on the basis of the $^{13}$C and $^1$H n.m.r. spectroscopic data in the literature$^{146,152}$ for the N-methyl-5-and 6-nitro-indazoles and isoindazoles. The explanation for structural assignments in the n.m.r. spectra of the benzimidazole derivatives also apply to these compounds.

The signal for the H-6 proton can be used to distinguish between the 5-nitroindazole and 5-nitroisoindazole isomers. The chemical shift value of H-6 is approximately 0.2 p.p.m. downfield of the equivalent proton in the isoindazole isomer.$^{146,152}$ In these N-alkyl-5-nitro isomers, this difference is reversed, i.e. the isoindazole is approximately 0.2 p.p.m. downfield of the equivalent proton in SN$_1$ substituted indazole. Also, the signal for H-7 is approximately 1.2 p.p.m. upfield of the equivalent protons in isoindazole analogues. We suggest that these effects are explained by the presence of a second 5-nitroindazole nucleus present in SN$_1$ product [93] because the signal for H-7 and H-6 would be in the shielding ring current of this aromatic indazole ring. The $^1$H n.m.r. spectrum of this indazole [93] is also simplified as compared with the other isoindazole [94] spectrum. Despite this, the chemical shift values of this SN$_1$ product [93] agree with those of indazole,$^{146,152}$ rather than the isoindazole series. The signals in the spectrum indicated that the other isomer is the isoindazole [94] (Table 2.12).

For the 6-nitro-indazole and isoindazoles, the $^1$H n.m.r. spectroscopic data agreed with similar isomers reported in the literature$^{146,152}$ (Table 2.13). The integration of proton signals in the $^1$H n.m.r. spectrum of one isomer suggested the presence of another 6-nitroindazole nucleus as discussed for the 5-nitroindazole analogue.

Interpretation of the $^{13}$C n.m.r. spectra supported the assignment of the above structures. The reported$^{151}$ chemical shifts values for C-3, C-7 and C-8 clearly distinguish the isoindazoles from the indazoles. These carbons are underlined in the tables (Table 2.14 and 2.15) of $^{13}$C n.m.r. data.

The i.r. spectra of the SN$_1$ products showed no absorption band for the C-NO$_2$ at $\nu_{\text{max}}$ 1550 and 1350 cm$^{-1}$. 
further supporting the assignment of the structure.

Although the isoindazole analogue 6-nitroindazole could not be separated pure, its structure was deduced by elimination of signals due to the $S_{N1}$ product.

Mass spectrometry can also be used to determine the structure. However, 5- and 6-nitroindazoles are reported to have similar mass spectra, and they showed that unsubstituted indazole and benzimidazole rearrange to a common structure prior to fragmentation. Consequently, it is of little use in structure determination. The fragmentation observed for the $S_{N1}$ product is shown in scheme 2.9.

![Scheme 2.9: Fragmentation pattern of N-substituted nitroindazoles.](image)

**Steric and Electronic Effects in $S_{RN1}$ Reactions of Diazoles**

The $S_{RN1}$ reactions of diazoles (discussed above) showed similar steric effects to that observed for the nitroimidazoles; i.e., the reactions proceeding via the bulky $\text{Me}_2\text{CNO}_2$ radicals were slower than those proceeding by the less sterically hindered $p$-nitrobenzyl radicals.

We have proposed that the nitroimidazole 143a reactions proceeded because the intermediate radical-anion formed has the unpaired electron in a $\pi^*$ SOMO which is considerably
lower in energy than the corresponding C-N $\delta^*$ SOMO. We also suggested that this radical-anion was further stabilized by conjugation to the nitro group. In these heterocycles, conjugation is extended (i.e., energy further lowered) into the benzene ring and also to the nitro group where present, therefore also favouring the formation of radical-anions and hence, the reaction.

Radical reaction also occurred with imidazole (equation 2.46) which showed that the reaction will occur in the absence of a nitro group or further extended benzo-conjugation. This provides further evidence that the unpaired electron in the radical-anion of these diazole species resides in a $\delta^*$ SOMO of sufficiently low energy to allow the reaction to proceed, and implies that any further conjugation only facilitates the reaction.

**$S_{N1}$ reaction on 1-(1-methyl-1-nitroethyl)-benzimidazole [89]**

The $S_{N1}$ substitution reaction observed in the preparation of 1-(1-methyl-1-nitroethyl)benzimidazole [89] (equation 2.47) is unusual because loss of nitrite anions in $S_{N1}$ reactions is not common and nitrite ion is not a good leaving group. However, some examples have been cited in the literature.\textsuperscript{153,154}

Alston et al.\textsuperscript{154} reported the loss of nitrite anion during an enzyme inhibition reaction as shown in scheme 2.10.

\[
\text{CH}_3\text{CHO} + \text{Me-C-NO}_2^+ \rightarrow \text{H}^+ + \text{Me-C-NO}^+ \rightarrow \text{Me-CN}^+ + \text{H}_2\text{O} \rightarrow \text{Me-CN} \rightarrow \text{inactivate enzyme}
\]

**Scheme 2.10 : Loss of nitrite anion from a nitroethane/flavin adduct.**

Likewise, Ono et al.\textsuperscript{153} have recently reported nucleo-
philic substitution of nitro group from tertiary nitro compounds in the presence of a Lewis-acid catalyst, SnCl$_4$(equation 2.80).

\[
\begin{align*}
R_3C & \overset{\text{N}}{\longrightarrow} \overset{\text{O}}{\text{N}} & \overset{\text{O-SnCl}_4}{\longrightarrow} & R_3C^+ + \text{NO}_2^- + \text{SnCl}_4 \\
R & = \text{Me, Ph} \\
A^- & = \text{SPh}
\end{align*}
\]

(2.80)

A number of reactions were carried out under different reaction conditions in an attempt to emulate the observed $S_N$1 reaction in 1-(1-methyl-1-nitroethyl)benzimidazole [89] (equation 2.47).

Acid catalysed hydrolysis of 1-(1-methyl-1-nitroethyl) benzimidazole [89] gave benzimidazole and acetone (scheme 2.11) while in the presence of a base, there was no reaction. These reactions suggest that loss of nitro group from the tertiary centre of 1-(1-methyl-1-nitroethyl)benzimidazole [89] is catalysed by the acid as reported by Ono. The mechanism of the hydrolysis (scheme 2.11) is probably similar to that proposed by Alston et al. in their enzyme studies (scheme 2.10). The acetone produced was trapped as 2,4-dinitrophenyl hydrazone (42%) (equation 2.81).

The formation of acetone in these reactions prompted us to study the reverse reaction between benzimidazole and excess acetone in the presence of a weak acid, p-toluene sulphonic acid (p-TSA). A blue/green product was isolated which was not the $S_N$1 product, and even after extensive characterisation, the structure could not be determined.
Conclusion

$N$-Alkylation by radical, radical-anion reactions ($S_{RN1}$ and oxidative addition reactions) were successfully extended from 4(5)-nitroimidazoles to other diazoles and nitro-diazoles to give a wide range of interesting novel compounds.

Some $N$-alkylation reaction with 2-substituted-2-nitropropanes (or the anions of 2-nitropropane) showed interesting $S_{N1}$ substitution reactions to give additional novel compounds. The $S_{N1}$ reactions were observed only in products that were able to maintain their aromaticity in the transition state of the $S_{N1}$ mechanism. It was not possible to emulate this $S_{N1}$ reaction starting with the $N$-alkylated product and the corresponding diazole. The reactions in-

Scheme 2.11: Loss of nitrite anion from 1-(1-methyl-1-nitroethyl)benzimidazole
volving the p-nitrobenzyl chloride and the diazole anion proceeded by an $S_N$2 and/or an $S_{RN}$1 mechanism.

Radical $N$-alkylation of substituted diazole gave similar ratios of $N$-alkylated isomers to electrophilic $N$-alkylations. No regio-selectivity of alkylation of the ambident $N$-centres of substituted diazoles was observed by both mechanisms.

These reactions showed that $S_{RN}$1 reactions involving $N$-centres observed in 4(5)-nitroimidazole anions were not just "one-off" examples, but that they can be applied to other $N$-centred anions.
General. - DMF and DMSO were distilled at low pressure from calcium hydride and stored over molecular sieves. M.p.s. were recorded on a Kofler block and are uncorrected. I.r. spectra were determined as Nujol mulls on a Pye Unicam PU 516 spectrometer. $^1$H n.m.r. spectra were determined at 90 MHz on a Perkin-Elmer R32 spectrometer or at 60 MHz on a Varian EM 360A instrument: using tetramethyl silane (TMS) as an internal standard. N.m.r. analyses of reaction mixtures were carried out using a known amount of an internal standard ($p$-dimethoxybenzene or $p$-dinitrobenzene). $^{13}$C n.m.r. spectra were carried out on a Bruker WP-80 spectrometer. Mass spectra were carried out on Kratos MS 80 instrument. Analyses were performed by Microanalytical Department of Manchester University. Preparative and analytical thin layer chromatography were performed using Merck alumina 60 PF$_{254}$ (Type E) and Merck silica gel 60 PF$_{254}$. Column chromatography was performed using Hopkin and William alumina 'CAMAG' M.F.C. neutral, Brockman activity/and Merck silica gel 60, 70-230 mesh. Flash chromatography was accomplished using Merck silica gel, 230-400 mesh.

Nitrogen was dried and deoxygenated by passing it through a series of wash bottles containing Fiesers solution, conc. sulphuric acid, and potassium hydroxide pellets.

Common abbreviations used in the experimental sections:

- **DMF**: N,N-Dimethylformamide
- **DMSO**: Dimethyl sulphoxide
- **MgSO$_4$**: Anhydrous magnesium sulphate
- **t.l.c.**: Thin layer chromatography
- **O$_2$**: Oxygen gas
- **N$_2$**: Nitrogen gas
- **CDCl$_3$**: Deuterated chloroform
- **$^6$**: Chemical shift (ppm)
1. 1-(p-Nitrobenzyl)-4-nitroimidazole [65] (General Procedure for SRN¹ Reactions)

Potassium t-butoxide (2.97g, 26.5mmol) was suspended in dry DMSO (10ml) and added to a solution of 4(5)-nitroimidazole (2.0g, 17.7mmol) in dry DMSO (50ml) under nitrogen and under anhydrous conditions. The mixture was stirred under nitrogen for 30min to allow complete de-oxygenation. p-Nitrobenzyl chloride (4.55g, 26.5mmol) in DMSO (10ml) was added to the solution which rapidly turned red in colour. The reaction mixture was then irradiated with two 150-W fluorescent discharge lamps (mercury blended tungsten universal mounted (MBTU) lamps emitting light maximally at 430nm) from a distance of 10cm. After 8h the red colour changed to light orange and the reaction was terminated by adding water (50ml). The aqueous solution was extracted with CH₂Cl₂, the CH₂Cl₂ extracts were washed with water (7 x 50ml) to remove DMSO, dried, (MgSO₄) and evaporated to dryness to yield pure 1-(p-nitrobenzyl)-4-nitroimidazole [65] (100%). The pure crystals were further recrystallised from ethyl acetate - light petroleum (b.p. 40-60°C) (1:1) (3.3g, 75%); m.p. 138-139°C. [Found: C, 48.8; H, 3.3; N, 22.6. C₁₂H₈N₄O₄ requires C, 48.39; H, 3.25; N, 22.58%]; δH (CDCl₃) 5.73 (2H, s, CH₂); 8.00 (1H, d, C₂-H); 8.33 (1H, d, C₅-H) and 7.78-8.40 (4H, ABq, phenyl H); δC 49.59 (t, CH₂); 113.28 (d, 2-C), 119.46 (d, 5-C); 123.12 (d, phenyl-2-C); 128.04 (d, phenyl-3-C); 141.93 (s, 4-C) and 147.13 (s, phenyl-4-CNO₂); m/z [Found: M⁺, 248.0547. C₁₂H₈N₄O₄ requires M⁺, 248.0545].136(100), 133(77), 89(36), 48(48), 65(14).

2. General Procedure for Light Catalysis and Inhibitions Studies of SRN¹ Reactions

The general procedure was followed except as outlined in each method given below.

a) Inhibition studies with p-dinitrobenzene or di-t-butyl-nitroxide added to the reaction mixture immediately prior to the alkyl halides [82] and [58].

b) Inhibition studies with oxygen were carried out by replacing nitrogen gas by oxygen gas.
c) The studies of the effect of light catalysis were carried out by exclusion of light from the reaction which was effected by wrapping the flask in aluminium foil.

The results of these respective studies are shown in Tables 2.1 and 2.5.

3. Reactions between Nitroimidazoles [51] and [52] and substrates [82] and [58]

The following compounds were prepared using the general procedure for \( S_{RN} \) reactions.

a) 1-(1-Methyl-1-nitroethyl)-4-nitroimidazole [62]

The anion of 4(5)-nitroimidazole [51] (2.0g, 17.7mmol) was reacted with 2-bromo-2-nitropropane (4.46g, 26.5mmol; 1.5mol equiv.) to yield the pure (by t.l.c. and \(^1\)H n.m.r. spectroscopy) nitroimidazole [62] (3.27g, 92%). Recrystallisation from ethyl acetate-light petroleum (40-60°C) (1:1) gave analytically pure 1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62] (2.09g, 59%): m.p. 163-165°C; [Found: C, 36.1; H, 4.0; N, 28.0. \( \text{C}_6\text{H}_8\text{N}_4\text{O}_4 \) requires C, 36.0; H, 4.0; N, 28.0%]; \( \delta \)\( _{\text{H}} \) (acetone-\( d_6 \)) 2.50 (6H, s, Me), 8.40 (1H, d, 2-H), and 8.75 (1H, d, 5-H), \( \delta \)\( _{\text{C}} \) (acetone-\( d_6 \)) 26.13 (q, Me), 97.15 (s, \( \text{CMe}_2 \)), 120.28 (d, 5-C), 137.17 (d, 2-C), and 149.47 (s, 4-C); \( m/z \) [Found: \( M^+ \), 200.0545 (0.25%), \( \text{C}_6\text{H}_8\text{N}_4\text{O}_4 \) requires 200.0545], 154(100), 96(32), 69(99).

Repeat reactions for various reaction times were carried out in order to find a suitable time for inhibition studies. The reactions were carried out for 6h under the various conditions outlined in the general procedure for inhibition studies. The results of these inhibition studies are shown in Table 2.1.

The reaction between the anion, [51], and 2-bromo-2-nitropropane (1.5mol. equiv.) was carried out and the results are presented in Table 2.1.

b) 5-(4-Nitroimidazol-1-yl)-5-nitro-1,3-dioxane [64]

The anion, [51], 4(5)-nitroimidazole (2.0g, 17.7mmol) was reacted with dioxane [57] (5.0g, 23.6mmol) to yield a crude mixture (3.5g). Chromatography on neutral alumina with \( \text{CH}_2\text{Cl}_2 \) as the eluent, followed by recrystallisation from ethanol, gave crystals of pure 5-(4-nitroimidazol-1-
-yl)-5-nitro-1,3-dioxane (1.8g, 42%), m.p. 147-148°C [Found: C, 34.5; H, 3.3; N, 22.8. C_{7}H_{8}N_{4}O_{6} requires C, 34.4; H, 3.3; N, 22.95%].

b) 6-Nitroimidazole (acetone-d$_{6}$) 5.10 (6H, m, CH$_{2}$), 8.5 (1H, d, 2-H), and 9.10 (1H, d, 5-H); δ$_{C}$ (acetone-d$_{6}$) 68.08 (t, OCH$_{2}$C), 93.34 (t, OCH$_{2}$O), 90.08 (s, quat. C-NO$_{2}$), 119.96 (d, 5-C), and 136.86 (d, 2-C); m/z [Found: M$^{+}$, 244.039 (9%). C$_{7}$H$_{8}$N$_{4}$O$_{6}$ requires 244.0443, 198(55), 168(100), 152(34), 123(51), 96(21), 81(10), and 67(10).

c) The attempted preparation of 1-phenyl-4-nitroimidazole 4(5)-Nitroimidazole (2.0g, 17.7mmol) was reacted with phenyliodide (5.4g, 26.5mmol; 1.5mol equiv.) to give a crude mixture which was shown by $^{1}$H n.m.r. spectroscopy to consist of unreacted phenyl iodide (58%) and traces of unaltered 4(5)-nitroimidazole.

d) 2-Methyl-1(p-nitrobenzyl)-4-nitroimidazole [66]
The anion, [52], (2.0g, 15.7mmol) was reacted with p-nitrobenzyl chloride as outlined in the general $S_{RN1}$ reaction to yield (5.0g) crude product. Chromatography on neutral alumina with CH$_{2}$Cl$_{2}$-CHCl$_{3}$ (2:1) as eluting solvent, followed by recrystallisation from EtOAc, gave yellow crystals of the nitroimidazole [66] (3.01g, 73%), m.p. 181-183°C [Found: C, 50.8; H, 3.9; N, 21.4. C$_{11}$H$_{10}$N$_{4}$O$_{6}$ requires C, 50.4; H, 3.8; N, 21.4%]. δ$_{H}$ 2.35 (3H, s, Me), 5.50 (2H, s, CH$_{2}$), 7.04-8.10 (4H, ABq, phenyl-H), and 8.20 (1H, s, 5-H); δ$_{C}$ (CDCl$_{3}$) 13.18 (q, 2-Me), 50.63 (t, CH$_{2}$), 122.36 (d, phenyl-2-C), 129.46 (d, phenyl-3-C), 144.0 (s, 4-CNO$_{2}$), 146.58 (s, phenyl-4-CNO$_{2}$); m/z [Found: M$^{+}$, 262.0700 (100%). C$_{11}$H$_{10}$N$_{4}$O$_{6}$ requires 262.0702, 155(25), 136(89), 120(14), 106(54), 89(85), 78(48), and 63(9).

e) 2-Methyl-1-(1-methyl-1-nitroethyl)-4-nitroimidazole [63]
2-Methyl-4(5)-nitroimidazole [52] (2.0g, 15.7mmol) was reacted with 2,2-dinitropropane (3.16g, 23.6mmol) to yield a crude mixture (2.7g). Chromatography on neutral alumina with CH$_{2}$Cl$_{2}$ - CHCl$_{3}$ (2:1) as eluent, followed by recrystallisation from ethyl acetate, yielded colourless crystals of the nitroimidazole [63], (1.25g, 37%), m.p. 147-149°C [Found: C, 39.3; H, 4.7; N, 25.9. C$_{7}$H$_{10}$N$_{4}$O$_{4}$ requires C, 39.25; H, 4.71; N, 26.15%]; λ$_{max}$ 290nm (ε = 6 x 10$^{5}$);
$\gamma_{\text{max}}$ 3160, 3100, 1560 and 1350 cm$^{-1}$; $\delta^H$ (acetone-d$_6$) 2.32 (6H, s, Me$_2$), 2.45 (3H, s, 2-Me), and 8.50 (1H, s, 5-H); $\delta^C$ (acetone-d$_6$) 15.71 (q, 2-Me), 25.53 (q, Me$_2$), 97.74 (s, quat. CNO$_2$), 121.50 (d, 5-C), and 145.23 (s, 4-C); m/z [Found: M$^+$, 214.0703 (2%). $C_7H_{10}N_4O_4$ requires 214.0702], 168(15), 126(15), 96(100), and 69(15).

The reaction was carried out again using i) 2-bromo-2-nitropropane (1.5mol equiv.) and ii) 2-chloro-2-nitropropane (1.5mol equiv.) in place of 2,2-dinitropropane. The results are presented in Table 2.1.

g) 2-Methyl-1-[(1-Methyl-5-nitroimidazol-2-yl)-methyl]-4-nitroimidazole [67]

2-Methyl-4(5)-nitroimidazole [52] (250mg, 1.97mmol) was reacted with 2-(bromomethyl)-1-methyl-5-nitroimidazole, [59], (250mg, 1.14mmol) to yield crude product (262mg). Purification using alumina prep. t.l.c. with EtOAc-CH$_2$Cl$_2$ (9:1) as the eluent, followed by recrystallisation with EtOAc gave crystals of the nitroimidazole, [67] (183mg, 35%), m.p. 208-209°C [Found: C, 40.4; H, 3.63; N, 31.62. $C_9H_{10}N_6O_4$ requires C, 40.6; H, 3.79; N, 31.8%]; $\delta^H$ (CDCl$_3$) 2.40 (3H, s, 2-Me), 4.06 (3H, s, N-Me), 5.72 (2H, s, CH$_2$), 8.18 (1H, s, 5-H), and 8.48 (1H, s, 4'-H).

The reaction was repeated using the 5-nitroimidazole, [60], in place of 5-nitroimidazole, [59]. The results are reported in Table 2.1.

h) 1-[(1-methyl-5-nitroimidazol-2-yl)-methyl]-2-nitroimidazole [69]

2-Nitroimidazole (250mg, 2.21mmol) was reacted with the 5-nitroimidazole [60] (388 mg, 2.21mmol) to yield a crude mixture. Chromatography using silica gel prep. t.l.c. with EtOAc-CH$_2$Cl$_2$ (9:1) as eluent gave pure product. Recrystallisation from acetone-light petroleum (60-80°C) gave
yellow crystals of the nitroimidazole [67] (2.34mg, 42%), m.p. 168-169°C [Found: C, 38.3; H, 3.2; N, 33.6. C₈H₈N₆O₄ requires C, 38.1; H, 3.2; N, 33.3%]; 6H (acetone-d₆) 4.10 (3H, s, N-Me), 6.00 (2H, s, CH₂), 7.18 (1H, brs, 4-H), 7.48 (1H, s, 4'-H), and 7.82 (1H, s, 5-H); m/z 206 (M+ -NO₂, 100%), 160(24), 140(13), 94(17), 80(10) and 67(11).

The reaction was repeated using the 5-nitroimidazole [59], in place of 5-nitroimidazole [60] and the results are shown in Table 2.1.

4. The attempted preparation of 1-(1-methyl-1-nitroethyl)-2-nitroimidazole.

i) 2-Nitroimidazole (500mg, 4.42mmol) and KOBut (743mg, 6.64mmol) in dry DMSO (20ml) were stirred till dissolved under N₂. 2-Bromo-2-nitropropane (1.12g, 6.44mmol) was added to the solution and then irradiated as described in the general procedure for SN₁ reactions. The reaction was left stirring under N₂ for 48h. The normal work-up afforded a crude mixture, ¹H n.m.r. analysis of this mixture, using an internal standard yielded unaltered 2-bromo-2-nitropropane (22%) and 2,3-dimethyl-2,3-dinitrobutane (64%). The aqueous layer was acidified with ammonium hydrochloride and the precipitate filtered to give unreacted 2-nitroimidazole (200mg, 80%). The ¹H n.m.r. spectrum and t.l.c. was identical to that of the authentic sample.

ii) 2,2-Dinitropropane (445 mg, 3.32mmol) and 2-nitroimidazole (375mg, 3.32mmol) were reacted as in (i) above. Similar results were obtained with unreacted 2-nitroimidazole recovered (350mg, 70%) from the aqueous layer. The organic layer yielded 2,2-dinitropropane (18%) and 2,3-dimethyl-2,3-dinitrobutane (66%), determined by n.m.r. spectroscopy (using an internal Standard).

iii) The reaction was carried out as in (ii) but using HMPA instead of DMSO.

The normal work-up gave the same results. Acidification of the aqueous layer gave unreacted 2-nitroimidazole (360mg, 81%). An internal standard was added to the crude from the organic layer and ¹H n.m.r. analysis of the mixture showed 2,2-dinitropropane (19%) and 2,3-dimethyl-2,3-dinitrobutane (76%). The ¹H n.m.r. spectroscopic data of
these compounds were identical to those of the authentic samples.

5. Competitive $S_{RN1}$ Reactions

4(5)-Nitroimidazole (1.00g, 8.85mmol) and sodium hydride (318mg, 13.3mmol) were stirred in dry DMSO (25ml) under nitrogen until both reagents had reacted and dissolved. p-Nitrobenzyl chloride (1.52g, 8.85mmol) and 2-bromo-2-nitropropane (2.23g, 13.3mmol, 1.5mol equiv.) were added simultaneously to the reaction mixture. The reaction was then irradiated with 2 x 150W fluorescent lamps for 13h. The work-up was as described in the general procedure for $S_{RN1}$ reactions. The crude mixture was analysed by $^1$H n.m.r. spectroscopy using p-dimethoxybenzene as an internal standard.

The reaction was repeated with 2-methyl-4(5)-nitroimidazole (8.85mmol) in place of the 4(5)-nitroimidazole and equimolar amounts (8.85mmol) of p-nitrobenzyl chloride and 2-bromo-2-nitropropane. The results of both reactions are shown in Scheme 2.4.

6. General Procedure for Oxidative Addition of Nitroimidazole Anions to the Anion of 2-Nitropropane

a) Preparation of 1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62]

4(5)-Nitroimidazole [51] (1.0g, 8.85mmol) was added to a solution of sodium hydroxide (0.90g, 22.5mmol, 2.5mol equiv.) in water (10ml) and stirred under nitrogen at 0°C until the nitroimidazole dissolved. CH$_2$Cl$_2$ (30ml) and water was added to form a two-phase reaction mixture. The anion of 2-nitropropane (1.47g, 13.28mmol, 1.5mol equiv.) in water (10ml) was added to the solution followed by a solution of potassium ferricyanide (0.33g, 17.7mmol, 0.2 equiv.) in water (10ml), and then immediately followed by solid sodium persulphate (2.10g, 8.85mmol, 1 equiv.). The mixture was stirred for 1h, the CH$_2$Cl$_2$ and water layers were separated, and the aqueous fraction further extracted with CH$_2$Cl$_2$. The CH$_2$Cl$_2$ extracts were combined, washed with water, dried (MgSO$_4$), and evaporated to dryness to yield a crude product (1.11g). Chromatography on neutral alumina with EtOAc-Et$_2$O (4:1) as eluent gave the nitroimidazole [62] (0.60g, 34%) and 2,3-dimethyl-2,3-dinitrobutane (312mg, 20%)
based on the 4(5)-nitroimidazole). The products were characterised by comparison of $^1$H n.m.r. spectra and m.p.s. with authentic material (products from $S_{RN1}$ reactions).

The reaction was repeated several times under differing conditions; the reaction conditions used and the results are presented in Table 2.4.

b) 2-Methyl-1(1-methyl-1-nitroethyl)-4-nitroimidazole [63]

The reactions were carried out and worked up as reported above in the general procedure for oxidative addition reactions except that instead of 4(5)-nitroimidazole, 2-methyl-4(5)-nitroimidazole [52] was used. The results are shown in Table 2.4.

c) The attempted preparation of 1-(1-methyl-1-nitroethyl)-2-nitroimidazole

Using the general oxidative addition reaction method described above, 2-nitroimidazole (500mg, 4.42mmol) and sodium hydroxide (0.3g, 6.64mmol) in distilled water (20ml) were stirred until dissolved under N$_2$. Dichloromethane (40ml) was added and the two-phase solution cooled in an ice bath. The sodium salt of 2-nitropropane (740mg, 6.64mmol) in water (2ml) was added to the stirred solution, followed by potassium ferricyanide (0.3g, 0.884mmol) in water (2ml) and sodium persulphate (1.05g, 4.42mmol). The reaction was stirred for 45 minutes.

The normal work-up afforded a crude mixture (250mg), which was shown by $^1$H n.m.r. spectroscopy to be 2,3-dimethyl-2,3-dinitrobutane (43%) and a trace of 2,2-dinitropropane (5%).

The aqueous layer was acidified with ammonium hydrochloride and the filtered residue showed by $^1$H n.m.r. spectroscopy and t.l.c. to be unreacted 2-nitroimidazole (500mg 100%). $^1$H n.m.r. spectroscopic data were identical to those of authentic samples.

7. $S_{RN1}$ Reactions between 1-(1-Methyl-1-nitroethyl)-4-nitroimidazoles [62] and Nucleophiles

The reactions were carried out as detailed in the general procedures for $S_{RN1}$ reactions and the general procedure for light catalysis and inhibition studies of $S_{RN1}$ reactions. The reaction conditions and results are reported in Table 2.5.
a) Reaction between the 4-nitroimidazole, [62], and the anion of 2-nitropropane [61]

The nitroimidazole [62] (200 mg, 1 mmol) and the sodium salt of 2-nitropropane (334 mg, 3 mmol) were reacted to yield a crude mixture which was leached with CH$_2$Cl$_2$. The remaining crystals were pure unaltered 1-(1-methyl-1-nitroethyl)-4-nitroimidazole (56 mg, 28%). The CH$_2$Cl$_2$ solution was evaporated to dryness to yield pure 2,3-dimethyl-2,3-dinitrobutane (53 mg, 30%). Both compounds were characterised by comparison with authentic material by $^1$H n.m.r. spectroscopy and m.p.s.

Inhibition studies are carried out using DTBN (50 molar%), pDNB (40 molar%) and oxygen (dark). The results of the inhibition studies are reported in Table 2.5.

b) Reaction between the 2-methyl-4-nitroimidazole, [63], and the anion of 2-nitropropane

The reaction was carried out as above on the same scale and the same method of purification of products. The results of the two reactions (4.5 and 5 h) and inhibition studies are reported in Table 2.5.

c) Reaction between the nitroimidazole, [63], and 4(5)-nitroimidazole, [51].

The nitroimidazole, [63] (125 mg, 0.584 mmol) was added to the stirred solution of the sodium salt of 4(5)-nitroimidazole (66 mg, 0.584 mmol) and reacted for 14 h as described in the general procedure for $S_{RN1}$ reactions. The crude product was purified using alumina prep. t.l.c. with CH$_2$Cl$_2$-CHCl$_3$ (1:1) as the eluent to yield pure unaltered nitroimidazole [63] (59 mg, 26%) and 1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62] (47%). Both were characterised by comparison of t.l.c. R$_f$S, n.m.r. spectra, and m.p.s. with authentic materials.

d) Reaction between the nitroimidazole, [63], and benzene sulphinate

To the stirred solution of benzene sulphinate (84 mg, 0.596 mmol) in dry DMSO was added to the nitroimidazole, [63] (69 mg, 0.322 mmol) and reacted for 24 h as detailed in the general procedure for $S_{RN1}$ reaction. Work up of the reaction and evaporation to dryness of the CH$_2$Cl$_2$ layer gave unaltered nitroimidazole [63]. The aqueous layer was evapor-
ated to dryness to give benzene sulphinate (74mg, 88%). Both were characterised by comparison with t.l.c. R<sub>e</sub>s, n.m.r.
spectra and m.p.s. with authentic materials.

8. Preparation of 2-nitroprop-1-yl tosylate [80]

Tosyl chloride (0.5mol equivalents 13.6g, 35.7mmol) was added to warm pyridine (2.82g) and the mixture was cooled rapidly to 0°C in an ice bath until a salt was formed. 2-Nitropropan-1-ol (5.0g, 47.5mmol) was added to the salt and the semi-solid was left stirring overnight.

The resulting mixture was diluted with water, and extracted with ether (3 x 50ml). The organic extracts were combined, washed consecutively with dilute 2M sulphuric acid, sodium bicarbonate, water, dried (MgSO<sub>4</sub>) and solvent removed in vacuo. Chromatography on neutral alumina with CHCl<sub>3</sub> as eluent and recrystallisation afforded the tosylate [80] (5.7g, 88%); δ<sub>H</sub>(CDCl<sub>3</sub>) 1.57 (3H, d, -CH<sub>3</sub>), 2.56 (3H, s, CH<sub>3</sub> Ph-C~3 <sub>~</sub>, 4.44 (2H, t, -CH<sub>2</sub>-C), 4.56-5.07 (1H, m, -CH<sub>2</sub>-CH-NO<sub>2</sub>), and 7.51-8.0 (4H, ABq, phenyl H).

9. The attempted preparation of 1-(2-nitroprop-1-yl)-2-methyl-5-nitroimidazole

A mixture of 2-methyl-4(5)-nitroimidazole (1.4lg, 10mmol) and the tosylate [80] (5.0g, 20.4mmol) is heated with stirring at 140°C for 4h.

The resulting viscous oil was cooled, diluted with hot water (100ml) and adjusted to pH9 with 10% Na<sub>2</sub>CO<sub>3</sub> solution. The solution was extracted with CHCl<sub>3</sub> (3x100ml), the extracts were combined, washed with distilled water, dried (MgSO<sub>4</sub>) and solvent removed in vacuo to give a crude product. The <sup>1</sup>H n.m.r. spectrum of this crude did not allow identification.

10. The attempted preparation of 2-methyl-1-(nitromethyl)-5-
nitroimidazole

i) 2-Methyl-4(5)-nitroimidazole (1g, 7.87mmol) in toluene (25ml) was refluxed for 30 minutes. Bromonitromethane (1.2g, 8.66mmol) was added and left refluxing overnight. The precipitate which was observed since the beginning of the reaction was filtered and dried. <sup>1</sup>H n.m.r. spectroscopic analysis showed that it was unchanged nitroimidazole starting material (0.8g, 80%).
The filtrate was diluted with water (25ml) and neutralised with ammonium hydrochloride and then extracted with CHCl₃ (3x25ml). The normal work up afforded a crude mixture. The ¹H n.m.r. spectrum did not allow identification.

ii) The reaction was carried out again using the general procedure for SRN¹ reactions. The normal work up afforded a crude mixture. ¹H N.m.r. spectroscopy indicated imidazole starting material (45%).

11. Oxidative Addition of Diazo Anions to the Anions of 2-Nitropropane

The following compounds were prepared using the general procedure for oxidative addition reactions.

a) 1-(1-Methyl-1-nitroethyl)-imidazole [88]

Imidazole (1.0g, 14.7mmol) was dissolved in sodium hydroxide solution (1.5mol equivalent) as in the general procedure for oxidative addition reactions. The sodium salt of 2-nitropropane (2.45g, 22.1mmol; 1.5mol equiv.) was added to the reaction mixture followed by potassium ferricyanide (0.97g, 2.94mmol, 0.2mol equiv.) and solid sodium persulphate (3.49g, 14.7mmol). The work up was carried out as described in the general procedure to yield a crude product which was chromatographed on neutral alumina eluted with CH₂Cl₂:EtOAc (1:4), followed by recrystallisation from pet-ether (40-60°C) to give pale yellow crystals of 1-(1-methyl-1-nitroethyl)-imidazole (0.67g, 28%), m.p. 57-58°C. [Found: C, 46.1; H, 6.0; N, 27.5. C₆H₅N₃O₂ required C, 46.4; H, 5.85; N, 27.0%]; v max (nujol) 3120 (aryl H), 1550 and 1340cm⁻¹ (C-NO₂ stretching); δH (CDCl₃) 2.26 (6H, s, (CH₃)₂), 7.1 (2-H, brs, H-4 and H-5), 7.73 (1H, s, H-2), m/z [Found: M⁺ 155.0695 (1.57%). C₆H₅N₃O₂ requires 155.0699], 109(100), 82(30), 68(10), 55(21).

b) 1-(1-Methyl-1-nitroethyl)-benzimidazole [89]

The anion of benzimidazole [84] (1.0g, 8.47mmol) in sodium hydroxide solution (0.51g, 12.7mmol, 1.5mol equiv.) was reacted with the sodium salt of 2-nitropropane (1.41g, 12.7mmol, 1.5mol equiv.) to give a crude product. (An ¹H n.m.r. spectrum of the mixture was obtained before separation). Separation by column chromatography on neutral alumina using CH₂Cl₂:CHCl₃ (1:1) as eluent afforded two products: the titled product [89] (0.84g, 48%), m.p. 69-70°C. [Found: C, 58.3; H, 5.1; N, 20.3. C₁₀H₁₀N₃O₂ requires C, 58.53;
Various reagent ratios were used to find out the optimum reaction conditions (Table 2.7). The yields of products were calculated using $^1$H n.m.r. spectroscopy, with an internal standard. The results are shown in Table 2.7.

c) 1-(1-Methyl-1-nitroethyl)-5-nitrobenzimidazole [91]

5-Nitroimidazole (1.0g, 6.1mmol) was dissolved in aqueous sodium hydroxide solution (0.37g, 9.2mmol, 1.5mol equiv.). The sodium salt of 2-nitropropane was added to the solution followed by potassium ferricyanide (0.40g, 1.22mmol, 0.2mol equiv.) and sodium persulphate (1.45g, 6.1mmol). The reaction was stirred for 75min as in the standard oxidative addition reaction above. After an initial inefficient column chromatography, the crude mixture was chromatographed on alumina preparative t.l.c. plates with CH$_2$Cl$_2$:pet-ether (40-60°C) as eluent to give two products. Recrystallisation from CHCl$_3$:pet-ether (40-60°C) afforded the titled compound [91] (0.52g, 34%), m.p.211-212°C. [Found: C,47.85; H,4.0; N,22.2. C$_{10}$H$_{10}$N$_4$O$_4$ requires C,48.00; H,4.02; N,22.39]; $\nu_{max}$ (nujol) 3160-3100 (aryl H), 1610 (\(\angle C=\angle C^{arom}\)), 1550 and 1340 (C-NO$_2$ aliphatic), 1520 and 1310, and 720-740cm$^{-1}$. The $^{13}$C and $^1$H n.m.r. spectroscopic data are presented in Table 2.10 and Table 2.11. $m/z$ [Found M$^+$ 250.0702 (0.73%), C$_{10}$H$_{10}$N$_4$O$_4$ requires 250.0696]. 204(100), 163(52), 158(77), 157(20), 133(17), 118(13), 117(46), 90(55).

The other compound was identified as 1-(1-Methyl-1-nitroethyl)-6-nitrobenzimidazole [92] (0.35g, 23%) m.p.129-130°C. [Found: C,48.1; H,4.0; N,22.2. C$_{10}$H$_{10}$N$_4$O$_4$ requires C,48.00; H,4.02; N,22.39%]. The $^1$H and $^{13}$C n.m.r. spectro-
scopic analyses are presented in Tables 2.10 and 2.11.

d) 1-1(-Methyl-1-nitroethyl)-5-nitroisoindazole [94] and 2,2-di-(5-nitroindazol-1-yl)-propane [93]

The anion of 5-nitroindazole [86] (1.0 g, 6.1 mmol) in potassium hydroxide solution was reacted with the sodium salt of 2-nitropropane (0.52 g, 9.2 mmol, 1.5 mol equiv.) as outlined in the general oxidative addition procedure above.

The crude product was separated by prep. alumina t.l.c. to afford two products. Both were recrystallised from chloroform-pet-ether (40-60°C) to give (i) the isoindazole [94] crystals (0.64 g, 42%), m.p. 135-136°C. \([\text{Found: } C, 48.0; H, 3.9; N, 22.0. ]\ C_{10}H_{10}N_{4}O_{4} \text{ requires } C, 48.00; H, 4.02; N, 22.39\%]; v_{\text{max}} \text{ (nujol) } 3160-3100 (aryl H), 1610 (>\text{C} \equiv \text{C}; \text{arom.}), 1550 \text{ and } 1340 (\text{C-NO}_{2} \text{ aliph.}), 1570-1350 \text{ cm}^{-1} (\text{C-NO}_{2} \text{ arom.}).

The n.m.r. spectroscopic data are shown in Tables 2.12 and 2.14 of the discussion. \(m/z\) [Found: \(M^+\), 250.0702 (3.7\%) \(C_{10}H_{10}N_{4}O_{4}\) requires 250.0701], 204(100), 158(47), 118(54), and 90(36).

The other product, 2,2-di-(5-nitroindazol-1-yl)-propane [93] was separated as pale yellow crystals (0.27 g, 32%), m.p. 159-161°C. \([\text{Found: } C, 55.5; H, 3.85; N, 22.65. ]\ C_{17}H_{14}N_{4}O_{4} \text{ requires } C, 55.73; H, 3.85; N, 22.94\%]; \text{C and } H \text{ n.m.r. spectroscopic data are presented in Tables 2.14 and 2.12 respectively. } m/z [\text{Found: } M^+, \text{ 366 (0.72\%)}, M^+-162(100), 163(9), 158(11), and 118(8).]

e) 2,2-di(6-nitroindazol-1-yl)-propane [95]

The anion of 6-nitroindazole [87] (1.0 g, 6.1 mmol) in potassium hydroxide solution was reacted with the sodium salt of 2-nitropropane (1.02 g, 9.2 mmol, 1.5 mol equiv.) as described in the general oxidative addition procedure. The crude product was very difficult to separate using column chromatography, therefore prep. t.l.c., and reverse phase chromatography were used to obtain the two products [95] [96] observed using t.l.c. and \(1H\) n.m.r. spectroscopy of the crude mixture. Only one of the isomers could be isolated pure, [95] the other remained as a mixture. Both (the separated product [95] and the mixture) were recrystallised and analysed. The titled compound [95] was obtained as yellow crystals (0.65 g, 29\%), m.p. 210-213°C, \([\text{Found: } C, 55.25; \text{H, 3.25; N, 22.65. } C_{17}H_{14}N_{4}O_{4} \text{ requires } C, 55.25; H, 3.25; N, 22.65\%].}
The n.m.r. spectroscopic analysis of the mixture (presented in Tables 2.13 and 2.15) confirmed the presence of the indazole [95] and the isoindazole isomers [96] (see discussion section).

12. $S_{RN}1$ Reactions between Diazole Anions and 2-substituted-2-nitropropanes

The diazole anions ([83]–[87]) were reacted with 2-substituted-2-nitropropanes (Me$_2$C(X)NO$_2$ with X = Cl[54], Br[55] and NO$_2$[56]) in DMSO (or HMPA) as outlined in the general procedure for $S_{RN}1$ reaction. The yields of the reactions are presented in Table 2.6 and i.r. and n.m.r. spectra of the 1-(1-methyl-1-nitroethyl)-derivatives ([88]–[94]) were identical to those prepared via the oxidative addition method.

13. $S_{RN}1$ Reactions between Diazole Anions and p-nitrobenzyl chloride

The following reactions were carried out and worked up as reported in the general procedure for $S_{RN}1$ reactions.

a) 1-(p-Nitrobenzyl)-5-nitrobenzimidazole [99]

The anion of 5-nitrobenzimidazole [85] (1.0g, 6.1mmol) was reacted with p-nitrobenzyl chloride (1.05g, 6.1mmol) to yield a product mixture. Column chromatography on neutral alumina with CHCl$_3$:pet-ether (1:2) as eluent, gave separation of the mixture. The two products were recrystallised (EtOAc-Pet-ether) and analysed to give i) 1-(p-nitrobenzyl)-5-nitrobenzimidazole [99] (0.53g, 29%), m.p. 196-198°C [Found: C, 55.95; H, 3.3; N, 18.6. C$_{14}$H$_{10}$N$_4$O$_4$ requires C, 56.38; H, 3.38; N, 18.79%.] m/$_z$ [Found: M$^+$ 298.0709 (100%). C$_{14}$H$_{10}$N$_4$O$_4$ requires 298.0702], 163(14), 136(63), 117(11), 90(32). ii) 1-(p-nitrobenzyl)-6-nitrobenzimidazole [100] (0.55g, 30%), m.p. 187-188°C. [Found: C, 56.1; H, 3.3; N, 18.5. C$_{14}$H$_{10}$N$_4$O$_4$ requires C, 56.38; H, 3.38; N, 18.79%] m/$_z$ [Found: M$^+$ 298.0695 (100%). C$_{14}$H$_{10}$N$_4$O$_4$ requires 298.0702], 136(56), 90(36). $^1$H and $^{13}$C n.m.r. spectral data for both compounds, [99] and [100], are reported in Tables 2.10 and 2.11.

b) 1-(p-Nitrobenzyl)-5-nitroindazole [101]

The anion of 5-nitroindazole [86] (1.0g, 6.1mmol) was...
reacted with p-nitrobenzyl chloride (1.05g, 6.1mmol). The resulting crude mixture was separated by column chromatography on neutral alumina to afford two products after recrystallisation EtOAc-Pet-ether (60-80°C), i) the titled compound [101] (0.6g, 33%), m.p. 159-161°C, [Found: C,56.5%; H,3.2; N,19.0. \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires C,56.38; H,3.38; N,18.79%]. m/z \( \text{Found: M}^+ 298.0677 \) (100%). \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires 298.0702], 136(35), 90(20). ii) 1-(p-nitrobenzyl-5-nitroisoindazole [102] m.p. 179-180°C, [Found: C,55.90; H,3.2; N,18.7. \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires C,56.38; H,3.38; N,18.79%], m/z [Found: \( \text{M}^+ 298.0694 \) (100%). \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires 298.0702], 136(30), 106(23), 90(23). \( ^1\)H and \( ^13\)C n.m.r. spectral data are reported in Tables 2.12 and 2.14.

c) 1-(p-nitrobenzyl)-6-nitroindazole [103] and 1-(p-nitrobenzyl)-6-nitroisoindazole [104]

The titled compounds were prepared by reacting the anion of 6-nitroindazole [87] (1.0g, 6.1mmol) with p-nitrobenzyl chloride (1.05g, 6.1mmol). The crude mixture was separated by column chromatography on neutral alumina. Recrystallisation from CHCl₃/Hexane afforded the indazole [103] as colourless needles (0.77g, 42%), m.p. 184-185°C, [Found: C,56.3; H,3.2; N,19.0. \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires C,56.38; H,3.38; N,18.79%]. \( \nu \) max (nujol) 3030, 3040 (aryl H), 1610, 1600 (\( \begin{array}{c} \text{C}=\text{C}\end{array} \text{arom.} \)), 1520, 1360, 1340 (C-NO₂ stretching arom.) and 740 cm⁻¹; \( ^1\)H and \( ^13\)C n.m.r. spectroscopic data are presented in Tables 2.13 and 2.15. m/z [Found: \( \text{M}^+ 298.0702 \) (100%) \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires 298.0701], 251(14), 136(65), 106(58), 90(61), 89(75).

The second product, 1-(p-nitrobenzyl)-6-nitroisoindazole [104] was separated as bright yellow needles (0.82g, 45%) m.p. 217-219°C. [Found: C,56.5; H,3.2; N,18.8. \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires C,56.38; H,3.38; N,18.79%]. \( \nu \) max (nujol) 3040 (aryl H) 1610, (\( \begin{array}{c} \text{C}=\text{C}\end{array} \text{arom.} \)), 1520, 1380, and 1340 cm⁻¹ (C-NO₂ stretching arom.). \( ^1\)H and \( ^13\)C n.m.r. spectroscopic data are reported in Tables 2.13 and 2.15. m/z [Found \( \text{M}^+ 298.0702 \) (100%) \( \text{C}_{14}\text{H}_{10}\text{N}_{4}\text{O}_4 \) requires 298.0713] 251(17), 136(28), 106(21), 90(18), 89(25).

d) Attempted preparation of 1-(p-nitrobenzyl)-imidazole

The anion of imidazole [83] (1.0g, 14.7mmol) was re-
acted with p-nitrobenzyl chloride (2.5g, 14.7mmol). The crude mixture obtained was separated by chromatography on neutral alumina using CH$_2$Cl$_2$:EtOAc 1:4 as eluent to give a product which was recrystallised from ethyl acetate to give cream coloured crystals (0.87g, 38%) m.p. 240-242°C; $^1$H and $^{13}$C n.m.r. spectra and the elemental analysis did not support the structure of the titled compound.

e) Attempted preparation of 1-(p-nitrobenzyl)-benzimidazole

Various attempts to prepare the titled compound resulted in a brown decomposed oil. It was not possible to analyse the oil.

14. Attempted preparation of 2,2-di-(benzimidazol-1-yl)-propane [90] from 1-(1-methyl-1-nitroethyl)-benzimidazole [89]

a) 1-(1-Methyl-1-nitroethyl)-benzimidazole [89], (0.5g, 4.88mmol) was stirred in CH$_2$Cl$_2$/CHCl$_3$ (4:1) (20ml) under N$_2$ for 30min. Benzimidazole (0.576g, 4.88mmol) was added to the suspension and left stirring for 2h. The reaction mixture was diluted with water (15ml), washed with 2M sodium hydroxide solution (15ml×2) followed by water. The organic layer was dried (MgSO$_4$) and evaporated to dryness in vacuo to give 32% of the unreacted benzimidazole [89]. The $^1$H n.m.r. spectrum and t.l.c. was identical to that prepared previously.

b) As above but with methanol:water (3:2) as solvent and in the presence of an acid (5 drops of conc.HCl). Chloroform was added to the reaction after 4h, and worked up as above. The $^1$H n.m.r. spectrum also showed the presence of a signal at 2.1 ppm, acetone was suspected. The next reaction was repeated using the same procedure and the resulting organic layer after base wash was added to 2,4-dinitrophenylhydrazone (20ml) to form the hydrazone derivative. After slight warming of the reaction mixture the precipitate formed was filtered and recrystallised from ethanol/water (42%) m.p. 110-118°C, lit. 155 128°C.

c) As in a) above but with methanol:water (3:2) and in the presence of a base, 2M aq. sodium carbonate solution (5 drops) was added to the solution. Work up of reaction as above, and addition of 2:4 DNP gave no precipitation of the acetone derivative.
15. Attempted synthesis of 2,2-di-(benzimidazol-1-yl)-propane [90] via Lewis acid catalysed $S_N^1$ reaction

The benzimidazole [89] (0.2g, 0.98mmol) was added to the anion of benzimidazole [84] (0.23g, 1.95mmol, 2mol equiv.) in dichloromethane (20ml) followed carefully by stannic chloride (2mol equiv.). The mixture was left stirring at room temperature for 26h.

The reaction was filtered to remove the white precipitate of the Lewis acid complex. The dichloromethane layer was worked up as in experiment 14 to give unreacted starting material [89] (58%). The $^1$H n.m.r. spectrum and t.l.c. agreed with the authentic material [89].

16. Attempted preparation of 1-(1-methyl-1-nitroethyl)-benzimidazole [89] from benzimidazole and acetone

Into a round bottomed flask equipped with a Dean-Starke water separator, a condenser and drying tube was introduced benzimidazole (2.0g, 16.7mmol), benzene (100ml), excess acetone (20ml) and $p$-toluenesulphonic acid ($p$TSA). The mixture was refluxed for 48h. As the flask cooled, the precipitate was filtered and $^1$H n.m.r. spectroscopic analysis showed unchanged benzimidazole (1.9g, 95%).

The reaction was set up again using a Soxhlet liquid-liquid extractor in place of the Dean-Starke water separator. Anhydrous copper (I) chloride was introduced into the thimble of the extractor. Benzimidazole was refluxed in (dried and distilled) acetone (200ml) with ($p$-TSA-catalyst) for 48h. A blue coloration was noted in the reaction as it refluxed after 12h, the colour deepened after 48h. The product collected after evaporation of the blue solution gave a blue/green solid with a peculiar $^1$H n.m.r. spectrum. It was not possible to assign the structure. The 'blue-green' solid was recrystallised from dichloromethane to give blue crystals, the $^1$H n.m.r. spectrum showed possibly benzimidazole or a coupled derivative of benzimidazole. The u.v. spectrum showed $\lambda_{\text{max}}^\text{ethanol} = 590, 558$ and 281nm for this compound in ethanol, $\lambda_{\text{max}} = 573.0, 304.0$nm in the presence of base; in the presence of acid, $\lambda_{\text{max}} = 280.0$nm.
CHAPTER 3
SYNTHESIS AND MODE OF ACTION STUDIES


Butler et al.13 have suggested that the presence of an electron-withdrawing group on the C-2 side chain could improve activity. Consequently, the synthesis of compounds as represented by the general structures [108] and [109] were planned. These analogues have received no attention in the literature, particularly compounds of the general structure [108] with \( X = \text{NO}_2, \text{Me}_2\text{CNO}_2, \text{C(Br)NO}_2 \), and [109] with \( Y = \text{Br}, \text{Cl}, \text{NO}_2 \).

The synthesis of the analogues [108] and [109] was envisaged by two routes:

i) Introduction of a functionalised methyl or isopropyl group at the C-2 position of 1-methyl-2-substituted-5-nitroimidazoles [110] (equation 3.1).

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{Me} & \quad \text{Me} \\
\text{Z} & \rightarrow \quad \text{O}_2\text{N} \\
\text{N} & \quad \text{Me} \\
\text{CH}_2\text{X} & \\
\end{align*}
\]

[110] \( Z = \text{H}, \text{I}, \text{NO}_2, \text{SO}_2\text{Me} \)


\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{Me} & \quad \text{Me} \\
\text{Me(CMe}_2\text{H)} & \rightarrow \quad \text{O}_2\text{N} \\
\text{N} & \quad \text{Me} \\
\text{CH}_2\text{X(CMe}_2\text{X)} & \\
\end{align*}
\]

The discussion of the synthesis of these compounds is in 2 parts:
3.1 Synthesis of α-substituted 1,2-dimethyl-5-nitroimidazoles
3.2 Synthesis of α-substituted 2-isopropyl-1-methyl-5-nitroimidazoles.
3.1 Synthesis of \( \alpha \)-substituted 1,2-dimethyl-5-nitroimidazoles

The synthesis of 2-(halomethyl)-1-methyl-derivatives can be envisaged from two possible synthetic pathways:

Route A: From 2-(hydroxymethyl)-1-methyl-5-nitroimidazole
Route B: From 1,2-dimethyl-5-nitroimidazole (dimetridazole).

The synthesis of 2-(bromomethyl)-1-methyl-5-nitroimidazole ([108], X=Br) and its chloro derivative ([108], X=Cl) from 2-(hydroxymethyl)-1-methyl-5-nitroimidazole ([108], X=OH) have been patented by Merck and Co. These compounds serve as appropriate intermediates in the preparation of new \( \alpha \)-substituted 1,2-dimethyl-5-nitroimidazoles.

Scheme 3.1: Reaction pathway to 2-(hydroxymethyl)-, 2-(bromomethyl)-, and 2-(chloromethyl)-1-methyl-5-nitroimidazole
3.1.1 From 2-(hydroxymethyl)-1-methyl-5-nitroimidazoles [112]

The preparation of 1-methyl-5-nitroimidazole [111] has been discussed in the introduction and is outlined above in scheme 3.1.

Grindley and Pyman 157 first reported the condensation of 1-methyl-5-nitroimidazole with aqueous formaldehyde but failed to isolate the product. Sehgal and Agrawal 157 reported the hydroxymethylation of the C-2 position of this compound with 40% aqueous formaldehyde to the extent of 30% by heating at 140°C for 6h. 2-(Hydroxymethyl)-1-methyl-5-nitroimidazole [112] was prepared by condensation of the imidazole [111] with paraformaldehyde in DMSO. The best yield was obtained when 1-methyl-5-nitroimidazole [111] was condensed with paraformaldehyde at the lower temperature of 110-117°C for 53h in DMSO in a sealed tube. The product was separated from the starting materials by column chromatography. Steam distillation has been used 157,158a to effect separation; however, attempts to utilize this method resulted in low yields of the required product. During the investigation, it was found that high temperatures (150°C) resulted in charring with a resultant low yield of the required product, and at temperatures below 110°C, mainly unchanged starting materials were recovered.

Treatment of the hydroxymethylated product [112] with thionyl chloride in refluxing toluene produced 1-methyl-2-chloromethyl-5-nitroimidazole [60] in 72% yield as reported in the literature 6,156 (scheme 3.1). The bromo analogue [59] was similarly prepared as above (scheme 3.1). When the reaction was carried out in toluene, a side reaction was observed, in which the toluene is brominated as in equation 3.3. Consequently the reaction was performed in benzene.

\[
\begin{align*}
\text{CH}_3\text{SOBr}_2 & \rightarrow \text{CHBr}_2 \\
\end{align*}
\]

(3.3)

3.1.2 From 1,2-Dimethyl-5-nitroimidazole

An alternative approach to the synthesis of 2-(bromo-methyl)-, and 2-(chloromethyl)-5-nitroimidazole is via 1,2-.
-dimethyl-5-nitroimidazole [2] as outlined in Route B, scheme 3.1. 1,2-Dimethylimidazole [2] is a potential intermediate because the C-2 methyl group is theoretically amenable to chemical modification; such as bromination and oxidation. Aiming at the functionalization of the C-2 methyl group on 1,2-dimethylimidazole [2], bromination with N-bromosuccinimide (NBS) was investigated. NBS is a useful synthetic reagent, which, in the presence of a radical initiator, (light or peroxides), selectively brominates weak allylic C-H bonds. 1,2-DMNI [2] was reacted with NBS under reaction conditions suitable for radical bromination (dry non-aprotic solvent, e.g. carbon tetrachloride, (CCl₄), light catalysis and benzoyl peroxide.) Various attempts, carried out to obtain a monobrominated product at the "benzylic" position, were unsuccessful. A mixture of compounds [113], [114] and [115] was always present in the crude mixture as indicated by ¹H n.m.r. spectroscopy. The compounds could not be separated for identification by normal purification procedures.

The most widely accepted mechanism involves molecular bromine, produced and maintained in low concentrations as shown in equation 3.6 in scheme 3.2.

The 3 possible products [113]-[115] could arise if bromine radical (bromine) was permitted to accumulate, then, ring as well as allylic brominations were possible. The ring bromination could take place by aromatic electrophilic substitution which is unlikely in the absence of a strong Lewis base, or by radical substitution. A similar crude mixture and difficulty of separating the required monobrominated product has been reported for NBS bromination shown in equation 3.8.

When N-chlorosuccinimide (NCS) was used as the halogenating agent, only unaltered starting material was obtained.

One of the protons of 1,2-DMNI [2] is weakly acidic due to the electron-withdrawing nature of the nitro group.
and the imidazole ring itself (c.f. p-nitrotoluene\textsuperscript{159c}). In the presence of a base 1,2-DMNI [2] gave a pink to violet coloured solution. The colour confirmed the presence of a conjugated product, which could arise from an anion formation at the C-2 position of the imidazole [2] (equation 3.9).

Various attempts to brominate this anion (which was generated using bases such as potassium t-butoxide, sodium hydroxide (\text{NaOH}), pyridine, triethylamine, sodium carbonate)
with brominating agents such as bromine, NBS, copper bromide all gave unaltered starting material.

Oxidation of 1,2-DMNI [2] with selenium dioxide was also attempted but no reaction was observed. 

**Synthesis using 2-(halomethyl)-1-methyl-5-nitroimidazole**

The 2-(halomethyl)-1-methyl-5-nitroimidazoles lend themselves to chemical modification via the labile halo atom. The reactions of these compounds with tertiary amines, thiolates and the anion of 2-nitropropane are presented in scheme 3.3.

![Scheme 3.3: Reaction pathway with 2-(Chloromethyl)-1-methyl-5-nitroimidazole [60] as the reaction intermediate](image-url)
2-(Chloromethyl)-1-methyl-5-nitroimidazole [60] was reacted with triethylamine and N,N-dimethyl-octylamine by nucleophilic displacement of the chloro substituent by the basic amine to give the corresponding quaternary ammonium compounds (scheme 3.3) in good yields. The products were characterised by $^1$H n.m.r. and mass spectroscopy and elemental analysis.

The reaction between 2-(halomethyl)-5-nitroimidazoles and thiolate anions, used as syntheses, are reported here, the full mechanism and inhibition studies are discussed later (chapter 3, Part B). Both the 2-(bromomethyl)- and 2-(chloromethyl)-derivatives were used in these preparations. 2-(Chloromethyl)-5-nitroimidazole [60] was reacted with the sodium salt of thiophenol to give (1-methyl-5-nitroimidazole-2-yl)-methyl phenyl sulphide [119] (74%) as shown in scheme 3.3. No disulphide was observed in the $^1$H n.m.r. spectrum of the crude product. Similarly, the reaction between 2-(chloromethyl) derivative [60] and both the anions of 2-thiopyridine and 2-thiopyrimidine gave the corresponding 2-pyridyl[120] and 2-pyrimidyl[121] sulphides in 74% and 63% yield, respectively. The mechanism of these reactions is discussed in Part B of this chapter.

2-(Chloromethyl)-5-nitroimidazole [60] was reacted with the anion of 2-nitropropane [61] under conditions suitable for $S_{RN1}$ reactions under an atmosphere of dry, oxygen free nitrogen to give 2-(1-methyl-5-nitroimidazole-2-yl) prop-1-ene in 46% yield. The product obtained arose from rapid elimination of HNO$_2$ from the expected $S_{RN1}$ product as shown in scheme 3.4. Shortly after the beginning of this investigation, Surzur et al. reported\textsuperscript{140} the reaction and product.

Although no inhibition studies were carried out in this work, the $S_{RN1}$ mechanism as outlined in scheme 3.4 was proposed by Surzur et al.\textsuperscript{140} on the basis of inhibition studies (scheme 3.4). The other product of the reaction was 2,3-dimethyl-2,3-dinitrobutane. (see chapter 2).

2-(Chloromethyl)-5-nitroimidazole [60] was also successfully reacted with the anion of 4(5)-nitroimidazole to give the corresponding $S_{RN1}$ product in 35% yield. The mechanism of reaction was discussed in Chapter 2.
Scheme 3.4: $S_{RN1}$ mechanism

3.1.4 The attempted preparation of 1-methyl-2-(nitromethyl)-5-nitroimidazole

i) via 2-(bromomethyl)-1-methyl-5-nitroimidazole [59]

Substitution of halide ion, present in the aliphatic side chain of an aromatic compound by a nitro group, can be effected using silver nitrite in ether.\textsuperscript{160} The method has been used successfully to prepare phenylnitromethane in 75\% yield (equation 3.14).

\[ \text{2-(Bromomethyl)-1-methyl-5-nitroimidazole [59]} \text{ reacted with silver nitrite in ether to give a mixture of} \]
products (equation 3.15) in low yield.

Various attempts using different solvents and temperatures also gave the same mixture of products in low yield. The two products arise from the ambident nitrite anion, i.e. reaction via the O- or N-centres. Kornblum et al.\textsuperscript{160} reported that the yield of the nitro compound falls progressively and the yield of alkyl nitrite rises as silver nitrite is treated with primary, secondary and tertiary halide.

ii) Reaction between 1,2-dimethyl-5-nitroimidazole and propyl nitrate

Nitration of active methylene compounds in the presence of a base and alkyl nitrates dates back to the 19th century. Thiele\textsuperscript{161} converted cyclopentadiene into the sodium salt of nitrocyclopentadiene by treatment with ethyl nitrate and sodium ethoxide (equation 3.16). The synthetic value of the method has been widely exploited.

\[ \text{Thiele's reaction} \]

The reaction of o- and p-substituted-toluene and heterocyclic compounds e.g. (equations 3.17 and 3.18) have been reported.\textsuperscript{109} The synthetic method was applied to the synthesis of 1-methyl-2-(nitromethyl)-5-nitroimidazole but only unaltered starting material was recovered.

Attempts to nitrate p-nitrotoluene in sodamide or potassium amide have also been reported\textsuperscript{109} unsuccessful. Under basic conditions, p,p'-dinitrobibenzyl is formed (equation
Russell and Janzen\textsuperscript{159c} reported that the spontaneous disproportionation of \( p \)-nitrotoluene and its derivatives in basic solution is not an isolated phenomenon, and can be expected whenever hydrogen atoms are alpha to a \( \alpha \) group that promotes the acidity of the hydrogen atoms and an easily oxidized anion. Although such a dimer is possible with the nitroimidazole, there was no indication of its formation.

### 3.2 Synthesis of \( \alpha \)-substituted 2-isopropyl-1-methyl-5-nitroimidazoles

The syntheses of these compounds, [109] with \( X=\text{Br, NO}_2 \) are discussed.

1) \( S_{N\text{Ar}} \) Substitution at C-2

The nucleophilic aromatic substitution (\( S_{N\text{Ar}} \)) reactions of thiophenes bearing electron-withdrawing groups, usually nitro groups, by various nucleophiles (e.g. alkoxides, thiolates, amines) has been reported by Spinelli\textsuperscript{162} and co-workers. Norris\textsuperscript{104} also reported the reaction of 2-nitropropan-2-ide [61] with nitrothiophenes (e.g. equation 3.20).

By analogy with 2-substituted-5-nitrothiophenes, it was hoped that the equivalent 5-nitroimidazoles would undergo \( S_{N\text{Ar}} \) substitution at C-2 when reacted with the anion of
nitroalkanes, thereby providing a new route to the required analogues (equation 3.21).

\[ \text{Scheme 3.5: Reaction pathway via 4-iodo-1-methyl-5-nitroimidazole} \]
Imidazole was iodinated using two equivalents of iodine in the presence of aqueous sodium hydroxide at room temperature in good yields. The probable mechanism of reaction is as follows (Scheme 3.6):

Scheme 3.6: Probable mechanism of iodination reaction

2,4(5)-Di-iodoimidazole was nitrated by mixed acids as described in the introduction section to give 2-iodo-4(5)-nitroimidazole in good yields (72%). N-Methylation of the nitrated product [122] was obtained using dimethylsulphate. Recrystallisation of the crude mixture gave mainly the required product, the 5-nitro isomer, in 63% yield.

When the anion of 2-nitropropane (and nitroethane) was reacted with 2-iodo-5-nitroimidazole, unaltered nitroimidazole and 2-iodo-2-nitropropane were obtained; when the reacting nucleophile was the anion of nitroethane, no iodine abstraction was observed. Towards the end of this piece of work, a reference was cited disproving the structure of 2,4(5)-di-iodoimidazole. These workers reported that the correct structure is actually 4,5-di-iodoimidazole. This new assignment of structure was proved using X-ray crystallography, isotope labelling experiments, mass spectral analysis and $^{13}$C n.m.r. spectroscopy. However, allowing for the corrected structure, 4-iodo-1-methyl-5-nitroimidazole, nucleophilic aromatic substitution of the iodine in the 4-position should still occur. This centre is also well
'set' for a possible $S_{N}Ar$ reaction. As mentioned above, only the starting nitroimidazole and 2-iodo-2-nitropropane were isolated.

A possible mechanism for the formation of 2-iodo-2-nitropropane is shown in equation 3.22. The anion [124] would be partially stabilized by the nitrogens in the ring and the nitro group. Nitroanions are poor nucleophiles and iodine is susceptible to X-philic (halogenophilic) attack due to its $\delta^+$ nature in structure [124]. Consequently, a nitro group substituent in the 2-position was envisaged. The nitro group is a stronger electron-withdrawing group than iodine.

The overall synthetic route to 1-methyl-2,5-dinitroimidazole is illustrated in scheme 3.7. It is well known that direct nitration of imidazole and some of its derivatives leads to the introduction of the nitro group into
either position 4 or 5. The electrophilic substitution of azomycin [30] by an additional nitro group was carried out in the presence of acetic anhydride with fuming nitric acid\textsuperscript{76b} at 100°C to give 2,4(5)-dinitroimidazole in moderate yields (60%).

Dimethyl sulphate failed to react with 2,4(5)-dinitroimidazole under neutral reaction conditions, even after 36h. It is probable that the nucleophilicity of the lone pair of electrons on the unsubstituted (pyridine-like) nitrogen atom is diminished due to the electron-withdrawing effect of the two nitro groups \cite{125}. However, when 2,4(5)-

\begin{center}
\includegraphics[width=0.5\textwidth]{fig.png}
\end{center}

- dinitroimidazole was stirred with excess of diazomethane at room temperature for 36h, the reaction went to completion as shown by t.l.c. analysis. The products, 1-methyl-2,5-dinitroimidazole and its 4-isomer were identified by $^1$H n.m.r. spectroscopy and comparison with other 1-methyl-4- and 5-nitroimidazoles.

The reaction of 1-methyl-2,5-dinitroimidazole with the anion of 2-nitropropane gave unreacted starting material, but unlike the reaction of the same anion with iodoimidazole derivative, 2-2-dinitropropane was not isolated. The reason for lack of reaction is not known considering that the starting material is well set up for a possible S\textsubscript{N}Ar reaction (c.f. Norris\textsuperscript{104} equation 3.20). However, the reaction was not investigated thoroughly because 1-methyl-2,5-dinitroimidazole is not readily available. The yield of 2-nitroimidazole (azomycin) is moderate (49%) but the work up is very tedious which explained why it is commercially very expensive.

Synthesis via 1-methyl-2-methylsulphonyl-5-nitroimidazole

A new nitroimidazole, satranidazole [9], recently introduced into the market was prepared by reaction of 1-methyl-
-2-methylsulphonyl-5-nitroimidazole [126] with imidazoline anion (equation 3.23). Since then, a range of new compounds have been reported\textsuperscript{164} which have been prepared using this synthetic intermediate [126].

The success of aromatic nucleophilic substitution of the methylsulphonyl group at C-2 of nitroimidazole which has been reported by Nagarajan et al.\textsuperscript{164} prompted an investigation with the aim of using the anion of 2-nitropropane as the nucleophile.

Investigation was commenced by the synthesis of 1-methyl-2-methylsulphonyl-5-nitroimidazole [126].\textsuperscript{164}e The reaction pathway is illustrated in scheme 3.8.

\begin{equation}
\begin{aligned}
\text{O}_2\text{N} & \quad \begin{array}{c}
\text{N} \\
\text{Me} \\
\text{N}
\end{array} \\
\text{Me} & \quad \begin{array}{c}
\text{SMe} \\
\text{Me} \\
\text{N}
\end{array} \\
\text{Me} & \quad \begin{array}{c}
\text{O}_2\text{N} \\
\text{Me} \\
\text{N}
\end{array} \\
\text{Me} & \quad \begin{array}{c}
\text{O}_2\text{N} \\
\text{Me} \\
\text{N}
\end{array} \\
\text{Me} & \quad \begin{array}{c}
\text{O}_2\text{N} \\
\text{Me} \\
\text{N}
\end{array}
\end{aligned}
\end{equation}

Scheme 3.8 : Reaction pathway for the synthesis and reaction of 1-methyl-1-methylsulphonyl-5-nitroimidazole [126]

1-Methyl-2-mercaptoimidazole was methylated using methyl iodide. Nitration of the methylated product, followed by oxidation with monoperphthalic acid, gave a mixture
of isomers of 1-methyl-2-methylsulphonyl-4, and 5-nitroimidazoles. Purification was effected by column chromatography on silica to give the required 5-nitroimidazole in 65% yield. The identity of the compounds were confirmed by $^1$H n.m.r. spectroscopy, and m.p. comparison with those in the literature. 164e

The reaction between 1-methyl-2-methylsulphonyl-5-nitroimidazole and the sodium salt of 2-nitropropane unfortunately gave unreacted starting material and 2-methylsulphonyl-2-nitropropane; a situation similar to that observed with iodoimidazole derivative. The structure of the latter compound was confirmed by m.p., and i.r. and $^1$H n.m.r. spectroscopic comparison with authentic material. 124b

The sulphonyl group has a poor -I effect and hence would not strongly favour an ipso-attack by an incoming nucleophile (i.e., less carbonium ion character on the carbon bearing the $\text{SO}_2\text{Me}$ group), and therefore, the first step of the $S_NAr$ mechanism which is usually rate-determining would be slow for $\text{SO}_2\text{Me}$ groups (as with the iodo derivatives); this step is promoted by groups with strong -I effect. An approximate order of the effect on the rate of $S_NAr$ substitution is $^{104}$: $\text{F} > \text{NO}_2 > \text{SO}_2\text{Ph} > \text{Cl}, \text{Br}, \text{I} > \text{N}_3 > \text{SO}_2\text{R} > \text{NH}_2$. This is in effect, an approximate order for the electron-withdrawing (-I) ability of these groups to initiate the first step of $S_NAr$ reactions.

The electron-withdrawing groups attached to the sulphur centre (the 5-nitroimidazole ring, and the two oxygens of the sulphonyl group) in compound [126] created a partial positive charge on the sulphur, and thus makes it susceptible to nucleophilic X-philic attack. Consequently, it is probable that due to the slow rate-determining step in $S_NAr$ (poor -I effect) by the $\text{SO}_2\text{Me}$ group, the X-philic reaction via the partially positive sulphur-centre becomes more favourable (equation 3.24).

![Image of chemical reaction](https://example.com/chemical_image.png)
In an attempt to confirm the intermediacy of the anion at the C-2 position of the l-methyl-5-nitroimidazole, \[111\] was reacted with 2,2-dinitropropane in the presence of a base, sodium hydride, and light catalysed for 24h. Unaltered imidazole \[111\] and 2,3-dimethyl-2,3-dinitrobutane were isolated, but not the expected product (equation 3.25).

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{Me} \\
\text{Me} & \quad \text{NO}_2 \\
\text{Me} & \quad \text{NO}_2
\end{align*}
\]

For the 2,3-dimethyl-2,3-dinitrobutane to be formed, the anion of 2-nitropropane must have been generated during the reaction which can then react via the \( S_{RN1} \) mechanism (see chapter 2). Possible mechanisms are proposed in scheme 3.9. The nitroimidazole anion probably abstracted a nitro group from 2,2-dinitropropane to form l-methyl-2,5-dinitroimidazole (equation 3.26). A precedent for this abstraction was shown by Russell et al. \[130a\] in their work with enolate anions and 2-substituted-2-nitropropanes (see equation 3.32).

\[
E^\Theta + \text{Me}_2\text{C}(X)\text{NO}_2 \rightarrow E-X + \text{Me}_2\text{CNO}_2^\Theta
\]

\( E = \text{PhC(OLi)} \rightarrow \text{CH}_2'\text{tBuC(OLi)} = \text{CH}_2 \)

\( X = \text{Cl}, \text{NO}_2 \)

The anion of 2-nitropropane thus generated can transfer an electron to 2,2-dinitropropane (equation 3.28), which would initiate a \( S_{RN1} \) mechanism leading to formation of 2,3-dimethyl-2,5-dinitrobutane. Alternatively, an electron could be transferred to l-methyl-2,5-dinitroimidazole \[127\] (equation 3.27) since the latter is a strong electron acceptor (c.f. \( \Pi \)-dinitrobenzene).
The 2-nitro-2-propyl radical formed can react with another anion of 2-nitropropane (equation 3.30) via the non-chain oxidative dimerisation mechanism\(^\text{118}\) to give the observed product with 1-methyl-2,5-dinitroimidazole acting as the final electron acceptor (equation 3.31).

The imidazole radical-anion formed is probably lost in
the basic solution which would explain why this product was not observed.

The overall reaction can be summarised in equation 3.33.

\[
\begin{align*}
2 \text{O}_2N\text{Me}^- + 2 \text{MeNO}_2 & \rightarrow 2 \text{O}_2N\text{MeNO}_2^- + 2 \text{Me}_2\text{C} = \text{NO}_2^- \\
\end{align*}
\]

More work is required to confirm the proposed mechanism. The reaction (equation 3.33) is similar to the reverse of that observed with the methylsulphonyl group (equation 3.24) described previously. The reaction (equation 3.33) also supported the fact that no 2,2-dinitropropane was observed in the reaction of the anion of 2-nitropropane with 1-methyl-2,5-dinitroimidazole.

The attempted synthesis of \(\alpha\)-substituted 2-isopropyl-1-methyl-5-nitroimidazole via functionalization at C-2

An alternative synthetic approach to target compounds of the type [109] was envisaged using ipronidazole [4] as the starting material.

The synthetic pathways for the synthesis of these compounds is presented in scheme 3.10.

Iproniazide was synthesised as follows: Commercially available 2-isopropylimidazole was nitrated by the method of Pyman in 30% yield (lit. 32%). The use of nitric acid alone gave a similar yield (22%). Changing the order of addition of mixed acids and 2-isopropylimidazole did not affect the overall yield of the reaction. A blue colour was observed as the reaction mixture was poured into cold water. The solution was carefully neutralised and extracted with chloroform. \(^1\)H n.m.r. spectroscopic analysis of the chloroform layer showed a mixture of the nitrated imidazole and another compound identified as 2-nitro-2-nitrosopropane.
In a separate run, the precipitate was filtered immediately and shown to be dimer of 2-nitro-2-nitrosopropane. It is not clear how this compound was formed but a possible mechanism is shown in scheme 3.11.

The 2-isopropyl-4(5)-nitroimidazole was methylated using diazomethane and the mixture of isomers obtained separated by column chromatography to give the required 5-nitro isomer (60%) and the 4-nitro isomer (8%).

2-Isopropyl-1-methyl-5-nitroimidazole was reacted with NBS in dry CCl₄. The best reaction conditions were obtained when the NBS was added portion-wise to the reaction mixture and light catalysed for 8h.

N.m.r. spectroscopic and t.l.c. analyses of the crude product showed a mixture of products which were separated only after tedious column chromatography using flash silica. The two compounds [128] and [129] were confirmed by ¹H n.m.r. spectroscopy (¹H n.m.r. data shown below). The olefin [129] failed to crystallise and further purification for further
analysis was not attempted. The molecular ion (M<sup>+</sup>) in the mass spectrum was not confirmed for the bromo-derivative [128] because of the labile bromine atom. Polarographic studies of compound [128] confirmed the presence of the nitro group.

The formation of the compound [129] can be envisaged by elimination of HBr from the required product [128], (equation 3.34).

\[
\begin{array}{c}
\text{[128]} \\
\text{[129]}
\end{array}
\]

\[
\text{[128]} \xrightarrow{-\text{HBr}} \text{[129]}
\]

The reaction of the bromo-derivative [128] with the anion of 2-thiopyridine will be discussed later.

Conclusion

α-Substituted 1-methyl-5-nitroimidazoles were successfully prepared via 2-(chloromethyl)-1-methyl-5-nitroimidazole. However, the attempted synthesis of 1-methyl-2-(nitromethyl)-5-nitroimidazole and the preparation of 1-methyl-2-(bromo-methyl)-5-nitroimidazole by bromination of 1,2-dimethyl-5-nitroimidazole failed.

Synthesis of [109] via direct substitution at C-2 has proved unsuccessful. Attempted substitutions of the iodo- and methylsulphonyl- derivatives by the anion of 2-nitropropane via S<sub>N</sub><sup>Ar</sup> mechanism led to the abstraction of the substituent by the nucleophile to give 2-substituted-2-nitropropane. 1-Methyl-2-(1-methyl-1-bromoethyl)-5-nitroimidazole [128] has been prepared in low yield but the compound proved to be unstable.
Part B

Model in vitro Mode of Action Studies with Thiolates

Winkelmann et al.\(^\text{17}\) have synthesised and studied the structure activity relationship of a number of \(\alpha\)-substituted 1,2-dimethyl-5-nitroimidazoles [108]. They divided the nitroimidazoles into three main groups based on their antimicrobial activity.

1) 5-Nitroimidazoles which possess good leaving groups have low biological reactivity, [108] with \(X = \text{Cl, Br, PhSO}_2\text{-O-, CF}_3\text{CO}_2\text{-, etc.}\). They\(^\text{17}\) suggested that these are metabolised too rapidly before reaching the site of action due to their instability and reactivity, and hence exhibit weak or no biological activity.

2) 5-Nitroimidazoles with poor leaving groups, [108] with \(X = \text{H, PhO, PhS, } -\text{NCH}_3, \text{ etc.}, \) show weak biological activity because the C-X bond is resonance stabilized and hence more difficult to metabolise, i.e., they reach the site of action but no activity (C-X bond is difficult to break).

3) Those with moderate leaving groups, [108] with \(X = S-\text{pirinidazole), } -\text{OCONH}_2 \) (ronidazole), \(-\text{O}^+\text{Me} \) (flexnizidazole) usually show high activity. Winkelmann et al.\(^\text{17}\) indicated that the cleavage of \(-\text{CH}_2\text{X} \) bond is important for activity.

Part of the aim of this project was to test these hypotheses using 4 different studies and to correlate the results from these studies, and possibly to gain an insight into the mode of action of these drugs. The studies used include: 1) model in vitro experiments in solution reactions that tended to mimic reaction environments in biological systems. 2) E.s.r. spectroscopy to confirm the structure of, and possibly the dissociation of the intermediate radical-anions formed. 3) In vitro antimicrobial testing against both anaerobic and aerobic micro-organisms and finally 4) polarographic measurements of nitroimidazoles (see chapter 4).
Reaction between 5-Nitroimidazoles [108] and Thiolates

Nitroimidazole radical-anions have been reported by various authors\textsuperscript{24,41,166} (see introduction). A recent article by G.T. Miwa et al.\textsuperscript{166} reported that protein alkylation by nitroimidazoles proceeded via a reductive, rather than an oxidative, mechanism. They\textsuperscript{166} also showed that cysteine thiol is the site of nitroimidazole alkylation on proteins. Consequently, model \textit{in vitro} studies involved the reactions between nitroimidazoles [108] and different thiols in the presence of light to facilitate single electron transfer and in an appropriate solvent system that may possibly mimic the biological environment.

Model \textit{in vitro} reactions between $\alpha$-substituted 1,2-dimethyl-5-nitroimidazoles [108] and thiolates

An important characteristic of the action of a drug is the solubility in water or organic solvent. Water solubility plays a key role as to whether a drug can be tested in model \textit{in vitro} experiments using systems which mimic biological ones. Consequently, investigations were carried out in protic solvents (water:methanol, 1:3), although the reactions of some of the thiolates were initially conducted in dipolar aprotic solvents.

The reaction between $\alpha$-substituted 1,2-dimethyl-5-nitroimidazoles (where the substituent is an electron-withdrawing group or atom) and thiolate anions may proceed via a number of reaction mechanisms.

Thiolates are very strong nucleophiles\textsuperscript{167} and may be expected to participate readily in bimolecular nucleophilic displacements (S$_{N}$2) reactions. On the other hand, thiolate anions are easily oxidized to the corresponding thiyl radicals which rapidly combine to give the disulphide.\textsuperscript{115} (see introduction).

The presence of the nitro group profoundly influences the reactivity of nitroimidazoles. The electron density on the $\alpha$-substituent on the C-2 methyl group is markedly decreased due to the inductive electron-withdrawing effect of the nitro group. This allows the possibility of abstraction of the $\alpha$-substituent by a suitable nucleophile. In addition the whole molecule may be reduced by single electron transfer to the nitro group to form the radical-anion.
In the present mode of action study, thiolates [135]-[137] of similar activity to cysteine were used but without the other reactive centres of the latter, although cysteine itself was used to confirm that it does react with these nitroimidazoles. These thiolates were also chosen because they were readily available. The anion of 2-nitropropane, a poorer nucleophile than the thiolates, was also included for comparison of reactions. The nitroimidazoles chosen were representative of each group of activity postulated by Winkelmann et al. 17 as described above.

The investigation was begun by synthesising the required nitroimidazole derivatives in each group. The syntheses were reported in Chapter 3, part A:

**Group 1**

- ![Chemical structure](image1)

**Group 2**

- ![Chemical structure](image2)

**Group 3**

- ![Chemical structure](image3)
2-(Chloromethyl)-1-methyl-5-nitroimidazole [60] was reacted with phenyl thiolate in 64% yield (scheme 3.1). No diphenyl disulphide was observed by the $^1$H n.m.r. spectrum of the crude product or on t.l.c. The possibility of the reaction proceeding by a $S_{RN1}$ mechanism was investigated using diagnostic tests for the mechanism. The results of these experiments are shown in Table 3.1.

Table 3.1 Inhibition studies of the reaction between 2-(chloromethyl)-1-methyl-5-nitroimidazole and phenylthiolate

<table>
<thead>
<tr>
<th>Reaction Condition</th>
<th>Time</th>
<th>% Yield$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard, $^b$ MeOH/H$_2$O $N_2$</td>
<td>1h</td>
<td>74$^E$</td>
</tr>
<tr>
<td></td>
<td>30min</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>10min</td>
<td>96</td>
</tr>
<tr>
<td>Standard + t-(Bu)$_2$NO$^-$</td>
<td>10min</td>
<td>97</td>
</tr>
<tr>
<td>Standard + O$_2$, dark</td>
<td>10min</td>
<td>94</td>
</tr>
<tr>
<td>Standard, DMSO</td>
<td>4h</td>
<td>63$^E$</td>
</tr>
</tbody>
</table>

$^a$. % Yields were calculated by $^1$H n.m.r. spectroscopy using an internal standard

$^b$. Standard reaction is carried with the phenylthiolate [135] in MeOH:water (3:1) under $N_2$ and photolysed.

$^r$. % Yield after recrystallisation

No inhibition was observed in this reaction (Table 3.1) showing that the reaction does not proceed by the $S_{RN1}$ mechanism. Similar results of inhibition studies (Table 3.2) showed that the reaction between 2-(chloromethyl)-1-methyl-5-nitroimidazole and pyrimid-2-yl thiolate (scheme 3.1) also did not proceed by an $S_{RN1}$ mechanism. The order of nucleophilicity and electron donating ability for the thiolates under study is:
Table 3.2

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Time</th>
<th>% Product [121]</th>
<th>% recovered starting material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard conditions</td>
<td>10mins</td>
<td>76</td>
<td>8</td>
</tr>
<tr>
<td>+ (Bu⁺)₂NO⁻</td>
<td>10mins</td>
<td>74</td>
<td>10</td>
</tr>
<tr>
<td>+ O₂ + dark</td>
<td>10mins</td>
<td>73</td>
<td>14</td>
</tr>
</tbody>
</table>

The reaction was carried out as described in the experimental section. The % yields were calculated using ¹H n.m.r. spectroscopy.

The reactions were complete, or almost complete, in 10min, and no disulphide was reported. The lack of disulphide in these reactions would preclude the X-philic mechanism¹¹⁸,¹²⁰c (equation 3.43).

Mechanisms by which the reaction can proceed are:

SN₁ mechanism

An intermediate carbonium ion is formed if an SN₁ mechanism is assumed. The carbonium ion would be unstable due to the close proximity of two positively charged carbon atoms (equation 3.41).

\[
\text{SN}_2 \quad (3.41)
\]

SN₂ mechanism

Due to the nature of the thiolate, an SN₂ attack on the nitroimidazole can be either on the C-2 methyl carbon to give a chloride anion (equation 3.42), or on the chlorine (equation 3.43). The resulting carbanion would be resonance stabilized into the imidazole ring and the nitro group.
The conjugated carbanion [132] has been shown previously to be highly coloured ($\lambda_{\text{max}} = 528 \text{nm}$), and has been shown as an intermediate in various reactions and studies although various attempts to react the carbanion were unsuccessful. It is possible that the reaction between thiolates and nitroimidazoles proceed by this route (equations 3.43 and 3.44). Some evidence suggests that the presence of this nitroimidazole anion in strong basic solution is precluded by alternative competing reactions.

Another possible mechanism is non-chain single electron transfer, $S_{\text{ET}}^2$ as proposed by Russell et al. (scheme 3.13) in which the radical-anion and the thyl radical are held in a solvent cage. The initial s.e.t. is rapid and is not light catalysed (these reactions are complete in 10 min. and are not inhibited by $S_{\text{RN}}$ inhibitors). For a $S_{\text{ET}}^2$ process to proceed, the stability of the nitroimidazole radical-anion should be such that decomposition to the nitro radical [131] can occur before the initially formed thyl radical and imidazole radical-anion have time to diffuse apart.

Evidence supporting the possibility of an $S_{\text{ET}}^2$ mechanism over $S_N^2$ are: firstly, the nitroimidazole is a strong electron acceptor and forms a radical-anion (e.g., $\text{spectro-}$
Scheme 3.13: Possible s.e.t. mechanisms of reaction between nitroimidazole [60] and thiolates

scopy) and the thiolates are strong electron donors, therefore, a single electron transfer is highly favoured. Secondly, a deep violet colour was obtained transiently during the reaction indicating a radical-anion or charge-transfer complex between thiolate and nitroimidazole (though this
could also support the carbanion intermediate [132] in the dehalogenation-addition ($S_N^1$) mechanism, and lastly, the presence of 2,3-dimethyl-2,3-dinitrobutane when the anion of 2-nitropropane was used as an oxidizing agent. The 2,3-dimethyl-2,3-dinitrobutane can only be formed by a reaction of 2-nitropropane and 2-nitropropyl radicals (see Chapter 2).

Since inhibition is not observed in these studies with phenylthiolate, it can be assumed, if s.e.t. is taking place, that the rate of dissociation of the radical-anion is faster than the rate of diffusion (scheme 3.13), i.e. the imidazole radical-anion and the thyl radical are sufficiently reactive to react faster than they can diffuse apart from the solvent cage. The alternative is that the reaction is not $S_{ET}^2$ but $S_N^1$.

The reaction between the anions of 2-nitropropane, 2- and 4(5)-nitroimidazoles and 2-(chloromethyl)-1-methyl-5-nitroimidazole proceeds by an $S_{RN}^1$ mechanism and those of thiolates proceed by a $S_{ET}^2$ or $S_N^2$ mechanism. The reason for the different mechanisms is not clear but the following explanation could be postulated. The thiolate anions are much stronger nucleophiles than the anions of 2-nitropropane and nitroimidazoles, consequently, nucleophilic displacement by a $S_N^2$ mechanism would be favourable with the thiolates. However, both the thiolates and the anions of 2- and 4(5)-nitroimidazoles have been shown to act as electron donors (see chapter 2), and 2-(chloromethyl)-1-methyl-5-nitroimidazole has been shown to be a good electron acceptor by e.s.r. spectroscopy (see later). Thus, a single electron transfer is possible in all these reactions. In the case of the weaker nucleophiles, the rate of $S_N^2$ substitution is very slow (in fact, $S_N^2$ reactions at the tertiary carbon centre are hindered by steric hindrance) and electron transfer mechanisms dominate.

Inhibition studies diagnostic of a $S_{RN}^1$ mechanism confirmed this mechanism for the reaction between the nitroimidazole substrate [60] and the anion of 2-nitropropane.
Thus, as shown in scheme 3.13, it can be inferred that the rate of diffusion must be faster than the rate of dissociation ($k_{\text{diff}} > k_{\text{diss}}$) in the reactions of the weaker nucleophiles. As the rate of dissociation of the radical-anion is independent of the nucleophile or the resulting radical, it must be concluded that the thiolates reaction does not proceed by a $S_{\text{ET}}$ mechanism. Therefore, our evidence strongly suggests that the reaction between the nitroimidazole substrate [60] and thiolates proceeds by a $S_{\text{N2}}$ mechanism.

In conclusion, these results suggest that the group of $\alpha$-substituted 5-nitroimidazole with good leaving groups will react rapidly with thiolates and other strong nucleophiles. It is therefore likely that in vivo, these compounds react with nucleophiles before reaching the site of activation and hence appear to be largely inactive (see chapter 4).

**Reaction between Ronidazole [3] and (1-methyl-5-nitroimidazol-2-yl)-methyl pyrid-2-yl sulphide, and phenylthiolate**

The reaction between (1-methyl-5-nitroimidazol-2-yl)-methylpyrid-2-yl sulphide [120] and phenylthiolate after 6h gave diphenyl disulphide (45% based on the thiolate) and unaltered imidazole [120] (83%) (equation 3.45).

\[
\text{PhSSPh} \quad (45 \text{% based on the thiolate})
\]

(3.45)

The reaction between a molar equivalent of ronidazole and phenyl thiolate gave diphenyldisulphide (3%) and unaltered ronidazole (98%) after 10min (equation 3.46), and after 5h gave 2-(hydroxymethyl)-1-methyl-5-nitroimidazole [112] in 43% yield (equation 3.47) and PhSSPh (50%).

The use of 2mol equivalent of the thiolate increased the rate of reaction. The low yields suggest the formation of other products which were not isolated. To obtain evidence regarding the mechanism of the reaction, inhibition was carried out under an atmosphere of oxygen in place of
nitrogen, and in absence of light. The results are shown in Table 3.3.

Table 3.3 Results of reactions between ronidazole and phenylthiolate including inhibition studies

<table>
<thead>
<tr>
<th>Mol. Reaction equiv. condition</th>
<th>Unaltered Hydroxy</th>
<th>PhSSPh</th>
<th>PhSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1 h·N₂,10min 1 h</td>
<td>98</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>1 h·N₂,5h</td>
<td>42</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>2 h·N₂,12h</td>
<td>0</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>2 dark,N₂,1h</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>2 dark,N₂,5h</td>
<td>M²</td>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td>2 dark,O₂,5h</td>
<td>M²</td>
<td>M</td>
<td>3</td>
</tr>
<tr>
<td>2 dark,O₂,5h</td>
<td>M²</td>
<td>M</td>
<td>0</td>
</tr>
</tbody>
</table>

a Reactions were carried out in MeOH:H₂O (3:1) and under light catalysis. b % Yields are isolated yield. M² —mixture of ronidazole [3] and hydroxy compound [112].

d Yield of disulphide was based on the thiol.

The results in Table 3.3 confirmed that the formation of diphenyldisulphide is inhibited. The α-substituent, -OCONH₂, in ronidazole is a very poor leaving group and therefore, the radical-anion of ronidazole would be expected to be relatively stable, i.e., will not readily dissociate. In fact, e.s.r. spectroscopy confirmed that the radical-
anion of ronidazole is stable at room temperature in solution. Therefore, the rate of diffusion of the ronidazole radical-anion is almost certainly faster than its rate of dissociation, i.e. the radical-anion and thiyl radical diffuse apart faster than they react (see scheme 3.13).

The delayed action for the hydrolysis of ronidazole in these reactions is not clear. No hydrolysis was observed until after 1h. For hydrolysis to occur during the first

\[
\begin{align*}
\text{Ronidazole} & \xrightarrow{\text{SPh(Or'OH)}} \text{Ronidazole radical-anion} \\
\text{Ronidazole radical-anion} & \xrightarrow{\text{H}_2\text{O}} \text{Ronidazole hydroxylated product} + \text{SPh} \\
\text{Ronidazole} & \xrightarrow{\text{NH}_2\text{CO}_2\text{H}} \text{Ronidazole hydroxylated product} + \text{NH}_3 + \text{CO}_2
\end{align*}
\]

1h, 2mol equivalent of the thiolate is required. The hydrolysis is also slowed down in the dark as shown in Table 3.3. When the reaction was photolysed, only the hydroxymethyl [112] product was isolated at 5h, while in the dark, for the same reaction, a mixture of both the hydroxymethylated product [112] and unreacted ronidazole was obtained.

The stability of the pirindazole radical-anion can also explain the lack of substitution product (equation 3.48). Diphenyl disulphide is almost certainly produced by a s.e.t. redox reaction (equation 3.49).

\[
\begin{align*}
\text{Pirindazole} + \text{PhS}^- & \xrightarrow{\text{PhS}^-} \text{PhSSPh} \\
\text{PhS}^- + \text{PhS}^- & \xrightarrow{\text{[PhSSPh]}} \text{[PhSSPh]}^+ \\
\text{[PhSSPh]}^+ + \text{[120]} & \xrightarrow{\text{[120]}} \text{[120]}^+ + \text{PhSSPh}
\end{align*}
\]
An attempt to reduce pirinidazole [120] using sodium dithionite during its reaction with phenyl thiolate anion to catalyse substitution resulted in only diphenyl disulphide and no product resulting from the imidazole. Sodium dithionite has been used by various authors to reduce ronidazole, \[ ^{166}\] metronidazole \[ ^{24,41}\] and other nitroimidazoles \[ ^{41}\] to radical-anions in mode of action studies in model biological systems. The lack of the required product in this study is probably due to the stability of the radical-anion.

The reaction between ronidazole, and pirinidazole, and the anions of 2-nitropropane resulted in isolation of unaltered imidazole and 2,3-dimethyl-2,3-dinitrobutane. In both cases, the yield of the latter is less than 20%, and though no inhibition studies were carried out, the reaction probably proceeds by s.e.t. mechanism as shown for formation of diphenyl disulphide in equation 3.49.

**Reaction between 1,2-dimethyl-5-nitroimidazole and (group 3) phenylthiolate**

The reaction between 1,2-dimethyl-5-nitroimidazole and phenylthiolate in the dark gave unaltered 1,2-dimethyl-5-nitroimidazole (86%), phenyl thiol (66%) and phenyl disulphide (7%). This result supported the above result and suggested that a non-chain dimerisation mechanism (equation 3.49) may be in operation.

**Conclusion**

The studies show that both groups 2 and 3 give slow redox reactions and no substitution reactions. \( \alpha \)-Substituted nitroimidazoles with moderate and poor leaving groups both give stable radical-anions. This indicates that the nature of the \( \alpha \)-substitution does not especially influence the redox behaviour of the nitroimidazole. Therefore, the proposal that group two (moderate leaving groups) may owe their superior activity to dissociation of the radical-anion is not correct. In conclusion, it seems reasonable to propose that the low activity of group one is due to rapid reaction with nucleophiles before reaching the redox site but that the difference between group two and three, which both form stable radical-anions, cannot be explained by leaving group ability.
E.s.r. Spectroscopic Studies of α-substituted 1,2-Dimethyl-5-nitroimidazoles

2-Bromomethyl- and 2-chloromethyl-1-methyl-5-nitroimidazole ([59] and [60]) have been shown to undergo $S_{RN1}$ substitution with nitronates\(^{143a}\) and nitroimidazole anions\(^{143a}\) (Chapter 2). Therefore, it is interesting to use e.s.r. spectroscopy to study the radical-anions which are intermediates in the $S_{RN1}$ reactions of these α-substituted 1,2-dimethyl-5-nitroimidazoles.

The e.s.r. spectroscopic studies of some α-substituted 1,2-dimethyl-5-nitroimidazoles synthesised by us were carried out at Leicester University by Prof. M.C.R. Symons. The results of the e.s.r. studies, including the biological implications for these compounds are discussed.

The e.s.r. spectrum of the radical-anions of the bromoimidazole [59] (obtained at low temperature 77K) showed significant coupling between the C-Br $\sigma^*$ and $\pi^*$ - molecular orbitals of the radical anion [59]$^-$ as was observed for [p-O$_2$N-C$_6$H$_4$-CH$_2$Br]$^*$.\(^{136}\) For any coupling to occur, the C-Br bond must overlap hyperconjugatively with the $\pi$-system indicating that the bromine adopts the $90^\circ$ out-of-plane site. This delocalisation is probably due to electron-donation from the ring into the C-Br $\sigma^*$ molecular orbital rather than the opposite.

On annealing to 170K the radical-anions for the bromo derivative [59] dissociate to features assignable to the corresponding radical [131] thereby providing evidence for the dissociation step in the proposed $S_{RN1}$ mechanism and for the intermediate radical. This loss of halide anion was not detected in the chloro derivative [60], despite the higher solvation energy for Cl$^-$, suggesting that delocalisation of the SOMO onto chlorine is less than onto bromine.

\[\text{[131]}\]
This dissociation of the radical-anion provides useful evidence for the key step (scheme 3.4 equation 3.11) in the 
SRN1 mechanism. However, previous work\textsuperscript{136} showed that 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 [59] undergoes dissociation. For the corresponding nitrofuran derivatives, only the iodo radical-anions dissociated, while no dissociation was observed for $[p$-$N$-$C$_6$H$_4$-CH$_2$Br]$^-$ or $[p$-$N$-$C$_6$H$_4$-CH$_2$I]$^-$ radical-anions.

Studies\textsuperscript{36} of the e.s.r. spectrum of the radical-anions of ronidazole [4] in liquid-phase at room temperature indicated that the radical-anions are reasonably stable suggesting that dissociation to the corresponding radicals\textsuperscript{131} and anions ($^-$OCONH$_2$) does not take place. The aminocarbonylate anion ($^-$OCONH$_2$) is a poor nucleofuge and therefore this result is not surprising. Also, neither the hydroxide anions nor NMe$_3^-$ are not lost from the radical-anions of the hydroxy and NMe$_3$ derivatives ([112] and [116]).

The e.s.r. spectra of the radical-anions of the 4- and 5-nitroimidazoles indicate that they have similar structures and distribution of electron-density, whereas the e.s.r. spectra of the 2-nitroimidazoles indicate a different structure and electron-distribution. (see Chapter 2 for the e.s.r. studies of 2- and 4-nitroimidazoles). 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 radio-sensitizing and antimicrobial activity.

In fact, the compounds exhibit large differences in antimicrobial activity against anaerobic organisms.\textsuperscript{47} The 2- and 5-nitroimidazoles are significantly active while the 4-nitroimidazoles are almost inactive.

The observation from the e.s.r. studies therefore suggests that the differences in biological activity cannot be readily explained by the differences in radical-anion structure, and that other factors should be considered. Differences in reduction potentials have been widely proposed\textsuperscript{42b,47} to explain the low reactivity of the 4-nitro-
imidazoles relative to the high reactivity of the 2- and 5-
-nitro-derivatives. (see Chapter 4 for some representative
$E^\ddagger$ values). Also, if the intermediates responsible for the
antimicrobial activity are the hydroxylamines$^{47}$ as opposed
to the radical-anions, the difference in activity may be
due to differing DNA reactivity between 2-, 4-, and 5-hydroxyl-
aminoimidazoles.$^{8b,47}$

The $\alpha$-substituted 1,2-dimethyl-5-nitroimidazoles show
similar antimicrobial activity$^{17}$ and their radical-anion
structures are also similar. Thus, the nature of the $\alpha$-sub-
stituent does not appear to be important. The ability of
the 2-bromo methyl radical-anions [59] $^4$ to dissociate does
not appear to be important to the reactivity, suggesting
that the 5-nitroimidazole radical-anion is the active biol-
ogical intermediate rather than the radical [131] formed by
loss of halide ion. These results support the observations
reported for the liquid-phase reactions; and are important
to the understanding of the mode of action of ronidazole
[4] and pirinidazole [120] which have clinical applications.
PART C Experimental

1. Preparation of 2-bromo-2-nitropropane

Sodium hydroxide (20g) and 2-nitropropane (44g, 0.49mol) were mixed in water (200ml) in a round bottomed flask until a single layer was formed. Bromine (108g, 36ml) was added slowly until mixture retained a slight brownish colour.

The resultant two phases were separated, the aqueous layer was discarded, and the organic layer washed successively with 10% aq. sodium thiosulphate solution, 5% aq. sodium hydroxide, and distilled water. The organic layer was dried (MgSO₄), and distillation in vacuo yielded 2-bromo-2-nitropropane as a colourless lacrymatory liquid (54g, 65%); b.p. 62-63°C (30mm) [lit. 106 b.p. 61-62° (29mm)]; \( \nu_{\text{max}} \) (neat) 1554 cm\(^{-1}\); \( \delta_H \) (CDCl\(_3\)) 2.21 (s).

2. Preparation of 2-chloro-2-nitropropane

2-Nitropropane (89g, 1mol) and aqueous sodium hydroxide (4g of NaOH in 400ml water) were mixed until one layer was formed. Chlorine was then passed through the reaction mixture until it was no longer absorbed. The excess chlorine was removed by passing nitrogen through the solution for 30 minutes. The two layers were separated. The organic layer was washed successively with 10% aq. thiosulphate solution, 5% aq. sodium hydroxide solution, and distilled water. The organic layer was dried (MgSO₄) and distilled in vacuo (30mm) to yield 2-chloro-2-nitropropane as a colourless liquid (94g, 76%), b.p. 48-50°C (30mm) [lit. 106 49° (30mm)]; \( \delta_H \) (CDCl\(_3\)) 2.14 (s).

3. Preparation of 2,2-dinitropropane

A mixture of 2-nitropropane (4.45g, 50mmol), sodium hydroxide (3.0g, 75mmol) and distilled water (25ml) was stirred until a homogenous solution was formed. Methylene chloride (25ml) was added and the mixture was cooled in an ice-water bath. A solution of sodium nitrite (14.0g, 0.2mol) in distilled water (25ml), a solution of potassium ferri-cyanide (3.30g, 10mmol) in water (10ml), and solid sodium persulphate (11.9g, 50mmol) were added consecutively to the stirred solution. The reaction temperature rose to 42°C before subsiding, and the mixture was stirred for 1h.

The aqueous phase was separated and was extracted
twice with CH₂Cl₂. The combined organic layers were washed with saturated sodium chloride solution, dried (MgSO₄), and distilled to give 2,2-dinitropropane (5.50 g, 82%), b.p. 68-70°C (10 mm) [lit.127 b.p. 56-60°C (3 mm)]; δ_H (CDCl₃) 2.15 (s).

4. Preparation of the sodium salt of 2-nitropropane

Sodium (5.8 g, 0.25 g atom) was slowly added to dry methanol (100 ml) with stirring under nitrogen. When all the sodium had dissolved, 2-nitropropane (22 g, 0.25 mol) was added and stirring continued for 30 minutes. The excess solvent was removed in vacuo at 60°C to yield a free flowing white powder (27.7 g, 100%); δ_H (D₂O) 2.0 (s).

5. Preparation of the lithium salt of 2-nitropropane

The above procedure was followed except that lithium (1.7 g, 0.25 g atom) was used in place of sodium. A white solid was obtained (23.2 g, 99%); δ_H (D₂O) 2.0 (s).

6. Preparation of the lithium salt of nitroethane

Lithium (1.39 g, 0.2 mol g atom) and nitroethane (15.0 g, 0.2 mol) were reacted as above to give a white solid (16.4 g, 100%); δ_H (D₂O) 1.8 (3H, d, CH₃), 6.25 (1H, q, CH).

7. Preparation of 4(5)-Nitroimidazole

Concentrated nitric acid (16 ml) followed cautiously by conc. sulphuric acid (16 ml), were added carefully to imidazole (8.0 g, 126 mmol) in a round bottomed flask immersed in an ice-salt bath. A vigorous reaction ensued and when this had subsided, the reaction mixture was refluxed with gentle stirring for 2h at 60°C.

The reaction mixture was cooled and poured into ice-cold water and neutralised carefully with sodium hydroxide. The precipitate of 4(5)-nitroimidazole was filtered and dried in a vacuum desiccator (17.2 g, 60%); m.p. 290-292°C [lit.73 m.p. 312-313°C]; ν_max (nujol) 3140 (NH), 1550 and 1370 cm⁻¹ (C-NO₂); δ_H (d₆-DMSO), 7.75 (1H, s, C₂-H) and 8.01 (1H, s, C₄(5)-H).

8. Preparation of 1-methyl-5-nitroimidazole

4(5)-Nitroimidazole (1.4 g, 12.4 mmol) and dimethyl sulphate (1.87 g, 14.9 mmol, 1.2 equiv.) were placed in a round bottomed flask. The mixture was refluxed on a steam bath for 1h. The resulting brown oil was triturated with ethyl
acetate and the precipitate filtered. The residue was dissolved in a minimum amount of water and basified with 2M aq. sodium hydroxide to pH 7-8. The solution was saturated with sodium chloride and extracted with chloroform (3x20ml), dried (MgSO₄), and the solvent removed in vacuo to yield a yellow solid. ¹H n.m.r. spectroscopy and t.l.c. analyses showed two products. The two products were separated using column chromatography on alumina with CHCl₃ as eluent. The major product was shown to be the required 5-nitro isomer (0.69g, 44%); m.p. 56-57°C [lit. 56 °C]; νmax (nujol), 3100 (aryl H), 1570 and 1360cm⁻¹ (C-NO₂); δH (CDCl₃) 3.96 (3H, s, N-Me), 7.48 (1H, s, C₃-H), and 7.85 (1H, s, C₄-H). The ¹H n.m.r. spectrum of the other compound was identical to that of authentic 1-methyl-4-nitroimidazole.

9. Preparation of 1,2-dimethyl-5-nitroimidazole
a) Potassium hydroxide (5g), distilled water (5ml) and ethanol (25ml) were introduced in a round bottomed flask of a diazomethane generator under anhydrous conditions. Diazald (21.4g, 0.1mol) and diethyl ether (130ml) were introduced into the dropping funnel connected to the flask. The receiving flask was immersed in an ice-salt bath. The reaction flask was heated at 60-65°C and the content of the dropping funnel gradually added to the flask. The yellow diazomethane in diethyl ether was collected.
b) 2-Methyl-4(5)-nitroimidazole (4g, 31.5mmol) was added to the solution of diazomethane in diethyl ether prepared above, followed by a catalytic amount of methanol (1ml). The reaction was stirred under anhydrous conditions. The resulting orange solution was evaporated to dryness in vacuo to give a crude solid which was recrystallised from aqueous methanol to give colourless needles of 1,2-dimethyl-5-nitroimidazole (3.4g, 76%); m.p. 136-138°C [lit. 66 °C]; νmax (nujol) 3100 (aryl H), 1570 and 1370cm⁻¹ (C-NO₂); δH (CDCl₃) 2.48 (3H, s, C₃-H), 3.94 (3H, s, N-Me) and 7.91 (1H, s, C₄-H); m/z [Found: M⁺ 141.0518 (100%) C₅H₇N₃O₂ requires 141.0538], 95(50), 80(5), 53(11).

10. Preparation of 1,2-dimethyl-4-nitroimidazole
Dimethyl sulphate (1.5ml) was added to a stirred solution of 2-methyl-4(5)-nitroimidazole (3.0g, 23.6mmol) in
10% aqueous sodium hydroxide solution (15ml). Further sodium hydroxide solution (15ml) and dimethyl sulphate (1.5ml) were added to the reaction.

The solid which separated was filtered and washed with diethyl ether. The filtrate was extracted with chloroform (2x50ml). The organic layers were combined, washed with water (1x50ml), dried (MgSO₄), and the solvent removed in vacuo to yield a crude crystalline residue. The filtered residue and that from the chloroform layer were combined and re-crystallised from hot water to yield colourless crystals of 1,2-dimethyl-4-nitroimidazole (2.2g, 66%); m.p. 178-180°C [lit. 66 m.p. 182-183°C]; ν max (nujol), 1540 and 1350cm⁻¹ (C-NO₂); δ H (CDCl₃) 2.40 (3H, s, C₂-Me), 3.73 (3H, s, N-Me) and 8.0 (1H, s, C₅-H).

11. Preparation of 2-(hydroxymethyl)-1-methyl-5-nitroimidazole

1-Methyl-5-nitroimidazole (12.7g, 0.1mol), paraformaldehyde (13.97g, 0.46mol) and DMSO (102ml) were introduced into a Carius tube and sealed. The sealed tube was heated at 115-117°C for 53h. The tube and contents were allowed to cool to room temperature. The tube was opened and the solution was evaporated to dryness. The residue was purified by column chromatography on alumina with CHCl₃, followed by CHCl₃: methanol (MeOH) as eluent. The separation afforded unreacted starting material (1.9g, 15%), and a pale yellow solid which was recrystallised from toluene to give pale yellow needles of 1-methyl-2-(hydroxymethyl)-5-nitroimidazole (8.6g, 55%); m.p. 116-117°C [lit. 157b m.p. 110°C]; ν max 3140 (-OH), 1550 and 1380cm⁻¹ (C-NO₂); δ H (d₆-DMSO) 4.0 (3H, s, N-Me), 4.57 (2H, d, -CH₂-), 5.68 (1H, t, -OH) and 7.98 (1H, s, C₄-H): m/z [Found: M⁺ 157.0481(100%). C₅H₇N₃O₃ requires 157.0487], 156(19), 140(8), 127(32), 111(49), 94(9).

12. Preparation of 2-(chloromethyl)-1-methyl-5-nitroimidazole

2-(Hydroxymethyl)-1-methyl-5-nitroimidazole (1.0g, 6.36mmol) was dissolved in refluxing toluene (100ml). Thionyl chloride (20ml) was added cautiously to the refluxing solution and left to reflux for 20min. The solution was evaporated to dryness in vacuo and the resulting residue was leached with toluene to remove excess thionyl chloride leaving the hydrochloride salt of
the titled compound. The salt was dissolved in distilled water and basified to pH 8-9 with aqueous sodium bicarbonate solution and then extracted with CHCl₃ (3x100ml). The organic extracts were combined, washed with water, dried (MgSO₄), and removal of solvent as above gave a crude solid which was recrystallised from acetone/MeOH to give yellow crystals of the titled compound (0.8g, 72%); m.p. 61-62°C m.p. not reported in the ref. to this preparation\(^{156}\); \(\nu\) max (nujol) 3200 (aryl H), 1560 and 1310 cm\(^{-1}\) (C-NO\(_2\)); \(\delta\) H (CDCl\(_3\)) 4.0 (3H, s, N-Me), 4.8 (2H, s, -CH\(_2\)-), and 8.00 (1H, s, C\(_4\)-H); m/z [Found: M\(^+\) 175.0137 (26%) and 177.0118 (8%). C\(_5\)H\(_6\)N\(_3\)O\(_2\)\(^{35}\)Cl and C\(_5\)H\(_6\)N\(_3\)O\(_2\)\(^{37}\)Cl requires 175.0149 and 177.0119 respectively], 140(100), 94(11).

13. Preparation of 1-methyl-5-nitroimidazol-2-yl-methyl N,N,N-trimethyl methanimium chloride\(^6\)

2-(Chloromethyl)-1-methyl-5-nitroimidazole (600mg, 3.42mmol) was dissolved in 40% ethanolic trimethylamine (100ml). The solution was stirred for 2 days and the extent of the reaction was monitored by t.l.c. The solution was evaporated to dryness and the residue was leached with dry diethyl ether to give a residue which was recrystallised from acetone to give white crystals of the titled compound [116] (550mg, 68%); m.p. 201-202°C; [Found: C, 37.95; H, 6.8; N, 22.2; Cl, 14.25, C\(_8\)H\(_{15}\)N\(_4\)O\(_2\) Cl requires C, 40.9; H, 6.4; N, 23.9; Cl, 15.1%]; \(\nu\) max (KBr) 3160 (aryl H), 3080-3000 (-C-H), 2800 (N-Me), 1535 and 1360 cm\(^{-1}\) (C-NO\(_2\)); \(\delta\) H (D\(_2\)O) 3.38 (9H, s, NMe\(^3\)), 4.19 (3H, s, N-Me), 5.91 (2H, s, -CH\(_2\)-) and 8.29 (1H, s, C\(_4\)-H); m/z [Found: M\(^+\) 234.0872 (0.17%). C\(_8\)H\(_{15}\)N\(_4\)O\(_2\)Cl, requires 234.0883], 141(100).

14. Preparation of (1-methyl-5-nitroimidazol-2-yl)-methyl dimethyl octylammonium chloride

2-(Chloromethyl)-1-methyl-5-nitroimidazole (1g, 5.70mmol) was added with stirring, to a solution of N,N-dimethyl octylamine (5g, 32mmol) in methanol (50ml) and diethyl ether (50ml). The reaction was monitored by t.l.c. and terminated when all the starting material had reacted (2 days).

The reaction mixture was evaporated to dryness and the excess N,N-dimethyl octylamine was leached out with diethyl
ether. The resulting buff-coloured solid was recrystallised from acetone/ether to give colourless crystals of the titled compound [117] (1.25g, 66%); m.p. 210-211°C; [Found: C, 53.8; H, 8.75; N, 16.8; Cl, 11.0. C_{14}H_{26}N_{4}O_{2} Cl requires C, 54.08; H, 8.71; N, 16.82; Cl, 10.67%]; \( \nu \) max (KBr) 3100 (aryl H), 2930-2960 (C-H), 2860 (N-CH3), 1560 and 1370 cm\(^{-1}\) (C-N02), \( \delta \) H (0.91 (3H, s, -(CH2)7-Me), 1.32 (14H, broad, (CH2)6), 3.61 (6H, s, \( \delta \) Me2), 4.2 (3H, s, NMe), 5.6 (2H, t, -CH2N) and 8.1 (1H, s, C4-H).

15. Preparation of 2-(bromomethyl)-1-methyl-5-nitroimidazole 6,156
2-(Hydroxymethyl)-1-methyl-5-nitroimidazole (1.0g, 6.34mmol) and thionyl bromide (20ml) in toluene (100ml) were reacted using the same procedure as above.

The residue obtained after work up was purified by column chromatography on alumina using CHCl3:MeOH (98:2) as eluent. Recrystallisation of product from the column in pet-ether (40-60°C)/MeOH gave lacrymatory crystals of the bromo compound (1.6g, 76%); m.p. 75-76°C; \( \nu \) max (KBr) 3100 (aryl H), 3030 (N-Me), 1530 and 1370 cm\(^{-1}\) (C-N02); \( \delta \) H (CDCl3) 3.92 (3H, s, N-Me), 4.46 (2H, s, C-CBr) and 7.88 (1H, s, C4-H); m/z [Found: M" 220.9634 (6%) C_{6}H_{6}N_{3}O_{2}Br requires 220.9624. No accurate mass for isotope Br due to a major reference peak.] 219(5), 140(100), 94(31).

16. Attempted bromination of 1,2-dimethyl-5-nitroimidazole
a) With N-bromosuccinimide
1,2-Dimethyl-5-nitroimidazole (500mg, 3.55mmol), N-bromosuccinimide (633mg, 3.56mmol), and dibenzoyl peroxide (250mg) were dissolved in dry carbon tetrachloride (CCl4) (100ml) in a 3-necked round bottomed flask equipped with a double surface reflux condenser, a silica-gel drying tube and a magnetic stirrer bar. The mixture was stirred, refluxed and irradiated with two 150-W fluorescent lamps from a distance of 10cm, for 16h.

The resulting mixture was filtered to remove the precipitate. The filtrate was washed consecutively with a weak solution of sodium metabisulphite, aqueous sodium carbonate, and distilled water. The organic layer was dried (MgSO4) and evaporated to dryness in vacuo.

The \( \text{^1H n.m.r.} \) spectrum of the crude product indicated
unchanged starting material, the required product, and two other brominated imidazole products. (See discussion, chapter 3). Separation by column chromatography on alumina with CHCl₃ as eluent was not successful.

Other attempts, using 2mol equivalents of N-bromosuccinimide and portion-wise addition of bromine (1mol equiv.) gave similar mixtures.

b) With N-bromosuccinimide in the presence of a base

Carbon tetrachloride (50ml) followed by 1,2-dimethyl-5-nitroimidazole (284mg, 2mmol) were added to a solution of potassium (10mg, 0.256g atom) in dry t-butanol (20ml). A violet solution was obtained which was stirred for 30min. N-Bromosuccinimide (198mg, 1.11mmol) was added in small portions and after addition, the reaction was left for 7h under an atmosphere of nitrogen.

The resulting yellow solution was diluted with distilled water and neutralised with dilute hydrochloric acid to pH 7. The CC₁₄ layer was dried (MgSO₄) and solvent removed in vacuo to give a pale yellow solid (92mg, 32%). The ¹H n.m.r. spectrum was identical to that of the nitroimidazole starting material.

c) Using Copper (II) bromide

Copper (II) bromide (2.79g, 125mmol) was suspended in ethyl acetate (25ml) and 1,2-dimethyl-5-nitroimidazole (107mg, 7.5mmol) in dry CHCL₃ (25ml) was added to the refluxing mixture. There was no colour change and the reaction was stopped after 5h.

The reaction mixture was poured into ice-cold water, extracted with chloroform (2x25ml) and evaporated to give unchanged starting material. The ¹H n.m.r. spectrum and m.p. were identical to that of authentic material.

17. U.V. Measurements of the Anion of 1,2-Dimethyl-5-nitroimidazoles

i) 1,2-Dimethyl-5-nitroimidazole (1,2 DMNI) (1mg, 7.04umol) was dissolved in methanol (10ml) in a volumetric flask. After a 1.5 x 10² dilution, the u.v. spectrum of the solution was recorded on U.V. 160.

To this solution was added each of the following solutions in turn: 2-3 drops of aq. 2M NaOH, aq. 2M HCl, aq. 2M NaOH
18. The attempted preparation of 1-methyl-2-(nitromethyl)-
-5-nitroimidazole from 1,2-dimethyl-5-nitroimidazole

a) Using sodamide base

Dry liquid ammonia was prepared by double distillation
in essentially the same manner as that used by Bunnett and
Hrutfiord. Two one-litre flasks, connected by a drying
tube packed with silica-gell and 4A molecular sieves and
equipped with large dry-ice condensers, were pre-dried by
flaming thoroughly whilst being evacuated with dry nitrogen
gas. Ammonia gas was then passed through NaOH pellets and
condensed into the first flask where it was dried further
by addition of sodium metal until the blue colour persisted
for several minutes.

The ammonia was then allowed to condense into the sec­
ond flask via the drying tube. This flask and its condenser
were made with reversed joints to avoid condensation running
down into the flask through the joints. The ammonia was
again pre-dried with sodium metal. The initial flask was
disconnected by pinch clips.

Dry ammonia (100ml) was prepared in the above manner.
Ferric nitrate (one crystal, reaction catalyst) was added
followed by sodium metal (69mg, 17.7mmol). The metal was
added over a period of 15min and the resulting sodamide/NH₃
mixture was stirred a further 30min using a magnetic stirrer.
During this time, the blue colour turned to silver grey.

1,2-Dimethyl-5-nitroimidazole (1.0g, 7.1mmol) in tetrahydro­
furan (THF) was added and immediately the solution turned
violet-black in colour. The reaction mixture was cooled to
-33°C. n-Propyl nitrate (2.23g, 21mmol, 3mol equivalents)
was then added and the mixture was stirred for 30min.

Ammonium chloride (964mg, 17.7mmol) was added to quench
the sodamide. Diethyl ether (100ml) was added and the mix­
ture was allowed to evaporate to dryness overnight. The
residue was extracted twice with chloroform/water (50ml of
each) and the layers of the individual extracts were sepa­
rated. The combined CHCl₃ extracts were washed with 2MHC1
(2x50ml), dried (MgSO₄), and evaporated in vacuo to yield
unreacted starting material (0.2g, 20% recovery).
The m.p., t.l.c., i.r. and $^1$H n.m.r. spectra were identical to those of authentic material.

The aqueous phases were combined and the resulting precipitate was filtered and dried. $^1$H n.m.r. analysis of the crude deep violet precipitate (not very soluble in common $^1$H n.m.r. solvents, CDCl$_3$, d$_6$-DMSO or D$_2$O) could not be identified. The precipitate was soluble at pH 1 and at alkaline pH's but precipitated at pH 6-7. Attempts to identify the compound were not successful. A duplicate run gave similar results.

b) i) Using trimethylamine base

Dry trimethylamine (50ml) was pipetted into a 3-necked round bottomed flask equipped with a nitrogen inlet and outlet. A gentle stream of nitrogen was passed through for 5min. and maintained throughout the reaction. 1,2-Dimethyl-5-nitroimidazole (1g, 7.1mmol) was added and the mixture was stirred for 30min. The usual purple colour of the anion of the imidazole was not observed, and the nitrogen flow was stopped due to evaporation of the trimethylamine. n-Propyl nitrate (2.24g, 21.3mmol) was added to the reaction and the mixture was stirred for 30min.

The resulting precipitate was filtered and dried and shown by $^1$H n.m.r. spectral data to be unreacted starting material (166mg, 17%).

The filtrate was neutralised with ammonium hydrochloride and then extracted with CHCl$_3$ (2x50ml). The organic extracts were combined, washed with distilled water (1x50ml), dried (MgSO$_4$), and the solvent removed in vacuo. Analysis using $^1$H n.m.r. spectroscopy indicated unreacted starting material (368mg, 37%; total recovery yield = 54%). The identity of the compound was confirmed by comparison with $^1$H n.m.r. spectrum of the authentic sample.

ii) Dry pyridine (50ml) was used instead of the volatile trimethylamine in exactly the same manner described above. Analysis of the crude product gave similarly recovered starting material (600mg, 60%).

19. The attempted preparation of 1-methyl-2-(nitromethyl)-5-nitroimidazole from 2-(bromomethyl)-1-methyl-5-nitroimidazole

Anhydrous acetonitrile (100ml) was pipetted into a 3-necked round bottom flask equipped with a dropping funnel,
a silica gel drying tube and a magnetic stirrer bar. The flask was wrapped in aluminium foil to exclude light and cooled in an ice-bath to 0°C. Silver nitrite (1.137g, 7.39mmol) was added followed by a catalytic amount of calcium hydride. The mixture was stirred for 15min. 2-(Bromomethyl)-1-methyl-5-nitroimidazole (1.8g, 8.18mmol) in dry acetonitrile (5ml) was added dropwise via the dropping funnel to the silver nitrite solution. The reaction mixture was stirred at 0°C for 24h.

The flocculent precipitate which formed was allowed to settle and the solvent decanted off. Evaporation of the acetonitrile in vacuo gave a gummy yellow residue, which was dissolved in H2O/CHCl3. The solid which separated was filtered and dried and shown (m.p. > 250°C) to be an inorganic salt.

The chloroform layer was washed with distilled water, dried (MgSO4), and evaporated to dryness in vacuo. Analysis using 1H n.m.r. spectroscopy of the crude (100mg) obtained indicated a mixture of products, the required 1-methyl-2-(nitromethyl)-5-nitroimidazole, its nitrite ester, and other impurities, (the 1H n.m.r. spectrum was compared with those of 2-(nitromethyl)-pyridine and the nitrite ester analogue.) Attempts at separation using alumina preparative t.l.c. led to decomposition.

A duplicate run was carried out in anhydrous ether. After the initial 24h at 0°C, the reaction was left stirring at room temperature for 48h. The reaction was stopped after a negative Beilstein test was obtained. The ether was removed in vacuo to give a crude sample (15mg). Analysis using 1H n.m.r. spectroscopy showed the required product. The reaction was not investigated further because of the low yields (15mg from 1.8g of starting material).

20. Reactions of 2-(Chloromethyl)-1-methyl-5-nitroimidazole

a) With the Lithium Salt of 2-Nitropropane

Dry DMF (30ml) was charged into a 3-necked round bottomed flask equipped with a magnetic stirrer bar, and a nitrogen inlet. Nitrogen gas was passed through for 30min, and maintained throughout the reaction. The lithium salt of 2-nitropropane (315mg, 2.84mmol, 2molar equivalents) was
added to the flask and stirred until dissolved. 2-(Chloromethyl)-1-methyl-5-nitroimidazole (250mg, 1.42mmol) was added to the solution. The reaction was carried out as described in the general 3_rn reactions (Chapter 2, part C) and was stirred under nitrogen for 12h.

The reaction mixture was poured into iced water and extracted into diethyl ether (3x30ml). The ether extracts were combined, washed with water (7x30ml), dried (MgSO₄), and the solvent removed in vacuo to give a crude mixture. The mixture was separated by column chromatography on alumina with chloroform as eluent to give 1-methyl-2-(2-methylprop-1-ethyl)-5-nitroimidazole (181mg, 46%); δ_H (CDCl₃) 2.22 (6H, d, CH₂), 3.97 (3H, s, N-Me), 6.05 (1H, brs, CH=C=CH₂) and 8.1 (1H, s, C₆-H).

b) With the potassium salt of 2-methyl-4(5)-nitroimidazole

2-Methyl-4(5)-nitroimidazole (250mg, 1.97mmol) and potassium t-butoxide (331mg, 2.95mmol) were added to degassed dry DMSO (50ml) in a round bottomed flask and yellow colour of the imidazole anion was immediately formed. The anion was left stirring for 30min and 2-(chloromethyl)-1-methyl-5-nitroimidazole (345mg, 1.97mol) was added to the yellow solution which rapidly turned red brown. The reaction was irradiated as described in Chapter 2, part C, and left stirring for 30h under nitrogen.

The reaction mixture was poured into ice-cold H₂O/CHCl₃, and extracted with CHCl₃ (3x50ml). The rest of the work up procedure was as described above. The crude product was separated on alumina preparative t.l.c. plates to afford a product which was recrystallised from ethyl acetate to give 2-methyl-1-[(1-methyl-5-nitroimidazol-2-yl)-methyl]-4-nitroimidazole (105mg, 20%); m.p. 208-209°C. δ₇ (acetone-d₆) 2.4 (3H, s, C₂-Me), 4.06 (3H, s, N-Me), 5.72 (2H, s, -CH₂-), 8.18 (1H, s, C₆-H) and 8.48 (C₅-H); m/z 220 (M⁺-NO₂, 100%), 140(26), 94(20).

c) With the Lithium Salt of 2-Nitroethane

The lithium salt of 2-nitroethane (138mg, 1.70mmol, 1.2mol equivalents) and 2-(chloromethyl)-1-methyl-5-nitroimidazole (250mg, 1.42mmol) were reacted together as described above. On adding the two reagents, an immediate purple colour was observed which turned yellow after two minutes. The reaction was stirred for 12h.
The normal work up gave a crude mixture, which could not be identified.

21. Preparation of 4(5)-di-iodoimidazole

Imidazole (1.7g, 25mmol) was dissolved in sodium hydroxide solution (1.0g in 150ml distilled H₂O) and cooled to 0-10°C in a 3 litre 1-necked flask equipped with two dropping funnels and a magnetic stirrer bar. Iodine (12.7g, 50mmol) was added portion-wise to the stirred solution. At the same time a sodium hydroxide solution (3g in 250ml distilled water) and pet-ether (200ml) were also added.

The reaction was stopped when the iodine colour disappeared. The aqueous layer was washed with pet-ether (3x200ml) and acidified with dilute hydrochloric acid to pH 6. The solution was left in the refrigerator overnight to complete precipitation. The precipitate was filtered and recrystallised from 50% aqueous ethanol to yield colourless needles 4(5)-di-iodoimidazole (7.76g, 97%); m.p. 180-182°C [lit. 172 m.p. 182°C]; \( \nu_{\text{max}} \) 3100cm⁻¹ (NH); \( \delta_H \) (CDCl₃/d₆-DMSO) 7.72 (s, C₂-H). The crystals were light sensitive and therefore, were wrapped in aluminium foil to exclude light.

22. Preparation of 4-iodo-5-nitroimidazole

4(5)-Di-iodoimidazole (8.0g, 25mmol) was added cautiously in portions, with stirring, to a mixture of conc. nitric acid and sulphuric acid (300ml, 1:1v/v), cooled in an ice-salt bath to -20°C. After the addition was completed, the mixture was warmed to 20-25°C, and stirred at this temperature for 4h.

The resulting clear oily liquid (which tended to darken with time) was poured, with stirring, into ice-water (100ml). The resulting precipitate was filtered and was repeatedly washed with 10% sodium iodide solution in order to remove free iodine. The yellow solid was recrystallised from 50% aqueous ethanol to give bright yellow crystals of 4-iodo-5-nitroimidazole (4.3g, 72%) m.p. 284-285°C [lit. 172 m.p. 281°C]; \( \nu_{\text{max}} \) 1540 and 1360cm⁻¹ (s) (C(NO₂) stretching); \( \delta_H \) (CDCl₃/d₆-DMSO) 7.89 (s, C₂-H).

23. Preparation of 4-iodo-1-methyl-5-nitroimidazole

4-Iodo-5-nitroimidazole (2.0g, 8.36mmol) and dimethyl
sulphate (1.33g, 10.56mmol) was refluxed for 90min. on a water bath. The resulting brown melt was allowed to cool and then triturated with ethyl acetate to give a solid. The solid was dissolved in water and neutralised with 2M aq. NaOH solution in order to neutralise unreacted dimethyl sulphate. The solution was saturated with sodium chloride and extracted with chloroform (3x50ml). The chloroform layers were combined, washed once with water, dried (MgSO₄), and evaporated to dryness in vacuo. The crude product was recrystallised from aqueous methanol to yield deep yellow needles of 4-iodo-1-methyl-5-nitroimidazole (0.6g, 63%) m.p. 151-152°C [lit. 172 m.p. 152°C], υ max 1550 and 1350cm⁻¹ (C-NO₂); δ_H (CDCl₃) 4.00 (3H, s, N-Me), 7.6 (1H, s, C₂-H).

24. Reactions of 4-iodo-1-methyl-5-nitroimidazole

a) With the sodium salt of nitroethane

4-Iodo-1-methyl-5-nitroimidazole (500mg, 1.98mmol) was dissolved in dry DMF (20ml) and sodium salt of nitroethane (29mg, 1.29mmol, 1.5mol equivalents) were added to the solution.

The reaction was stirred at room temperature and followed by t.l.c. An aliquot (5ml) of the reaction mixture was taken after 21h, after the normal work up as described above, the ¹H n.m.r. spectrum confirmed starting material. The reaction was terminated after 48h and gave mainly unreacted starting material. The ¹H n.m.r. spectroscopic data and t.l.c. were identical to that of the authentic material.

The reaction was repeated under light catalysis, with copper (I) chloride under light catalysis, and under reflux. All attempts gave recovered starting material.

b) With the sodium salt of 2-nitropropane

The titled compound (508mg, 2mmol) and the sodium salt of 2-nitropropane (333mg, 3.0mmol) were reacted together as described above.

The ¹H n.m.r. spectrum showed [δ_H (CDCl₃) 2.49, 4.02, and 7.62] recovered starting material and 2-iodo-2-nitropropane (δ_H 2.49) [lit. 106 δ_H 2.49].
25. Preparation of 2-aminoimidazolium sulphate 76

2,2-Diethoxyethylamine (25g, 187mmol), distilled water (40ml) and 5-methylisothiourea 76a (25g, 180mmol) were heated together on a water bath for 1h. The water was removed in vacuo (in the fume cupboard because of the unpleasant smell of the thiol) to give an oil.

The latter was treated with conc. hydrochloric acid (18ml) and the mixture maintained at 50°-60°C for 15mins. Distilled water (90ml) was added and again removed in vacuo. This procedure was repeated and the oily syrup obtained was dissolved in absolute ethanol (25ml) and an equal volume of anhydrous ether was added. The solution was left to crystallise overnight. The filtrate was left standing for 4 days to yield a second crop. The total yield after recrystallisation from ethanol was 7.5g (16%), m.p. 269-270°C [lit. m.p. 270°C (dec)]. The i.r. spectrum was identical to that of the authentic compound.

26. Preparation of 2-Nitroimidazole (Azomycin) 76

2-Aminoimidazolium sulphate (1.57g, 5.94mmol) was dissolved in distilled water (7ml) and 40% fluoroboric acid (10ml) in a 3-necked round bottomed flask equipped with a dropping funnel and a magnetic stirrer bar. The solution was cooled to -20°C in an acetone liquid N₂ bath. A solution of sodium nitrite (4.1g, 59.4mmol) in distilled water (10ml) was added dropwise to the cooled 2-aminoimidazolium sulphate solution. The mixture was stirred at -10°C for 30min and then added to a solution of copper (II) sulphate (29.7g, 119mmol) in distilled water (200ml). An additional amount of sodium nitrite (4.1g, 59.4mmol) was added to this mixture and stirred for 2h at room temperature. The pH of the mixture was then adjusted to approximately pH 2 with dilute nitric acid and extracted with ethyl acetate (200ml) in a liquid-liquid Soxhlet extraction apparatus for 48h.

The ethyl acetate layer was dried (Na₂SO₄) and the solvent removed in vacuo to give a yellow syrup which was triturated with water and filtered. The solid was washed with small amounts of diethyl ether and recrystallised from anhydrous ethanol to yield yellow crystals of azomycin (0.63g, 49%), m.p. 286°-288°C [Lit. 76b m.p. 287°C]. The i.r. spec-
trum was identical to that of the authentic compound. This method is not suitable for large scale preparations because of the large volume of solvent involved.

27. Preparation of 2,4(5)-dinitroimidazole

A mixture of fuming nitric acid (15ml) and acetic anhydride (5ml) was carefully prepared and cooled to 0°C in an ice-salt bath.

2-Nitroimidazole (1.13g, 10mmol) was added in small portions to the prepared mixture with stirring. The solution was heated for 2h at 100°C and then at 115°C for 30min.

The solution was cooled and poured into ice-cold water and then extracted with ethyl acetate (3x50ml). The organic layers were combined, dried (MgSO₄), and the solvent removed in vacuo to give a yellow residue which was washed with cold H₂O (2x25ml) to give 2,4(5)-dinitroimidazole (0.96g, 60%), m.p. 275-278°C [lit. 276c m.p. 278-280°C]; νmax 3100 (NH), 1540 and 1340cm⁻¹ (C-NO₂); δH (d₆-DMSO) 8.50 (1H, s, C₄(5)-H), 8.92 (1H, brs, NH).

28. Preparation of 1-methyl-2,5-dinitroimidazole

2,4(5)-Dinitroimidazole (0.9g, 5.7mmol) was stirred with an excess of diazomethane in anhydrous ether (200ml) for 30h with a catalytic amount of dry methanol (Ca 1ml).

The unreacted diazomethane was neutralised with sodium hydroxide solution. The solution was filtered and the residue recrystallised from ethanol. The filtrate was concentrated in vacuo to yield an oil which showed the presence of two compounds on alumina t.l.c. The oil was crystallised with absolute ethanol, and re-suspended in a small amount of chloroform. Separation on alumina preparative t.l.c. plate with CH₂Cl₂ as eluent afforded the two isomers. The faster eluting fraction was recrystallised from absolute ethanol to yield pale yellow crystals which were identified as 1-methyl-2,5-dinitroimidazole (0.87g, 88%); m.p. (147-149°C), νmax 1550 and 1350cm⁻¹ (C-NO₂); δH (d₆-acetone) 4.31 (3H, s, N-Me), 8.59 (1H, s, C₅-H).

29. The attempted preparation of 1-methyl-2-(1-methyl-1-nitroethyl)-5-nitroimidazole

1-Methyl-2,5-dinitroimidazole (100mg, 0.581mmol) was
dissolved in dry DMF (10ml) in a 3-necked round bottomed flask equipped with a silica gel drying tube, a nitrogen inlet and a magnetic stirrer bar. The flask was wrapped in aluminium foil. The lithium salt of 2-nitropropane (82.8mg, 87.2mmol) in dry DMF (5ml) was added to the reaction flask and the mixture was stirred under nitrogen for 45min. The crude product was analysed using $^1$H n.m.r. spectroscopy and t.l.c. on alumina confirmed that the crude product was unreacted starting material (49% recovery).

30. **Preparation of 1-methyl-2-(methylmercapto)-imidazole**

2-Mercapto-1-methylimidazole (10g, 87.7mmol) distilled methanol under an atmosphere of nitrogen, was introduced into a 3-necked flask (100ml). The mixture was cooled to 10°C and sodium hydroxide (16ml, 10N) was added. Methyl iodide (13.2g, 92mmol, 1.05mol equivalents) in methanol (20ml) was added to the stirred solution over a period of 15min while the temperature was kept below 15°C. The reaction mixture was stirred under nitrogen at room temperature for 15h.

The solvent was removed in vacuo and the residue was dissolved in distilled water (100ml) and extracted with dichloromethane (3x100ml). The organic extracts were combined, washed with saturated NaCl solution, dried (MgSO$_4$), and solvent removed in vacuo to yield crude 2-(methylmercapto)-1-methylimidazole; $^1$H (CDCl$_3$) 2.64 (3H, s, S-Me), 3.79 (3H, s, N-Me) and 6.9 (1H, s, C$_4$-H). The oily crude (10.5g) was nitrated immediately.

31. **Preparation of 2-(methylmercapto)-1-methyl-4(5)-nitroimidazole**

The crude product from the reaction above was added dropwise, with stirring, to conc. nitric acid (1.42 specific gravity) (50ml) at 80°C. The internal temperature was maintained between 80-90°C. Copious evolution of oxides of nitrogen was observed. The mixture was heated at 90-95°C for one hour.

The mixture was cooled, poured into ice, treated with acetic acid (30ml) and neutralised to pH 8 with NaOH solution (30%) at 0°C. The mixture was extracted into CH$_2$Cl$_2$ (3x100ml), dried (Na$_2$SO$_4$), and evaporated to dryness.
vacuo to give a crude product (4.5g). Analysis of the crude product using $^1$H n.m.r. spectroscopy indicated two-isomers with the 5-nitro isomers as the major product. The isomers were not separated from the crude at this stage but used directly for the next step in the synthesis.

32. Preparation of 1-methyl-2-(methylsulphonyl)-5-nitroimidazole\(^1\)

The above crude (4.5g) was dissolved in dichloromethane (150ml) cooled in an ice-bath and was added to a solution of monoperphthalic acid (95.4ml, 65mmol) in diethyl ether (100ml) at 0°C. The mixture was stirred overnight (12h) at room temperature and gently refluxed for 2h. The precipitated phthalic acid was filtered and washed with CH$_2$Cl$_2$. The filtrate was cooled in ice and stirred with potassium hydrogen carbonate solution until a small aliquot of the organic layer did not give a positive test with starch and potassium iodide solution. The organic layer was separated, dried (Na$_2$SO$_4$), and the solvent removed in vacuo to give a crude product (4.23g). Purification by column chromatography on alumina with CHCl$_3$:MeOH (98:2) as eluent and recrystallisation from CH$_2$Cl$_2$-hexane gave pale yellow crystals of the titled compound (3.2lg, 65%); m.p. 92-94°C; [lit. 124 m.p. 90-92°C]

$^1$H (CDCl$_3$) 3.4 (3H, s, SO$_2$Me), 4.22 (3H, s, N-Me) and 7.84 (1H, s, C$_4$-H); and the 4-nitro isomer (490mg, 10%), $^1$H (CDCl$_3$) 3.45 (3H, s, SO$_2$Me), 4.02 (3H, s, N-Me) and 7.92 (1H, s, C$_4$-H).

33. Reaction of 1-methyl-2-(methylsulphonyl)-5-nitroimidazole

a) With the sodium salt of 2-nitropropane - General-Method

Clean sodium hydride (140mg, 5.85mmol) was suspended in dry DMF (5ml) in a 3-necked flask and cooled to 10°C. Nitropropane (433mg, 4.87mmol) in DMF (5ml) was added to the suspension and the mixture was stirred at room temperature for 1h. 1-Methyl-2-(methylsulphonyl)-5-nitroimidazole (1g, 4.87mmol) in DMF (5ml) was then added and stirring was continued at room temperature for 14h.

The solvent was removed in vacuo, and the residue was triturated with water and extracted with CH$_2$Cl$_2$ (3x50ml). Normal work up, as above, afforded a crude product (634mg). Separation on alumina preparative t.l.c. using EtOAc: CH$_2$Cl$_2$
(4:1) as eluent gave three compounds; the unreacted methylsulphonyl compound (260mg, 26%), 2,3-dimethyl-2,3-dinitrobutane (40mg, 5%), $S_H$ (CDCl$_3$) 1.7 (s) and methyl 1-methyl-1-nitroethyl sulphone (483mg, 59%), m.p. 81-83°C, [lit. 135b m.p. 82-84°C] $S_H$ (CDCl$_3$) 2.03 (6H, s, Me$_2$C), and 3.2 (3H, s, SO$_2$Me). A duplicate run gave similar results.

i) Reaction in the dark under an atmosphere of oxygen

The reaction was carried out as described above in the dark. Analysis of the crude product using $^1$H n.m.r. spectroscopy indicated methyl 1-methyl-1-nitroethyl sulphone (30%), 2,3-dimethyl-2,3-dinitrobutane (5%) and the unreacted imidazole starting material (25%).

b) With the sodium salt of nitromethane

Using the reaction procedure described in the general method above, a solution of nitromethane (297mg, 4.87mmol) in dry DMF was added to a suspension of NaH (140mg, 5.85mmol) in DMF (15ml). 1-Methyl-2-(methylsulphonyl)-5-nitroimidazole (1.0g, 4.87mmol) was added and the reaction mixture was left stirring at room temperature for 20h. Analysis using $^1$H n.m.r. spectroscopy of the crude, worked up in the usual manner, showed unreacted imidazole starting material (50%) and 1-methyl-5-nitroimidazole (23%). The $^1$H n.m.r. data of these compounds were identical to those of authentic samples.

34. Preparation of 2-isopropyl-4(5)-nitroimidazole

2-Isopropylimidazole (6g, 54.5mmol) was nitrated as described for the nitration of imidazole (chapter 2, part C). At the end of the reaction, the cooled mixture was poured slowly into ice-water, with stirring, and carefully neutralised to pH 8 with potassium carbonate. The aqueous solution was extracted with CHCl$_3$ (4x100ml). The organic extracts were combined, washed with saturated brine solution (1x100ml), dried (Mg$_2$SO$_4$), and the solvent removed in vacuo to give crude 2-isopropyl-4(5)-nitroimidazole (as indicated by t.l.c. and $^1$H n.m.r. spectroscopy of the crude mixture) (2.5g, 30%). The crude product was used directly in the next stage of the synthesis. 2-Nitro-2-nitrosopropane was obtained as a by-product as the dimer (4.1g, 32%), m.p. 75-76°C [lit. 173 m.p. 76°C]; $\nu_{max}$, 1560cm$^{-1}$; $S_H$ (CDCl$_3$) 1.5 (s).
35. Preparation of 2-(isopropyl)-1-methyl-5-nitroimidazole (Ipronidazole)

2-Isopropyl-4(5)-nitroimidazole (595mg, 3.52mmol) was methylated with diazomethane [600mg in diethyl ether (100ml)] using the same procedure as described in chapter 2, part C. Evaporation of the diethyl ether afforded a crude product which was subjected to column chromatography on alumina with pet-ether:EtOAc (2:1) as eluent. Recrystallisation of the residue from the first fraction from pet-ether 40-60°C afforded prism shaped crystals of 2-(isopropyl)-1-methyl-5-nitroimidazole (387mg, 60%); m.p. 60-61°C; [lit. 166 m.p. 62°C]

H (CDC13) 1.35 (6H, d, C(Me)2), 2.92-3.33 (lH, m, -C(Me)2-H), 3.94 (3H, s, N-Me) and 7.81 (1H, s, C4-H); C (CDC13) 20.720 (2C, q, £!e 2), 26.734 (1C, d, C(Me)2-H), 32.748 (1C, q, N-Me), 132.580 (1C, d, C4-H), 139.140 (1C-brs, C5-NO2) and 158.166 ppm (1C, s, C2-H).

The 4-nitro isomer (49mg, 8%) was also obtained from the column chromatography.

36. The attempted preparation of 1-methyl-2-(1-methyl-1-bromo ethyl)-5-nitroimidazole

Dry carbon tetrachloride (25ml) was pipetted into a dry 3-necked round bottomed flask equipped with a reflux condenser, a magnetic stirrer bar, and a silica gel drying tube connected on to the condenser. N-Bromosuccinimide (111mg, 0.74mmol), calcium carbonate (20mg) and azoisobisbutyronitrile (AIBN)(15mg) were added to the flask. Ipronidazole (125mg, 0.74mmol) in carbon tetrachloride (5ml) was then added and the reaction was refluxed for 15h.

The precipitate was filtered and the filtrate evaporated to dryness in vacuo. The crude mixture was separated on alumina preparative t.l.c. plates with pet-ether:EtOAc (8:1) as eluent. Analysis using H n.m.r. spectroscopy of the fast running fraction showed it to be the required product with trace impurities (10mg), and that the second band was mainly unchanged starting material (46mg, 37%).

Several attempts were made to improve the yield of the reaction. The best method was when N-bromosuccinimide (2.0g, 11.27mmol, 1.2mol equivalents) was added portionwise to the refluxing solution of dibenzoyl peroxide (375mg, 1.55mmol) (in place of AIBN) and ipronidazole (1.7g, 10.1mmol) in dry CCl4 (150ml). The reaction was irradiated with 2x150 watts
fluorescent lamps from a distance of 10 cm and stirred under a stream of nitrogen for 8 h.

The reaction mixture was worked up as described above. The crude mixture was separated into three fractions using flash silica column chromatography with CCl₄:EtOAc:Pet-ether (1:0.5:2) as the eluting solvent. ¹H n.m.r. spectroscopic analysis showed that the first fraction consisted of unidentified impurities. Fraction 2 (243 mg): δH (CDCl₃) 2.44 (3H, s, -C-Me), 4.28 (3H, s, N-Me), 4.30 (2H, Abq, JHH ~ 8.8), and 7.86 (1H, s, C₄-H), could not be crystallised.

Fraction 3 consisted of the required product (442 mg, 18%): δH (CDCl₃) 2.24 (6H, s, C-Me₂), 4.19 (3H, s, N-Me) and 7.8 ppm (1H, s, C₄-H); m/z 168 [M⁺ - 79(81)] (100%) 122(31), 121(23).

37. Reaction between 1-methyl-2-(1-methyl-1-bromoethyl)-5-nitroimidazole and the sodium salt of 2-thiopyridine

Clean sodium hydride (21.6 mg, 0.75 mmol) was suspended in dry DMF (15 ml) in a 3-necked round bottomed flask fitted with a nitrogen inlet and a magnetic stirrer bar. 2-Thiopyridine (60 mg, 0.5 mmol) was added to the mixture under an atmosphere of nitrogen and stirred until it dissolved. 1-Methyl-2-(1-methyl-1-bromoethyl)-5-nitroimidazole was added to the yellow solution. The reaction mixture was irradiated with 2x150W fluorescent lamps. An aliquot was withdrawn after 20 min, and the DMF was removed in vacuo. The residue was dissolved in water and extracted with CHCl₃. The CHCl₃ extracts were washed with water, dried (MgSO₄), and evaporated to dryness in vacuo. Analysis using ¹H n.m.r. spectroscopy and t.l.c. showed starting material and the expected product.

The reaction was terminated after 4 h. Similar work up and analysis showed an impure mixture of compounds. The crude mixture was leached with pet-ether and evaporation of the ether gave 2,2¹-di-pyridyl disulphide (10 mg, 17%).

It was not possible to separate other products or the starting material from the crude mixture.

A duplicate run was carried out for 30min. The ¹H n.m.r. spectrum of the crude product (20 mg) showed mainly the disulphide and unchanged starting material. The reaction was not investigated further.
38. **Reactions of 1-methyl-5-nitroimidazole:**

a) **With 2,2-dinitropropane**

1-Methyl-5-nitroimidazole (250mg, 1.97mmol) and 2,2-dinitropropane (36mg, 2.36mmol) were stirred together in dry DMF (25ml) under an atmosphere of nitrogen for 30min. Clean sodium hydride (70mg, 2.95mmol) was added to the colourless reaction mixture. The yellow solution was irradiated with 2x150W fluorescent lamps as described in the general procedure for S$_{RN1}$ reactions (chapter 2, part C).

Evaporation of the ether layer to dryness in vacuo after the normal work up gave a crude product (60mg); $\delta$H (CDCl$_3$), 1.76 (s) and 2.18 (s). The $^1$H n.m.r. $\delta$-values were identical to those of 2,3-dimethyl-2,3-dinitrobutane and 2,2-dinitropropane.

The aqueous layer was evaporated to dryness in vacuo. The residue was dissolved in a minimum amount of water and extracted with CHCl$_3$. The solvent was removed in vacuo to give unchanged starting material (the imidazole) (120mg, 48%). The $^1$H n.m.r. spectrum and t.i.c. was identical to that of the authentic sample.

b) **With 2-bromo-2-nitropropane**

2-Bromo-2-nitropropane (493mg, 2.93mmol) was reacted with 1-methyl-5-nitroimidazole (372mg, 2.93mmol) as described above. The % yield of the products in the crude mixture from the ether layer was calculated using $^1$H n.m.r. spectroscopy to give 2,3-dimethyl-2,3-dinitrobutane (29%) and unchanged 2-bromo-2-nitropropane (25%). Treating the aqueous layer as described above gave unreacted imidazole (63%).

39. **General procedure for the reaction between 2-substituted 1,2-dimethyl-5-nitroimidazole and the anions of 2-nitropropane, and thiolates.**

Distilled solvent* (20ml) was pipetted into a 3-necked round bottomed flask equipped with a magnetic stirrer bar. The solvent was de-oxygenated by passing a stream of nitrogen through for 30min. The sodium salt of the thiol (or the thiol and an equivalent of sodium hydroxide), or the salt of 2-nitropropane, was introduced into the flask and

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* Solvent = Dipolar aprotic solvent, DMSO or polar solvent MeOH:H$_2$O (3:1)
stirring was commenced. After 15 min an equimolar amount of \( \alpha \)-substituted 1,2-dimethyl-5-nitroimidazolyl was added and the reaction mixture illuminated with two 150W fluorescent lamps.

After the determined time, the reaction mixture was poured into ice-water (50 ml) and extracted into dichloromethane (4x20 ml). The combined dichloromethane (CH\(_2\)Cl\(_2\)) layers were washed with distilled water and dried over MgSO\(_4\). Removal of solvent in vacuo yielded the crude product. The unreacted thiolate or the anion of 2-nitropropane remained in the aqueous extracts.

40. Reactions of 2-(Chloromethyl)-1-methyl-5-nitroimidazole

The following reactions were carried out using the general procedure described above. The quantities of starting materials, points of note, separation procedures and analytical data are presented.

a) With the sodium salt of phenylthiol

The sodium salt of phenylthiol (187 mg, 1.42 mmol) and 2-(chloromethyl)-1-methyl-5-nitroimidazole (250 mg, 1.42 mmol) were reacted using the standard procedure in DMSO. A deep violet colour developed which changed to brown after 1 h. Normal work up of the reaction mixture after 4 h and re-crystallisation from EtOAc/pet-ether (60-80°C) gave (1-methyl-5-nitroimidazol-2-yl)-methyl phenyl sulphide (226 mg, 64%); m.p. 109-110°C; \( \delta \)\(_H\) (CDCl\(_3\)) 4.17 (2H, s, -CH\(_2\)-), 3.87 (3H, s, N-CH\(_3\)), 7.27 (5H, ABq arom.), 7.83 (1H, s, C\(_4\)-H); m/z [Found: M\(^+\) 249.0575 (86%). C\(_{11}\)H\(_{11}\)N\(_3\)O\(_2\)S requires 249.0572], 203(41), 140(100), 67(22).

A similar reaction was carried out in MeOH:H\(_2\)O (3:1) for 1 h to give the identical product (240 mg, 68%).

Inhibition studies were carried out on the above reaction in MeOH:Water (3:1). The crude reaction mixtures were analysed using \(^1\)H n.m.r. spectroscopy with an internal standard of p-dimethoxybenzene and the results are shown in Table 3.1. The standard reactions using the general procedure were carried out for 10, 30 and 60 min. The 10 min reaction was found to be most satisfactory and was used as the standard time for the inhibition studies.
i) Exclusion of light and under an atmosphere of oxygen; the reaction was carried out as described for the standard reaction except that the flask was wrapped in aluminium foil and nitrogen was replaced by oxygen.

ii) Inhibition studies with p-dinitrobenzene or di-t-butyl nitrooxide were carried out by adding the required amounts of p-dinitrobenzene or di-t-butyl nitrooxide to the reaction mixture immediately prior to 2-(chloromethyl)-1-methyl-5-nitroimidazole. A duplicate run gave similar results.

b) With the sodium salt of 2-mercaptopyrimidine

   i) Using the standard method (expt.39), freshly prepared sodium salt of 2-mercaptopyrimidine (190mg, 1.42mmol) was reacted with 2-(chloromethyl)-1-methyl-5-nitroimidazole (250mg, 1.42mmol) in DMSO for 1h.

   Normal work up resulted in a crude product which was recrystallised from ethyl acetate/pet-ether (60-80°C) to give (1-methyl-5-nitroimidazol-2-yl)-methyl pyrimid-2-yl sulphide (256mg, 63%) m.p. 142-144°C.

   $\delta_H$ (CDCl$_3$) 8.47 (2H, d, N-C-H), 7.85 (1H, s, C$_4$-H), 6.9 (1H, t, N-C-C-H), 4.45 (2H, s, -CH$_2$) and 4.02 (3H, s, N-CH$_3$); m/z [Found: M$^+$ 251.0473 (5.2%). C$_9$H$_9$N$_5$O$_2$S requires 251.0477], 158(18).

ii. The reaction was repeated in MeOH:H$_2$O (3:1). The same product (280mg, 78%); m.p. 143-144°C, was obtained.

   The aqueous layer was adjusted to pH 1 and extracted with dichloromethane (2x20ml). The combined CH$_2$Cl$_2$ layers were washed with distilled water (2x20ml), dried (MgSO$_4$), and the solvent removed in vacuo to afford unreacted 2-mercaptopyrimidine (9.5mg, 6%). The $^1$H n.m.r. spectrum was identical to that obtained from an authentic sample.

   Inhibition studies were carried out as in Expt.40: (a) in the presence of 50molar % di-t-butyl nitrooxide, (b) in the absence of light and under an atmosphere of oxygen. The results are reported in Table 3.2.

c) With the sodium salt of 2-mercaptopyridine

   The sodium salt of 2-mercaptopyridine (189mg, 1.42mmol) and 2-(chloromethyl)-1-methyl-5-nitroimidazole (250mg, 1.42mmol) were reacted by the standard method for 90min.
The crude product, obtained from work up, was recrystallised from methanol to give (1-methyl-5-nitroimidazol-2-yl)-methyl pyrid-2-yl sulphide (132mg, 74%); $\delta_H$ (CDCl$_3$) 4.08 (3H, s, N-CH$_3$), 4.57 (2H, s, -CH$_2$-), 7.55-7.69 (3H, m, pyridine H), 7.94 (1H, s, C$_4$-H) and 8.45 (1H, dd, N-CH). A duplicate run gave (1-methyl-5-nitroimidazol-2-yl)-methyl pyrid-2-yl sulphide (56%).

d) With the sodium salt of cysteine

The sodium salt of cysteine (122mg, 0.855mmol, 1.2mol equiv.) and 2-(chloromethyl)-1-methyl-5-nitroimidazole (125mg, 0.71mmol) were reacted together as described in the standard reaction above. The reaction was monitored by t.l.c. The t.l.c. plates were developed in nbutanol:acetic acid:H$_2$O (60:15:25) and sprayed with ninhydrin solution. The t.l.c. of the reaction mixture after 3h consisted of 3 spots, two of which were unreacted starting materials, and possibly a new product. The reaction was terminated after 30h when all the cysteine had been consumed (no spot on t.l.c.).

The $^1$H n.m.r. spectroscopic analysis of the crude mixture showed the presence of a possible product and other unidentifiable impurities. Attempted separation of the crude mixture using preparative t.l.c. on silica plates with n-butanol:acetic acid:water (60:15:25) as eluent was not successful.

41. Reactions of Ronidazole

a) With the sodium salt of phenylthiol

The sodium salt of phenylthiol (93.7mg, 0.71mmol) was reacted with ronidazole (142mg,0.71mmol) in MeOH:H$_2$O (3:1) as described in the standard procedure for 10min. $^1$H n.m.r. spectroscopic analysis of the crude mixture after work up indicated unreacted ronidazole (139mg, 98%). The aqueous layer was acidified to pH 1 and extracted with CH$_2$Cl$_2$. Evaporation of solvent in vacuo gave unreacted thiol (40mg, 52%).

The reaction was repeated for 1h. The $^1$H n.m.r. spectrum of the crude mixture showed unreacted ronidazole and diphenyl disulphide. In order to confirm the presence of diphenyl disulphide, the crude mixture was leached with pet-ether (40-60°C) leaving unreacted ronidazole (59mg,
Evaporation of the pet-ether to dryness in vacuo and recrystallisation from ethanol gave diphenyl disulphide (35 mg, 45%); m.p. 59-61°C [lit. 58-60°C] \( \delta^H (\text{CDCl}_3) 7.17 \) (m). The reaction was repeated for 1 h using 2 molar equivalents of the sodium salt of phenylthiol. \(^1\text{H} \) n.m.r. spectroscopic analysis of the crude mixture showed the presence of ronidazole, a new product with a proton signal at \( \delta^H = 4.70 \), (2-hydroxymethyl)-1-methyl-5-nitroimidazole, and diphenyl disulphide. The aqueous solution was acidified to pH 1, and following the procedure as above, phenylthiol (69 mg, 44%) was obtained.

The reaction between molar equivalents of ronidazole and phenyl thiolate was repeated for 5 h using the procedure above. The crude product was leached with pet-ether to yield the disulphide (38.7 mg, 50%). \(^1\text{H} \) n.m.r. spectroscopic analysis of the residue indicated the presence of only 2-(hydroxymethyl)-1-methyl-5-nitroimidazole. Recrystallisation of the crude product from toluene afforded the imidazole (48 mg, 43%); m.p. 117-119°C (authentic sample m.p. 118-119°C); \( \delta^H (\text{CDCl}_3) 4.0 \) (3H, s, N-Me), 4.70 (2H, d, \(-\text{CH}_2-\)), 5.6 (1H, brs, \(-\text{CH}_2\text{-OH}\)) and 7.80 (1H, s, \( \text{C}_4\text{-H}\)); \( m/z \) [Found: \( \text{M}^+ 157.0492 \) (98%), \( \text{C}_5\text{H}_7\text{N}_3\text{O}_3 \) requires 157.0487], 140(5), 127(23), 111(43), 81(100).

A repeat reaction for 12 h afforded after recrystallisation, 2-(hydroxymethyl)-1-methyl-5-nitroimidazole (49 mg, 44%) and diphenyl disulphide (48 mg, 62%).

Inhibition studies were carried out; (a) in the absence of light by wrapping the flask in aluminium foil, and (b) in the absence of light and under an atmosphere of oxygen for 1 h and 5 h using the standard procedure above except that 2 mol equivalent of phenyl thiolate was used. The results are presented in Table 3.3.

b) With sodium salt of phenylthiol in the presence of a reducing agent, sodium dithionite

The sodium salt of phenylthiol (2 mol equiv.) was stirred in MeOH: \( \text{H}_2\text{O} \) (3:1) under nitrogen until dissolved. Ronidazole (142 mg, 0.71 mmol) and sodium dithionite (4 mol equiv.) were added simultaneously to the stirred solution. The reaction was irradiated as previously described and stirred for 18 h.
After the normal work up, the \(^1\)H n.m.r. spectrum of the crude mixture (130mg) indicated mainly diphenyl disulphide and traces of unidentified impurities.

c) With the sodium salt of 2-nitropropane

A reaction between ronidazole (142mg, 0.71mmol) and sodium salt of 2-nitropropane (158mg, 1.42mmol, 2mol equiv.) was carried out as described in the standard procedure for 1h but in the absence of light catalysis. The \(^1\)H n.m.r. spectrum of the crude mixture indicated unreacted ronidazole (88mg, 62%) and 2,3-dimethyl-2,3-dinitrobutane (7%). A similar mixture, plus a trace of the 2-hydroxymethyl imidazole, was obtained when the reaction was light catalysed for 12h.

42. Reactions of (1-methyl-5-nitroimidazol-2-yl)-methyl pyrid-2-yl sulphide

a) With the sodium salt of phenylthiol

Using the standard reaction, the sodium salt of phenylthiol (2mol.equiv.) was reacted with (1-methyl-5-nitroimidazol-2-yl)-methyl pyrid-2-yl sulphide (250mg, 1mmol) in MeOH:H\(_2\)O (3:1) for 6h. The crude mixture obtained was leached with pet-ether to leave unreacted starting material (104mg, 83%). Evaporation of the pet-ether gave diphenyl disulphide (49mg, 45%). Unreacted thiolphenyl (30mg, 27%) was obtained from the aqueous layer. Comparison of the \(^1\)H n.m.r. spectrum with previous samples confirmed identity of the compounds.

b) (i) With the sodium salt of 2-nitropropane

The sodium salt of 2-nitropropane (111mg, 1mmol) was reacted with (1-methyl-5-nitroimidazol-2-yl)-methyl pyrid-2-yl sulphide (250mg, 1mmol) in MeOH:H\(_2\)O (3:1) for 7h. The yields of products in the crude, were obtained using \(^1\)H n.m.r. spectroscopy and gave unreacted imidazole starting material (97%) and 2,3-dimethyl-2,3-dinitrobutane (17%).

(ii) The same reaction was carried out except that the reaction flask was wrapped in aluminium foil to exclude light. Analysis of the crude mixture using \(^1\)H n.m.r. spectroscopy and t.l.c. showed only unchanged nitroimidazole and no dimer of 2-nitropropane was obtained.

c) With the sodium salt of phenylthiol in the presence of sodium dithionite

The reaction in Expt.42a was repeated in the presence
of a reducing agent, sodium dithionite. The crude mixture (472mg) gave diphenyl disulphide as the main product, the other impurities could not be identified.

43. Reaction between 1,2-Dimethyl-5-nitroimidazole and phenyl thiolate

i) Using the standard reaction as described in expt.39, the sodium salt of phenylthiol (3mol equiv.) was reacted with 1,2-dimethyl-5-nitroimidazole (250mg, 1.77mmol) in MeOH:H₂O (3:1) for 6h. The crude mixture, obtained after work up, was leached with pet-ether (40-60°C) to leave a residue of unreacted starting material, 1,2-dimethyl-5-nitroimidazole (271mg, 99%). Evaporation of the pet-ether in vacuo gave diphenyl disulphide (1%). The products were identified by m.p. and ¹H n.m.r. spectroscopy. Phenylthiol (60%) was also recovered.

ii) A repeat reaction, carried out for 6h in the absence of light gave similar results and phenylthiol (70%) was also recovered. Likewise, when the reaction time was extended to 32h in the dark, the following yields were obtained using ¹H n.m.r. spectroscopy with an interval standard: 1,2-dimethyl-5-nitroimidazole (89%), diphenyl disulphide (7%). Phenylthiol (66%) was recovered from the aqueous layer.

44. Reaction between 1,2-dimethyl-5-nitroimidazole and phenylthiolate in deuterated agents

The sodium salt of phenylthiol (46mg, 0.356mmol, 4mol equiv.) and 1,2-dimethyl-5-nitroimidazole (12.5mg, 890umol) were reacted together in CD₃OD/D₂O (3:1) for 14 days in the dark in an n.m.r. tube. The reaction was followed by ¹H n.m.r. spectroscopy. The presence of unreacted 1,2-dimethyl-5-nitroimidazole and traces of disulphide was shown until the reaction was terminated. The reaction was not investigated further.
INTRODUCTION

The syntheses of nitroimidazole derivatives [50], [108] and [109] were discussed in Chapters 2 and 3, part A. The antimicrobial screening is now discussed.

\[
\begin{align*}
\text{N} & \text{CH}_2 \text{X} \\
\text{Me} & \text{[108]} \\
\end{align*}
\]

\[
\begin{align*}
\text{O}_2 \text{N} & \text{R} \\
\text{X} & \text{[50]} \\
\end{align*}
\]

Although the nitroimidazoles [50] are mostly 4-nitro derivatives, reported to have little or no biological activity\(^{13}\) in comparison with their 5-nitro analogues, they are novel, interesting compounds and were screened because biological activity of novel compounds should not be assumed until proven. Also, as mentioned earlier, these 4-nitroimidazoles are structural analogues of 2-substituted-2-nitropanes, e.g. bronopol which has been shown to possess activity against aerobic micro-organism.\(^{118b,120c}\)

The determination of the sensitivity of bacteria to antibiotics (or antimicrobial agents) in vitro has become a common laboratory procedure. The sensitivity is generally defined in terms of the concentration of the antibiotic which inhibits the growth of the chosen organism within the time limit, and under the conditions of the test. This concentration is usually referred to as the minimum inhibitory concentration (MIC).

Several methods of determining the MIC are in common use, but the results obtained for any given strain of organism by the different methods may show wide discrepancies,\(^{174}\) depending on the details of the method used and the choice of end-point. Consequently, comparisons of results obtained from different laboratories by the same method are not always justifiable, and the significance of the difference in the results obtained for the same or related strains may be uninterpretable. To avoid such problems of comparison of results from the present study with literature values, metronidazole, dimetridazole and some known nitroimidazoles were included in these assays as standards or control com-
pounds. Two methods to determine bacterial susceptibility to antimicrobial agents (MIC) are employed.

The first method involved the diffusion of the antimicrobial agent into a solid medium (seeded with the test organism) from a disc placed on the agar surface, or from a 'well' cut into the agar. This method is known as the agar diffusion method. The second employed visual observation of growth of the organism in broth containing serial dilution of the antimicrobial agent. This is the tube dilution method. The former method is economical in time and materials, and is particularly favoured for the present study because most of the novel nitroimidazoles are obtained only in small quantity and of varying water solubility. For the agar diffusion method, the inoculum could either be applied to the surface of solidified agar plate (surface culture method) or distributed well into the molten agar before plating (seeded agar method). The latter method was employed because it is more appropriate for comparative study of new compounds in order to obtain clear definition of the inhibition zones. The antimicrobial agent is applied to the surface of the agar using either the disc (agar disc diffusion method, ADD) or the well (agar well diffusion method, AWD). In the former method, the amount of drug contained by each disc depends on the volume retained by the disc after dipping and draining off excess fluid, while in the latter, a constant, relatively large amount of drug is introduced into the well, though volume of drug is not important, it does provide a constant reservoir for diffusion.

In both cases, the size of the zone of inhibition depends on two dynamic factors: the rate of diffusion of drug from the reservoir into the medium, and the rate of growth of the micro-organism. A zone of inhibition around the well or disc results because an interface exists beyond which organisms had reached visible growth proportions before inhibition occurred, and before which the organism was overwhelmed before visible growth was achieved. The size of the inoculum used in test plates is an important factor influencing the size of the zone of inhibition. Other variables include the time of diffusion of antimicrobial agents prior to commencing incubation and the time of read-
ing the zone of inhibition. This is of particular significance with unstable antimicrobials.

Despite the advantages of the agar diffusion method, the traditional method of investigating the effect of antimicrobial agents on specific organisms involves incorporation of the antimicrobial agent directly into liquid growth media \(^{175}\) (Tube dilution method). Technical and biological variables such as size of inoculum, growth phase affecting the diffusion method also apply. However, there is no problem of drug diffusion, as the drug is in constant environment of the organism. Another advantage of this method is that the bactericidal effect of the drug on the organism can be evaluated by attempting drug neutralisation (usually by dilution) whilst incubating.

In the current study, the activity of nitroimidazoles \(^{50}, {108}\), and diazole derivatives was studied relative to a range of Gram positive and Gram negative aerobic and anaerobic bacteria and against selected filamentous and cellular fungi.

Knight et al. adduced from the decrease in viscosity flow time and denaturation of DNA that reduced nitroimidazoles such as metronidazole destabilised DNA by strand separation, strand breakage of the helix coil of DNA \(^{176}\) (see Chapter 1).

One approach to the study of reduction products is to measure the half-wave potential (see also Chapter 1). The half-wave potential of most of the synthesised nitroimidazoles were measured (except where prevented due to lack of solubility). Electrolytic reduction of metronidazole and 1-(1,2-dimethyl-5-nitroimidazol-2-yl)-2-nitroimidazole \(^{70}\) were carried out in the presence of DNA.
PART A
MATERIALS AND METHODS
4.1 Materials
4.1.1 Organisms
Bacteria
Aerobes
- *Bacillus cereus* LUT 11755
- *Escherichia coli* NCTC 9001
- *Pseudomonas aeruginosa* NCIB 6749
Anaerobes
- *Clostridium sporogenes* NCIB 532
- *Cl. histolyticum* NCIB 503
- *Bacteroides fragilis* NCTC 8560
Fungi
- *Candida albicans* ATCC 10231
- *Aspergillus niger* ATCC 16404
4.1.2 Media
Nutrient Broth (NB)
For maintenance of cultures of bacteria
- Beef extracts 10g
- Neutralised bacteriological peptone 10g
- Sodium chloride 5g
- Distilled water 1 litre
Where appropriate, nutrient broth was solidified by the addition of agar (1.5% w/v; Oxoid technical grade) to give nutrient agar (NA).
Neurospora Broth
For maintenance of cultures of fungi
- Maltose 38.0g
- Yeast extracts 2.5g
- Mycological peptone 8.0 g
- Malt extracts 2.0g
pH adjusted to 5.0.
When neurospora agar was required, agar (20% w/v; Oxoid technical grade) was added to the broth.
Tryptone Tellurite Agar
To isolate pure yeast cultures from a contaminated culture of C. albicans
- Tryptone 10g
- Mycological peptone 10g
Sodium chloride 5g
Glucose 2g
Agar 20g
Distilled water 1 litre
pH 7.5.

This medium was sterilized at 121°C for 15 minutes at 15 lb pressure. When cooled to 50°C, 50ml of sterile horse serum, and 10ml of 1% potassium tellurite solution (pH 9-9.5) were added. Plates were poured and were usable for 2 weeks when stored at 4°C.

**Cooked meat medium**

General purpose medium for anaerobes

Heart muscle 454g
Peptone 10g
'Lab-lemco' powder 10g
Sodium chloride 5g
Dextrose 2g
Distilled water 1 litre

Adjusted to pH 7.2

**Chemically defined medium (CDM)**

Na$_2$HPO$_4$ 7.1g
KH$_2$PO$_4$ 1.3g
MgSO$_4$·7H$_2$O 0.25g/100ml
FeSO$_4$ 0.1g/100ml (use 1ml)
MnSO$_4$·4H$_2$O 0.1g/100ml (use 1ml)
Casamino acids 1g/190ml
Thymine 10ug/ml
Glucose 5g/l (use 400ml)

Final pH 6.8

These reagents were dissolved in required amount of distilled water and autoclaved individually. The cooled solutions were mixed aseptically. Thymine and glucose were sterilised by membrane filtration as concentrated aqueous solutions and added aseptically as required.

**Glucose free medium (gfm)**

Medium CDM without added glucose

**Winge's medium**

For maintenance of yeast cells of *C. albicans*

Glucose 20g
Yeast extracts 3g
Distilled water 1 litre
Winge solid agar was obtained by adding agar (1.5 w/v; Oxoid technical agar).

**Clostridium reinforced broth**
- Clostridium reinforced medium (Oxoid) and (lab m) 38.0g
- Distilled water 1 litre

When necessary this medium was solidified by the addition of agar (1.5% w/v; Oxoid technical agar) to give clostridium reinforced agar.

**Wilkins-Chalgren anaerobe agar**

This medium was used for the culture of moderate to fastidious anaerobes such as Bacteroides species.

**Basal medium:**
- Wilkins-Chalgren anaerobe agar (Oxoid) 43g
- Distilled water 1 litre

**Final medium:**
- Basal medium (sterile) 1 litre
- Vitamin K solution 10ml
- Horse blood 5% v/v

**Solvent:**
- Acetone:sterile distilled water (70:30) was used to make up solutions to be tested against the aerobes and dimethyl sulphoxide (DMSO): sterile distilled water (10:90) for the solutions to be tested against the anaerobes.

**Glassware**

All glassware was washed thoroughly and sterilized by autoclaving before use. Pipettes were sterilized by dry heat at 160°C for 120 minutes.

**Sterilisation of Media** Except where stated otherwise, media were sterilized by autoclaving at 121°C for 15min.
Structures and Names of Nitroimidazoles synthesised and screened

1,2-Dimethyl-5-nitroimidazole [2]

2-(Hydroxymethyl)-1-methyl-5-nitroimidazole [112]

2-(Chloromethyl)-1-methyl-5-nitroimidazole [60]

2-(Bromomethyl)-1-methyl-5-nitroimidazole [59]

1-Methyl-5-nitroimidazol-2-yl-methyl N,N,N-trimethyl methamium chloride [116]

(1-Methyl-5-nitroimidazol-2-yl)dimethyl octylammonium chloride [117]

Ipronidazole [4]

1-Methyl-2-(1-methyl-1-bromoethyl)-5-nitroimidazole [128]

1-Methyl-2-methylsulphonyl-5-nitroimidazole [126]

(1-Methyl-5-nitroimidazol-2-yl) methylphenyl sulphide [119]
(1-Methyl-5-nitroimidazol-2-yl)methyl pyrid-2-yl sulphide [120]

Ronidazole [3]

Azomycin [30]

Metronidazole [1]
(Gift from May and Baker)

1-(1-Methyl-1-nitroethyl)-2-methyl-4-nitroimidazole [63]

1-(1-Methyl-1-nitroethyl)-4-nitroimidazole [62]

1-Methyl-5-nitroimidazole [111]

1,2-Dimethyl-4-nitroimidazole [30]

1-(p-Nitrobenzyl)-2-methyl-4-nitroimidazole [66]
5-(4-Nitroimidazol-1-yl) 5-nitro-1,3-dioxane [64]

1-(1-Methyl-1-nitroethyl)-6-nitroisoindazole [96]

1-(1-Methyl-1-nitroethyl)-5-nitro-benzimidazole [91]

1-(1-Methyl-1-nitroethyl)-6-nitro-benzimidazole [92]

1-(1-Methyl-1-nitroethyl)-5-nitro-1,3-dioxane [64]

1-(1-Methyl-1-nitroethyl)-6-nitroisoindazole [96]

1-(1-Methyl-1-nitroethyl) benzimidazole [89]

1-(1-Methyl-1-nitroethyl)-5-nitro-benzimidazole [91]

1-(p-Nitrobenzyl)-nitroimidazole [65]

1-(p-Nitrobenzyl)-2-nitroimidazole [69]

1-(1,2-Dimethyl-5-nitroimidazol-2-yl) 2-nitroimidazole [70]

1-(1,2-Dimethyl-5-nitroimidazol-2-yl)-4-nitroimidazole [67]

1-(1-Methyl-1-nitroethyl)-6-nitro-benzimidazole [92]
2,2-(Di-5-nitroindazol-1-yl)-propane [93]

2,2-(Di-6-nitroindazol-1-yl)-propane [95]
4.2 METHODS

4.2.1 Maintenance of Organisms

(a) Aerobic bacteria

Master cultures of each organism were maintained by monthly subculture on nutrient agar slopes. Following subculture slopes were incubated at 37°C for 24h, after which two slopes for each organism were kept at 2-4°C in the dark as Master cultures. The rest, as working slopes, were used to provide a regular source of experimental cultures and were kept at room temperature (dark). Master and working cultures were checked for purity when subcultured to ensure strain stability during testing.

(b) Fungi

Master cultures were maintained by fourteen day subculture on Neurospora agar slopes in universal bottles. Following subculture slopes were incubated at 30°C for 48h and then stored as for bacteria above. The organisms were stained and observed microscopically to check the purity. For C. albicans, in addition to above, cultures were occasionally plated out onto Tryptone Tellurite Agar and the isolated colonies subcultured again onto Tellurite agar. Isolated colonies from this were finally subcultured onto Neurospora agar slopes for maintenance as before.

(c) Anaerobes

Clostridium species were maintained by 'deep subculture' into Clostridium Reinforced Broth at monthly intervals. Such cultures were incubated under anaerobic conditions for 48h at 37°C and then maintained at 2-4°C in the dark. Bacteroides fragilis was maintained by fourteen day subculture on solid blood agar plates and deep subculture into cooked meat medium. Plates/broths were incubated anaerobically for 48h at 37°C followed by storage under anaerobic condition at 2-4°C. All organisms were checked for purity by Gram stain and examination of colony morphology before subcultures were made and before use.

Anaerobic conditions suitable for the growth of these organisms is provided and maintained in an anaerobic jar which consists of the following:

\[ \text{H}_2/\text{CO}_2 \text{ from cylinder (flushing system) (BOC Ltd.)} \]

\[ \text{H}_2 \text{ from hydrogen paks (Oxoid)} \]
Indicator paper (Oxoid)

Palladium catalyst (regenerated after use).

The prepared agar and broth cultures were placed in the anaerobic jar (BBL) followed by two hydrogen paks (Oxoid), palladium catalysts, and an indicator paper. The anaerobic jar was then evacuated using a vacuum pump, and a gentle stream of $H_2/CO_2$ was allowed to pass through for a few minutes. The jar was then tightly sealed and incubated.

4.2.3 Preparation of Standard Inoculum for testing

(a) For aerobes

A mass of cells equivalent to about three colonies was selected from a 24h bacteria or fungi agar plate or slope, or $0.1\text{ml}$ broth culture. This was used to inoculate $100\text{ml}$ chemically defined medium (CDM) in a sterile conical flask which was incubated at $37^\circ C$ for 18h (48h for fungi at $30^\circ C$). Half of the cell suspension was discarded and fresh sterile medium added aseptically. The flask was then placed in a stationary water bath incubator at $37^\circ C$ ($30^\circ C$ for fungi) for 4 h. The cells were harvested by centrifugation ($5000$ rpm, MSE mistral 6L) at $4^\circ C$ for 15min, and the pellet of cells washed twice and finally were re-suspended in glucose free medium (gfm) to give an O.D. reading of 0.2 ($420\text{nm}$; Unican SP 500 spectrophotometer). A $1\times10^4$ dilution of this cell suspension was prepared. Using the Miles and Mistral method (1938), the surface counting of suspension gave approximately $1\times10^6$ cells/ml. of the original cell suspension for both bacteria and fungi.

For subsequent work, the pellet obtained after centrifuging was diluted with gfm to give an approximate O.D. reading of 0.2 and then used for assay. The standardized inoculum is used both for diffusion and tube dilution assays.

(b) For anaerobes

The standard inoculum used for the agar diffusion assays was a 48h broth culture of the anaerobe. For the tube dilution assay, 5ml of the 48h broth was adjusted to 0.5 McFarland standard turbidity using fresh sterilized clostridium reinforced medium for the clostridium species and nutrient broth for B. fragilis. The turbidity standard was prepared by diluting $0.5\text{ml}$ of $0.048\text{M}$ barium chloride ($1.175\%$ w/v $\text{BaCl}_2\cdot2\text{H}_2\text{O}$) with $99.5\text{ml}$ of $0.36\text{N}$ sulphuric acid
The solution was kept sealed in the dark at room temperature. The adjusted inoculum contained a total count equivalent to $10^8$ cells/ml. This was counter-checked by method of Miles and Mistra; the surface viable count after 48h incubation was $3 \times 10^7$ cells/ml.

4.2.4 Preparation of Antimicrobial Solutions

A high concentration of the antimicrobial agent was prepared in acetone:water (70:30 for aerobes) and DMSO:water (10:90 for anaerobes) and diluted by 2-fold serial dilution with the appropriate solvent mixture to give a range of solutions for testing. The stock solution was stored at 2-4°C and diluted to use accordingly. The range of concentrations used for the tube dilution assay was predetermined by the results from the minimum zone of inhibition obtained from the agar diffusion assay. The solvent mixture used for both anaerobe and aerobe did not inhibit the growth of the organism.

4.2.5 Inhibition of Microbial Growth

(a) Methods based on drug diffusion in agar

7.5ml of the standard inoculum was added to 500ml of molten medium maintained at 50°C and mixed. Aliquots (25ml) were introduced aseptically into 9cm sterile plates resting on a level surface and allowed to solidify. For the anaerobes, the plates, after solidifying were pre-reduced under anaerobic conditions in an anaerobic jar for 2h at room temperature. For the aerobes, the plates were pre-dried in the incubator for 30min.

i) Agar Disc Diffusion method (ADD)

Four sterile antibiotic assay discs (6mm, oxoid) were dipped into four different concentrations of the antimicrobial solution, excess solution was removed by pressing the disc to the edge of the tube, and the discs were firmly applied as 2 x 2 test discs on to the surface of the seeded agar plate. A positive control containing no antimicrobial solution, but the appropriate solvent used in making up the solution, was set up as part of the 2 x 2 discs. Four replicate plates were similarly prepared. For aerobes, the applied chemicals were allowed to prediffuse for 2h at room temperature before incubation at 37°C for bacteria or 30°C for fungi. The plates containing the anaerobes were incubated directly after preparation.
The diameter of zones of inhibition of each test organism were measured using vernier calipers.

ii) Agar Well Diffusion method (AWD)

Instead of the disc antibiotic assay used in ADD method, four evenly spaced wells were aseptically cut out of the seeded agar with a No.5 (6mm) cork borer. Using an Eppendorf pipette (V.A. Howe Ltd.), 100\(\mu\)l of four different sterile antimicrobial concentrations was introduced into each well. A positive control (solvent system only) was also included. Four replicate plates were similarly prepared. The plates were incubated and inhibition zone measured as in ADD method.

(b) Tube dilution assay

A doubling dilution series was made for each nitroimidazole and diazole [except a) when the nitroimidazole or diazole lacks solubility, or b) when the MIC by the agar diffusion method \(>2000\) \(\mu\)g/ml for the aerobe or \(>250\) \(\mu\)g/ml for the anaerobe] in nutrient (for bacteria) and neurospora broth (for \(\text{C. albicans}\)), and clostridium reinforced broth for clostridia species.

The dilutions were made in sterile test-tubes, with 2ml volumes in each. Each series of dilutions was made in triplicates. 0.1ml of the standardized inoculum was added to each tube. Appropriate controls were set up: a) containing no cells to check the sterility of the antimicrobial agents, b) containing no antimicrobial agent to check the viability of the organisms. The tubes were incubated at 37°C (30°C) for 24h (48h) under aerobic or anaerobic conditions. The MIC of the test substance was the lowest concentration which inhibited the growth of the organism used (medium remained clear).

(c) Minimum Bactericidal Concentration (MBC)

Following the results of the tube dilution assays above, subcultures were made from each tube showing no growth by transferring 0.01ml aliquot to and thoroughly streaking the surface of an appropriate agar plate (in triplicates). 0.01ml of 1:10\(^2\) and 1:10\(^3\) dilutions of the control tubes also similarly prepared.

The plates were incubated aerobically or anaerobically at 37°C, 24h (48h) for bacteria and 30°C, 48h for the yeast.
as described previously. The MBC is read as the lowest concentration of drug which showed no colonial growth on the agar plates.

4.2.6 Extended Diffusion Experiments

a) **Effect of Aerobic and Anaerobic Conditions on the Action of some nitroimidazoles against E. coli and Ps. aeruginosa**

4.5ml of standard inoculum of E. coli and Ps. aeruginosa was each used to seed 300ml quantities of nutrient agar containing 5mg/ml potassium nitrate. The medium was distributed into plates and allowed to set. Different concentrations of the antimicrobial agents [70] and [69] were applied to the seeded agar using the AWD method. Half of the plates were incubated aerobically and the other half anaerobically at 37°C for 18h. The mean diameter of the zones of inhibition were compared for both sets of plates.

**Further Investigation of the Sensitivity of C. albicans to 2-hydroxymethyl-1-methyl-5-nitroimidazole [112]**

In the agar diffusion method, an unusual zone of inhibition was observed for C. albicans relative to the hydroxy drugs [112], the zone consisted of 'white or colourless cells (W-type cells) on a bed of black cells' (B-type cells).

Re-incubation for further 24h showed dark but clear zones of inhibition. This was investigated further as follows:

b) **Re-examination of cells around the zone of inhibition of C. albicans after initial drug [112] treatment**

A section of agar was cut across the edge of the well of a 48h grown C. albicans which contained 5mg/ml and 20mg/ml hydroxy drug [112]. The section was transferred onto a sterile glass slide. Samples were obtained aseptically from the top- and under- surface of the section and treated as follows: Each type of sample was used to inoculate i) fresh agar plates with and without 5mg/ml hydroxy drug [112] by the stab technique. ii) neurospora broth with and without 5ml of 5mg/ml hydroxy drug [112].

The plates and tubes were incubated at 30°C for 48h. A similar set was incubated at 37°C. As a control, cells away from the zones of inhibition and around 'control well' were similarly investigated.
c) **Gradient plate technique**

Into a tilted square petri-dish (about 10° from the horizontal) was introduced molten neurospora agar containing 5mg/ml hydroxy drug [112] and allowed to gel. The same volume of molten agar was spread over the set agar with plate now horizontally and allowed to set. The plate was dried in the incubator and allowed to pre-diffuse for 2h at room temperature. The surface of the agar was then flooded with standardized **C. albicans** (O.D. = 0.2) and excess removed with a sterile pasteur pipette. The plates were incubated inverted at 30°C for 48h and the pattern of growth observed.

d) **Qualitative determination of the interaction between 2-(hydroxymethyl)-1-methyl-5-nitroimidazole [112] and some related compounds**

The method reviewed by Maccacaro (1961) in the investigation of synergistic and antagonistic interactions between any two compounds was adapted and utilized. Two wells of diameter (8mm) using a standard cork borer were cut into neurospora agar plates about 5cm apart. 100µl of each of the two solutions were placed into each well. After incubation, the shape of the zones of inhibition were compared. The standard compound used in all was hydroxy drug [112], the other compounds were imidazole, L-cysteine and N-acetylglucosamine.

4.2.7 **Microscopy**

a) **Light Microscopy**

Routine examination of cultures was carried out using a light microscope (Jena, Zeiss). Preparations were either examined by phase contrast microscopy or were dried, fixed and stained with Grams stain or Loeffler's methylene blue. Light micrographs were taken using FP4 film (Ilford).

From the unusual zone of inhibition obtained with **C. albicans** and the hydroxy drug [112] (see section 4.2.5) samples were 'teased out' of agar from the top - (white cells) and the under-surfaces (black cells) (of well containing 20mg/ml drug) and were first examined before and after staining. Light micrographs were taken using FP4 film (Ilford).
b) **Scanning Electron Microscopy (SEM)**

Cells were prepared for scanning electron microscopy by a method based on that of Bulman and Stretton. Glutaraldehyde (TAAB Laboratories Equipment Ltd.) was added to cultures of *C. albicans* to give final concentrations of 1.5% w/v. After 2 minutes contact, the cells were removed by centrifugation at 1,600g at 4°C for 15 minutes, and re-suspended in 2ml glutaraldehyde (5% w/v in 1/2 strength Ringer's solution for 16h at 4°C). The cells were then removed by centrifugation (1,600g for 15 minutes), washed twice with sterile distilled water, and re-suspended in water to give an O.D of 0.8-0.9 at 420nm. One drop of this was dried in air on a round coverslip (13mm diameter) then dehydrated over calcium chloride in a desiccator. Samples were glued onto metal stubs using fast-drying conductive silver paints, (Agar Aids, Stanstead) and left to allow the solvent to evaporate for at least 8h. They were then coated with gold palladium in a high vacuum sputter coating unit (Polaron Equipment Ltd.) to obtain a coating of 10nm thickness. Samples were viewed in an electron microscope (ISI.SS40). Photographs were taken using FP4 film (Ilford).

4.2.8 **Polarographic Reduction of Nitroimidazoles**

Polarography of 100μM of each antimicrobial agent (except where the antimicrobial is not soluble) in 0.2M Na₂HPO₄ and NaH₂PO₄ buffer pH 7.0 was carried out under O₂-free N₂ using a dropping Hg cathode (drop time 1s) and Ag/AgCl anode at 25°C. Voltage scanning was recorded from a Polarogram 500 (Polarrecord E Cord) and E½-values obtained from the trace.

a) **Preparation of DNA solution**

Calf-thymus (15mg) was dissolved in 15ml buffer (15mM NaCl, 1.5mM trisodium citrate, 1mM EDTA pH 7.2) with slow stirring over 30h at 4°C. A drop of dil. HCl was added to aid dissolution. The solution was dialysed for 65h at 4°C against 200ml of the buffer. The dialysed preparation used for all experimental work was diluted accordingly for each test drug, to give drug:nucleotide ratio of 1:1. The concentration of calf-thymus was assayed spectrophotometrically using $E_{260} = 6600M^{-1}cm^{-1}$. 
4.2.9 Electrolytic Reduction

Electrolytic reduction of metronidazole, nitroimidazoles [69] and [70], in the presence of DNA was carried out at 25°C under O₂-free N₂ at a constant voltage of -800mV ± 5mV using Ag/AgCl anode and a Hg pool cathode at an initial current of 20μA. The reduction vessel contained 100μM metronidazole,[69] or [70]), plus calf-thymus at a drug-nucleotide ratio of 1:1. Reduction of metronidazole was followed by a decrease in current to zero. 5ml aliquots were withdrawn from the vessel, before reduction, and subsequently at intervals during reduction to determine the extent of drug reduction by loss of absorbance at 318nm characteristic of its nitro group. The sample was then dialysed for flow time analysis. Reduction experiment was carried out for 3 days.

a) Viscometric analysis of DNA after reduction

Viscometry (flow time) measurements were carried out for dialysed aliquots of DNA, and DNA reduced in the presence of drug using an Ubbelohde-type miniature suspended level viscometer (BS/1P/SL.S/). Four replicate readings were obtained for each analysis.
4.3 RESULTS - PART B
4.3.1 Growth Inhibition

a) By the agar diffusion method

Results of screening synthesised nitroimidazoles, diazoles, and reference nitroimidazoles for possible activity against a selected range of aerobic and anaerobic organisms by the agar diffusion methods (ADD and AWD methods) are presented in Table 4.1. The anaerobes (Cl. sporogenes, Cl. histolyticum and B. fragilis) were more susceptible to these nitroimidazoles than the aerobes (B. cereus, E. coli, Ps. aeruginosa, A. niger and C. albicans). Of significance are the activities of 1- (p-nitrobenzyl) -2-nitroimidazole [69], 1-(1,2-dimethyl-5-nitroimidazol-2-yl) -2-nitroimidazole [70], 1-(1,2-dimethyl-5-nitroimidazol-2-yl) -4-nitroimidazole [67], 1-(p-nitrobenzyl) -2-methyl-4-nitroimidazole [66], 2-(hydroxymethyl)-l-methyl-5-nitroimidazole [112], bronidox derivative [64], and the 2-(halomethyl)-l- methyl-5-nitroimidazoles against the anaerobes (MIC from inhibitory zone diameter range 0.2-128μg/ml). Some of the MIC values, e.g. for nitroimidazole [70], (0.45μg/ml); [112], (0.2μg/ml) against Cl. sporogenes are comparable to the values obtained for some of the clinically used nitroimidazoles [e.g. metronidazole [1], (0.29μg/ml); dimetridazole [2], (0.26μg/ml)] while they are more active than others [e.g. Ipronidazole [4], (11.8μg/ml)]. 1-(p-Nitrobenzyl)-4-nitroimidazole [65] was found to be totally inactive against both the anaerobes and aerobes. Similarly, the diazoles showed no activity towards the test organisms (MIC by the agar diffusion method > 250μg/ml for the anaerobes and > 2000μg/ml for the aerobes).

Generally, of all the nitroimidazoles screened against aerobic bacteria, only the nitroimidazoles [112], [62], [63] and the 2-halo derivatives (the observed MIC range 181-1807μg/ml), had MICs from the agar diffusion methods less than 2000μg/ml.

A graphical representation of the influence of nitroimidazole concentrations on the growth of test organisms is presented in Fig. 4.1-4.4 for a few of the nitroimidazoles screened. The slopes of graph of the selected nitroimidazoles are similar for Cl. sporogenes and B. fragilis.
Table 4.1 *In vitro* activities of Nitroimidazoles against selected anaerobes and aerobes

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>MIC (µg/ml) by the Agar Diffusion (Well) Method</th>
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<tr>
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<td></td>
<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td><em>B. Cereus</em></td>
</tr>
<tr>
<td>2-Bromomethyl-1-methyl-5-nitroimidazole [59]</td>
<td>-</td>
<td>NO₂</td>
<td>Me</td>
<td>CH₂Br</td>
<td>181</td>
</tr>
<tr>
<td>2-Hydroxymethyl-1-methyl-5-nitroimidazole [112]</td>
<td>-</td>
<td>NO₂</td>
<td>Me</td>
<td>CH₂OH</td>
<td>774</td>
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<tr>
<td>2-Chloromethyl-1-methyl-5-nitroimidazole [60]</td>
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<td>CH₂Cl</td>
<td>153</td>
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<td>1,2-Dimethyl-5-nitroimidazole [2]</td>
<td>-</td>
<td>NO₂</td>
<td>Me</td>
<td>CH₃</td>
<td>&gt;2000</td>
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Table 4.1 contd.

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<th>Name of compound</th>
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<th>(R^2)</th>
<th>(R^3)</th>
<th>(R^4)</th>
<th>MIC (ug/ml) by the Agar Diffusion (Well) Method</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>B. Cereus</td>
</tr>
<tr>
<td>(1\text{-Methyl-5-nitroimidazole-2-yl methyl phenyl sulphone [119]})</td>
<td>(\text{NO}_2)</td>
<td>(\text{Me})</td>
<td></td>
<td>(\text{CH}_2\text{SPh})</td>
<td>&gt;2000</td>
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<tr>
<td>(1\text{-Methyl-5-nitroimidazole-2-yl methyl pyrid-2-yl sulphone [120]})</td>
<td>(\text{NO}_2)</td>
<td>(\text{Me})</td>
<td></td>
<td>(\text{CH}_2\text{SPyn})</td>
<td>&gt;2000</td>
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<tr>
<td>(1\text{-Methyl-5-nitroimidazole-2-yl methyl pyrimid-2-yl sulphone [121]})</td>
<td>(\text{NO}_2)</td>
<td>(\text{Me})</td>
<td></td>
<td>(\text{CH}_2\text{SPym})</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>(1\text{-1-Methyl-1-nitroethyl-4-nitroimidazole [62]})</td>
<td>(\text{NO}_2)</td>
<td></td>
<td>(\text{C}(\text{CH}_3)_2\text{NO}_2)</td>
<td>-</td>
<td>1720</td>
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<tr>
<td>(1\text{-1-Methyl-1-nitroethyl-2-methyl-4-nitroimidazole [63]})</td>
<td>(\text{NO}_2)</td>
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<td>(\text{C}(\text{CH}_3)_2\text{NO}_2)</td>
<td>(\text{CH}_3)</td>
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Table 4.1 contd.

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<th>Name of compound</th>
<th>R^1</th>
<th>R^2</th>
<th>R^3</th>
<th>MIC (μg/ml) by the Agar Diffusion (Well) Method</th>
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<td><strong>Aerobic Organism</strong></td>
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<td></td>
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<td>B. Cereus</td>
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</tbody>
</table>
Table 4.1 contd.

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>MIC (μg/ml) by the Agar Diffusion (Well) Method</th>
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<td>Aerobic Organism</td>
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<td>B. Cereus</td>
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<td></td>
<td></td>
<td>E. coli</td>
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<td></td>
<td></td>
<td></td>
<td>F. Acet.</td>
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<td></td>
<td></td>
<td></td>
<td>A. Nigran</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>C. Albicans</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. I. spor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. I. histio-s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lyticum fragilis</td>
</tr>
<tr>
<td>1-(1-Methyl-1-nitroethyl)benzimidazole (89)</td>
<td>-</td>
<td>-</td>
<td>C(CH₃)₂NO₂</td>
<td>-</td>
<td>&gt;2000 &gt;2000 &gt;2000 &gt;2000 &gt;2000 &gt;250 &gt;250 -</td>
</tr>
<tr>
<td>5-nitrobenzimidazole [91]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C(CH₃)₂NO₂</td>
<td>-</td>
</tr>
<tr>
<td>6-nitrobenzimidazole [92]</td>
<td>-</td>
<td>-</td>
<td>NO₂</td>
<td>C(CH₃)₂NO₂</td>
<td>-</td>
</tr>
<tr>
<td>1-(1-Methyl-1-nitroethyl)-NO₂</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C(CH₃)₂NO₂</td>
<td>-</td>
</tr>
<tr>
<td>5-nitroisoindazole (94)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;2000 &gt;2000 &gt;2000 &gt;2000 &gt;2000 &gt;250 &gt;250 -</td>
</tr>
</tbody>
</table>

- R², R₃, R₄ represent substituents on the respective positions of the indazole or benzimidazole rings.
Table 4.1 contd.

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>R¹ R² R³ R⁴</th>
<th>MIC (µg/ml) by the Agar Diffusion (Well) Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Aerobic Organism</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
</tr>
</tbody>
</table>
| 1-(1-Methyl-1-nitroethyl)-6-nitroisoindazole [96] | NO₂ | C(CH₃)₂NO₂ | ≥2000 | ≥2000 | ≥2000 | ≥2000 | ≥250 | ≥250 | -

![Chemical structures](attachment:image.png)
Figure 4.1 A comparison of the sensitivities of Cl sporogenes to some Nitroimidazoles using Agar Well diffusion method.
Sensitivity of Cl histolyticum to Nitroimidazoles

Figure 4.2 A comparison of the sensitivities of Cl histolyticum to some Nitroimidazoles using the Agar Well diffusion method. All points are replicates of four readings.
Sensitivity of B. fragilis to Nitroimidazoles

Figure 4.3 A comparison of the sensitivities of B. fragilis to some Nitroimidazoles using the Agar Well diffusion method. All points are replicates of four readings.
Sensitivity of *Clostridium sporogenes* to Nitroimidazoles

![Graph showing sensitivity of *Clostridium sporogenes* to Nitroimidazoles using Agar Well diffusion method.](image)

**Legend**
- □ Nitroimidazole [1]
- △ Nitroimidazole [126]
- ● Nitroimidazole [120]
- ○ Nitroimidazole [4]
- ▲ Nitroimidazole [3]

**Figure 4.4** A comparison of the sensitivity of *Clostridium sporogenes* to some Nitroimidazoles using Agar Well diffusion method.
than for *Cl. histolyticum*. For *Cl. sporogenes*, the slopes of activity for the nitroimidazoles [66] and [69] showed parallel relationship to that of metronidazole [1] while [70] deviates from this parallel comparison.

b) **By the Tube Dilution Assay**

The results from the agar diffusion assay pre-determined which of the synthesised nitroimidazoles and diazoles were screened by the tube dilution method. Only the nitroimidazoles with MIC from the agar assays less than 250μg/ml for *Cl. sporogenes* and *Cl. histolyticum* were screened, except 1-methyl-2-methylsulphonyl-5-nitroimidazole.

The results of the tube dilution assay for nitroimidazoles screened are presented in Table 4.2. The table also contains the results from the agar diffusion method for easy comparison. Except with metronidazole 1,2-dimethyl-, and 2-(chloromethyl)-5-nitroimidazoles, the MIC from the tube dilution assay are higher than the MIC from the agar diffusion method. (This difference range from approximately two- to 10-fold higher). While for 1-(p-nitrobenzyl)-2-methyl-4-nitroimidazole [66], 1-(1,2-dimethyl-5-nitroimidazol-2-yl)-2-nitroimidazole [70], 2-(hydroxymethyl)-1-methyl-5-nitroimidazole [112], 1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62] and Ipronidazole [3] the results from both assays are in the same order of magnitude. 1-Methyl-2-methylsulphonyl-5-nitroimidazole [126] did not give any appreciable reading by the agar diffusion method but gave MIC = 62.5μg/ml by the tube dilution method.

c) **By the Minimum Bactericidal assay**

The results of minimum bactericidal assay carried out after end point of tube dilution assay are presented in Table 4.2 alongside results from the tube dilution and agar diffusion assays for easy comparison.

In approximately fifty percent of the nitroimidazoles studied, the MBC agreed with the MIC values from the tube dilution assay, while the rest are higher.

Although, in certain cases, there is a large discrepancy in the three methods employed, the general trend of activity of these nitroimidazoles against each test organism remained (Table 4.2).
### Table 4.2 The sensitivities of Nitroimidazoles against Cl. sporogenes and Cl. histolyticum via different methods

<table>
<thead>
<tr>
<th>Organism</th>
<th>Name of compound</th>
<th>Method of obtaining the MIC (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Agar diffusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIC (well)</td>
</tr>
<tr>
<td>Cl. sporogenes</td>
<td>2-bromomethyl-1-methyl-5-nitroimidazole [59]</td>
<td>0.316</td>
</tr>
<tr>
<td></td>
<td>2-hydroxymethyl-1-methyl-5-nitroimidazole [112]</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>2-chloromethyl-1-methyl-5-nitroimidazole [60]</td>
<td>0.771</td>
</tr>
<tr>
<td></td>
<td>1,2-dimethyl-5-nitroimidazole [2]</td>
<td>0.256</td>
</tr>
<tr>
<td>Cl. histolyticum</td>
<td>(1-methyl-5-nitroimidazo1-2-yl) methylphenyl sulphide [119]</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>(1-methyl-5-nitroimidazo1-2-yl) methyl pyrid-2-yl sulphide [120]</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>(1-methyl-5-nitroimidazo1-2-yl) methyl pyrimid-2-yl sulphide [121]</td>
<td>7.5</td>
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<tr>
<td></td>
<td>Ipronidazole [4]</td>
<td>11.74</td>
</tr>
<tr>
<td></td>
<td>1-methyl-2-methylsulphonyl-5-nitroimidazole [126]</td>
<td>-</td>
</tr>
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<td></td>
<td>1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62]</td>
<td>128.5</td>
</tr>
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<td>1-(1-methyl-1-nitroethyl)-2-methyl-4-nitroimidazole [63]</td>
<td>62.5</td>
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<td>Bronidox [64]</td>
<td>32</td>
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<tr>
<td></td>
<td>1-(p-nitrobenzyl)-2-methyl-4-nitroimidazole [66]</td>
<td>2.49</td>
</tr>
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<td>1-(p-nitrobenzyl)-2-nitroimidazole [69]</td>
<td>1.40</td>
</tr>
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<td>1-(1,2-dimethyl-5-nitroimidazo1-2-yl)-2-nitroimidazole [70]</td>
<td>0.447</td>
</tr>
<tr>
<td></td>
<td>1-(1,2-dimethyl-5-nitroimidazo1-2-yl)-4-nitroimidazole [67]</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>Metronidazole [1]</td>
<td>0.293</td>
</tr>
<tr>
<td>Cl. histolyticum</td>
<td>2-(bromomethyl)-1-methyl-5-nitroimidazole [59]</td>
<td>4.28</td>
</tr>
<tr>
<td></td>
<td>2-(hydroxymethyl)-1-methyl-5-nitroimidazole [112]</td>
<td>0.434</td>
</tr>
<tr>
<td></td>
<td>2-(chloromethyl)-1-methyl-5-nitroimidazole [60]</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Ronidazole [3]</td>
<td>0.592</td>
</tr>
<tr>
<td></td>
<td>1-(1-methyl-5-nitroimidazo1-2-yl)-methylphenyl sulphide [119]</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>(1-methyl-5-nitroimidazo1-2-yl) methyl pyrid-2-yl sulphide [120]</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>1-methyl-5-nitroimidazo1-2-yl methyl pyrimid-2-yl sulphide [121]</td>
<td>7.84</td>
</tr>
<tr>
<td></td>
<td>1,2-dimethyl-5-nitroimidazole [2]</td>
<td>0.526</td>
</tr>
<tr>
<td></td>
<td>Ipronidazole [4]</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>1-(1-methyl-1-nitroethyl)-4-nitroimidazole [62]</td>
<td>51.1</td>
</tr>
<tr>
<td></td>
<td>1-(1-methyl-1-nitroethyl)-2-methyl-4-nitroimidazole [63]</td>
<td>19.45</td>
</tr>
<tr>
<td></td>
<td>Bronidox [64]</td>
<td>5.75</td>
</tr>
<tr>
<td></td>
<td>1-(p-nitrobenzyl)-2-methyl-4-nitroimidazole [66]</td>
<td>61.1</td>
</tr>
<tr>
<td></td>
<td>1-(p-nitrobenzyl)-2-nitroimidazole [69]</td>
<td>17.73</td>
</tr>
<tr>
<td></td>
<td>1-(1,2-dimethyl-5-nitroimidazo1-2-yl)-2-nitroimidazole [70]</td>
<td>5.33</td>
</tr>
<tr>
<td></td>
<td>1-(1,2-dimethyl-5-nitroimidazo1-2-yl)-4-nitroimidazole [67]</td>
<td>19.67</td>
</tr>
</tbody>
</table>
4.3.2 Extended Diffusion Experiments

a) Effect of aerobic and anaerobic conditions on the action of some nitroimidazoles against E.coli and Ps.aeruginosa

The activity of drug observed (as the measured diameter of zone of inhibition) against the test organisms is less under anaerobic than the aerobic conditions (Table 4.3). For example, no zone of inhibition was observed against Ps. aeruginosa with 1-(1-methyl-1-nitroethyl)-4-nitroimidazole at 2.5μg/ml under anaerobic conditions, while a zone of 8.0mm was recorded for the same drug concentration under aerobic conditions. A similar observation was recorded for E.coli and the hydroxy drug [112].

b) Further Investigation of the Sensitivity of C.albicans to 2-(hydroxymethyl)-1-methyl-5-nitroimidazole [112]

Instead of the normal clear zone of inhibition, a black/brown zone of inhibition was obtained when C.albicans was incubated in the presence of the drug [112] (Fig.4.5). Further incubation in the presence of the drug [112] (5mg/ml, 10 x MIC), cells from the black section of the zone failed to show any visible growth, even after one week; while cells from the white section of the zone grew showing the characteristics of the black zone. In the absence of the drug, subcultured cells from both sections grew typical colonies with normal appearance of C.albicans. (see also light microscopy).

c) Gradient Plate technique

Fig. 4.6 shows the effect of small change in concentration of compound [112] on culture growth as exhibited by the gradient plate method. The plate showed no coloration of yeast cells at low conc. of drug [112] (higher level of drug free agar), but as concentration is increased, the brown coloration gradually developed.

d) Quantitative determination of the Interaction between 2-(hydroxymethyl)-1-methyl-5-nitroimidazole and some related compounds

Table 4.4 and Fig. 4.7 summarise the interaction of some related compounds on the effects of the 2-hydroxymethylated nitroimidazole [112] (20mg/ml) on the growth of C. albicans in agar plates.

Imidazole (20mg/ml) reacted synergistically, while
Table 4.3 The sizes of the zones of inhibition produced by two nitroimidazoles [112] and [63] against E. coli and Ps. aeruginosa under aerobic and anaerobic conditions

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>conc. used (mg/ml)</th>
<th>E. coli</th>
<th></th>
<th>Ps. aeruginosa</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anaerobic</td>
<td>Aerobic</td>
<td>Anaerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td>2-hydroxymethyl-1-methyl-5-nitroimidazole [112]</td>
<td>2.5</td>
<td>0</td>
<td>7.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0</td>
<td>9.9</td>
<td>0</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.2</td>
<td>14.6</td>
<td>7.1</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>16.3</td>
<td>28</td>
<td>9.2</td>
<td>14.9</td>
</tr>
<tr>
<td>1-(1-methyl-1-nitroethyl)-4-nitroimidazole [63]</td>
<td>2.5</td>
<td>0</td>
<td>11.5</td>
<td>0</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0</td>
<td>13.1</td>
<td>0</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>7.7</td>
<td>15.8</td>
<td>11.5</td>
<td>15.03</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>10.0</td>
<td>19.6</td>
<td>14.4</td>
<td>20.77</td>
</tr>
</tbody>
</table>

Table 4.4 The interaction of some related compounds with 2-hydroxymethyl-1-methyl-5-nitroimidazole [112]

<table>
<thead>
<tr>
<th>'Well' in agar containing</th>
<th>20C</th>
<th>20I</th>
<th>20NAC</th>
<th>Cyst.1%</th>
<th>Type of Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Synergistic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neutral</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Antagonist</td>
</tr>
</tbody>
</table>

20C = 20mg/ml 2-hydroxymethyl-1-methyl-5-nitroimidazole [112]
20I = 20mg/ml imidazole
20NAC = 20mg/ml N-acetylglucosamine
Cyst.1% = Cysteine 1%
L-cysteine (at 1%) was antagonistic and N-acetylglucosamine had little or no activity on the effects of 2-hydroxymethyl drug [112] on C. albicans.

4.3.3 Microscopy

a) Light Microscopy

Fig. 4.8 shows light micrographs of C. albicans growing in agar plate. Cells in the area distant from 'experimental' wells consisted of yeast cells with some pseudohyphae.

Within the zone area ('experimental' wells) however, at low magnifications, cells consisted of a white lawn of growth on top of a bed of black growth (Fig. 4.5). Staining each type of cells and observing by high power microscopy (x40), the white lawn (W-type) were normal yeast cells (blastospores), and the black growth (B-type) were mycelia forms (Figs. 4.8 and 4.9).

b) Scanning Electron Microscopy

Scanning electron microscopy of cells in the area distant from the 'experimental' wells show mainly yeast cells with some pseudohyphae. The yeast cells were spherical with convoluted surfaces while the pseudohyphae were narrow and branched tubes (Fig. 4.10). In the presence of the drug [112], 5mg/ml and 20mg/ml, electron micrograph of cells in the B-region in the zone of inhibition show a mixture of yeast but mostly mycelia cells which have collapsed. The damage was more severe at the higher concentration of drug [112] (see Fig. 4.10) and the zone of inhibition was sparsely populated. The cells from the W-region were similar in appearance to cells obtained from the area distant from the 'experimental' wells. (Figs. 4.9 and 4.10).

A similarly damaged yeast and mycelia cells of C. albicans was observed when the experiment was repeated using the tube dilution assay. In the presence of 2-(halomethyl)-1-methyl-5-nitroimidazole (halo = Br, Cl) [59] and [60], necking of the yeast cells occurred but no damaged cells were observed (Fig. 4.11). With N-acetylglucosamine, more pseudohyphae were formed (Fig. 4.11) than with the 2-hydroxyimidazole [112].
Fig. 4.5 Effect of 2-(hydroxymethyl)-1-methyl-5-nitroimidazole [112] on C. albicans grown on Neurospora solid agar at 30°C for 24h. The zone of inhibition consisted of a 'white lawn of cells' (W-type) on a bed of black cells (B-type). After 48h incubation the cells cleared to give a 'dark' zone of inhibition.
Fig. 4.6 Effect of hydroxymethyl imidazole [112] on C. albicans using the gradient plate technique. Note the gradual development of 'coloration' as drug concentration increased.
Fig. 4.8 Light micrograph of \textit{C. albicans} from the 'well' containing the 2-hydroxymethyl drug [112] (20mg/ml) after 24h incubation at 30°C. Sample of \textit{C. albicans} from: a) the W-type

b) the B-type of cells in the zone of inhibition. x 400
Fig. 4.9 Light micrograph of _C. albicans_ obtained from

a) White lawn (W-type)

b) Bed of black (B-type) cells in the zone of inhibition of 'well' containing 2-hydroxyl drug [112] (20mg/ml) after 24h incubation at 30°C and stained (methylene blue) x 400.

The cells of W-type (a) are mostly yeast (round) cells while those of B-type are mostly mycelia.
Fig. 4.10 a) S.E.M. of *C. albicans* (control) grown on solid neurospora agar for 24h at 30°C. The growth was mostly round and wrinkled yeast cells x 9000.

b and c) S.E.M. of *C. albicans* grown in the presence of 2 hydroxymethyl drug [112] (5mg/ml) for 24h at 30°C. Electron micrographs show the presence of yeast and hyphae cells with ruptured walls. x 2,500 and x 9,900.
Fig. 4.10 d) S.E.M. of *C. albicans* grown on neurospora solid agar in the presence of 2-hydroxymethyl drug [112] (20mg/ml). Note the deflated yeast cells and hyphae x 3,000.

Fig. 4.11 a) S.E.M. of *C. albicans* grown in neurospora medium for 24h at 30°C in the presence of 2-(bromomethyl)-1-methyl-5-nitroimidazole [59] (20mg/ml). Electron micrograph shows chains of yeast cells x 2,500.

b) S.E.M. of *C. albicans* grown on neurospora medium in the presence of 2-(chloromethyl)-1-methyl-5-nitroimidazole [60] (20mg/ml) for 24h at 30°C. Note the 'necks' between yeast cells x 1,500.
Fig. 4.11 c) S.E.M. of C. albicans grown for 24h in the presence of N-acetylglucosamine in neurospora medium at 30°C. Micrograph shows pseudohyphae and elongated yeast cells separated by 'necks' x 2,500.

Fig. 4.12 The effect of solvent used in making up antimicrobial solution for testing on the growth of Cl. sporogenes.

a) Using solvent mixture DMSO:distilled water.
   20 : 80
   Note the effect of the centre disc containing solvent as control.

b) Using solvent mixture DMSO:distilled water.
   10 : 90
   The control centre disc (solvent system only) has no inhibitory effect on Cl. sporogenes.
4.3.4 Polarographic reduction of Nitroimidazoles

Half-wave potentials \((E_1/2)\) at pH 7.0 for all synthesised and reference nitroimidazoles, and diazoles (except where lack of solubility prevented assay) are presented in Table 4.5. All \(E_1/2\) values quoted are with respect to the Ag/AgCl electrode. For comparison, the literature half-wave potential of reference nitroimidazoles (where available) are shown in brackets.

4.3.5 Electrolytic Reduction

The U.V. measurement of aliquots withdrawn at intervals during electrolytic reduction of nitroimidazoles in the presence of DNA showed the disappearance of the signal characteristic of the nitro group at the appropriate wavelength with time. The absorbance reading was converted to concentration of unreduced nitroimidazoles using the standard curve technique. The absorbance and % reduced drug concentration (µg/ml) are presented in Tables 4.6 and 4.7 for nitroimidazoles [1] and [70] respectively.

a) Viscometric analysis of DNA after reduction

The effect of reduced metronidazole and 1-(1,2-dimethyl-5-nitroimidazol-2-yl)-2-nitroimidazole [70] on the flow-time of calf-thymus DNA is shown in Table 4.7. It is shown that the flow-time of DNA decreased as the concentration of reduced drugs [1] and [70] increased. (see Figs. 4-13 and 4.14).
Table 4.5 Relationship between $E^\frac{1}{2}$ and spectrum of antimicrobial activity of nitroimidazoles

<table>
<thead>
<tr>
<th>Nitroimidazole</th>
<th>$E^\frac{1}{2}$ (mV)</th>
<th>MIC (Tube dilution) against <em>Cl. sporogene</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nitro group Others on imidazole nucleus</td>
<td></td>
</tr>
<tr>
<td>[1]</td>
<td>-475, (382)$^{182}$ (500)$^{183}$</td>
<td>0.037</td>
</tr>
<tr>
<td>[2]</td>
<td>-470</td>
<td>0.032</td>
</tr>
<tr>
<td>[3]</td>
<td>-372</td>
<td>15.6</td>
</tr>
<tr>
<td>[4]</td>
<td>-420</td>
<td>31.2</td>
</tr>
<tr>
<td>[59]</td>
<td>-393</td>
<td>1.208</td>
</tr>
<tr>
<td>[60]</td>
<td>-385</td>
<td>0.386</td>
</tr>
<tr>
<td>[62]</td>
<td>-480</td>
<td>125</td>
</tr>
<tr>
<td>[63]</td>
<td>-488</td>
<td>125</td>
</tr>
<tr>
<td>[64]</td>
<td>-444</td>
<td>62.5</td>
</tr>
<tr>
<td>[65]</td>
<td>-</td>
<td>&gt;250</td>
</tr>
<tr>
<td>[66]</td>
<td>-448</td>
<td>3.9</td>
</tr>
<tr>
<td>[67]</td>
<td>-425, -600</td>
<td>3.17</td>
</tr>
<tr>
<td>[69]</td>
<td>-424</td>
<td>-376</td>
</tr>
<tr>
<td>[70]</td>
<td>-472, -360</td>
<td>-</td>
</tr>
<tr>
<td>[112]</td>
<td>-414</td>
<td>0.39</td>
</tr>
<tr>
<td>[30]</td>
<td>-390</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(-390)$^{183}$</td>
<td></td>
</tr>
<tr>
<td>[80]</td>
<td>-615</td>
<td>-</td>
</tr>
<tr>
<td>[88]</td>
<td>-</td>
<td>-660</td>
</tr>
</tbody>
</table>

The values of $E^\frac{1}{2}$ were measured relative to Ag/AgCl. Literature $E^\frac{1}{2}$ values are quoted in brackets, where no values are quoted, insolubility of the drug is the reason. Ref. 183 measured relative to saturated calomel electrode.
Table 4.6  The effect of reduced metronidazole [1] on the viscosity flow time(s) of calf-thymus DNA

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>Conc. of reduced drug [1] µg/ml</th>
<th>% reduced drug [1]</th>
<th>Flow time(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.503</td>
<td>0</td>
<td>0</td>
<td>127</td>
</tr>
<tr>
<td>2.221</td>
<td>0.293</td>
<td>12</td>
<td>125</td>
</tr>
<tr>
<td>1.272</td>
<td>1.231</td>
<td>49</td>
<td>121</td>
</tr>
<tr>
<td>0.658</td>
<td>1.845</td>
<td>74</td>
<td>118</td>
</tr>
<tr>
<td>0.339</td>
<td>2.164</td>
<td>86</td>
<td>116</td>
</tr>
<tr>
<td>0.176</td>
<td>2.327</td>
<td>93</td>
<td>115</td>
</tr>
<tr>
<td>0.095</td>
<td>2.408</td>
<td>96</td>
<td>115</td>
</tr>
</tbody>
</table>

Table 4.7  The effect of reduced nitroimidazole [70] on the viscosity flow time(s) of calf-thymus DNA

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>Conc. of reduced drug [70] µg/ml</th>
<th>% reduced drug [70]</th>
<th>Flow time(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.499</td>
<td>0</td>
<td>0</td>
<td>107</td>
</tr>
<tr>
<td>2.133</td>
<td>0.95</td>
<td>29</td>
<td>102</td>
</tr>
<tr>
<td>1.416</td>
<td>1.75</td>
<td>53.8</td>
<td>97</td>
</tr>
<tr>
<td>1.019</td>
<td>2.1</td>
<td>64.6</td>
<td>96</td>
</tr>
<tr>
<td>0.946</td>
<td>2.25</td>
<td>69.2</td>
<td>95</td>
</tr>
<tr>
<td>0.856</td>
<td>2.35</td>
<td>72.3</td>
<td>94</td>
</tr>
<tr>
<td>0.836</td>
<td>2.65</td>
<td>81</td>
<td>92</td>
</tr>
</tbody>
</table>
Effects of Nitroimidazole on Calf-thymus DNA

![Graph showing the effect of reduced Nitroimidazole on the Viscosity flow time (secs) of Calf-thymus DNA. The graph displays a linear relationship between the percentage of reduced Nitroimidazole and the viscosity flow time. As the percentage of reduced Nitroimidazole increases, the viscosity flow time decreases.]

Figure 4.13 The Effect of reduced Nitroimidazole [1] on the Viscosity flow time (secs) of Calf-thymus DNA
Effects of Nitroimidazole on Calf-thymus DNA

![Graph showing the effect of reduced Nitroimidazole on viscosity flow time of Calf-thymus DNA. The graph plots the percentage of reduced Nitroimidazole against the viscosity flow time (in seconds), indicating a linear decrease in viscosity flow time as the percentage of reduced Nitroimidazole increases.]
4.4 **DISCUSSION** - PART C

The synthesised nitroimidazoles and diazoles relate to one of the following 'parent' compounds: metronidazole, dimetridazole, 2-nitroimidazoles or 2-substituted-2-nitropane.

The initial screening of the α-substituted 1,2-dimethyl-5-nitroimidazole derivatives showed that in general the halogen and especially the thio substituents decreased the effect of 1,2-dimethyl-5-nitroimidazole against anaerobes (*Cl. sporogenes* and *Cl. histolyticum*) while the hydroxy group enhanced it (Table 4.2). This is probably due to the labile halogen i.e. their radical-anion readily breaks down or they are readily metabolised before reaching the site of action (e.g. DNA) (see also Chapter 3, part B). The thio group was reported by Winkelman et al. 17 to possess activity against *T. vaginalis* because they possess moderate leaving groups. From the in vitro light catalysed reactions (Chapter 3), the radical-anion of the thio derivatives did not decompose under the reaction conditions employed. This could in effect mean that the radical-anion is able to 'survive' during transport in the cytoplasm to the site of action in DNA. The decrease in activity from that of the 'parent' compound could be that the latter compound (dimetridazole) is smaller and thus, can diffuse more readily in the cell than its substituent derivatives.

The quaternary ammonium derivatives [116] and [117] were shown to be inactive against examples of both anaerobic and aerobic micro-organisms. This may be due to the stability of the C-N bond which renders their metabolism (and/or breakdown of their radical-anion at the site of action) more difficult. Another possible explanation for this lack of activity could be a lack of transport across the cell membrane of the organisms. Being positively charged, they might be bound to the negatively charged sites in the membrane and hence, not 'available' within the organism. In comparison with the ammonium imidazole [116], an increase in antibacterial effect was not observed (Table 4.1) by raising the lipophilicity as in the imidazole [117].

The 2-hydroxymethyl derivative [112], in addition to
its activity against both anaerobes and aerobes (Tables 4.1 and 4.2), has an interesting form of activity against C. albicans. This activity (black/brown zone of inhibition) was enhanced by imidazole but inhibited by L-cysteine. The inclusion of N-acetylglucosamine in the medium did not influence this effect of the hydroxy compound on C. albicans. Dimorphism in C. albicans was investigated by Wilson and he reported that N-acetylglucosamine is the minimal requirement for hyphae morphogenesis from the yeast form. This agrees with the increased hyphae observed under the electron microscope for C. albicans grown in the presence of N-acetylglucosamine (Nac) (Fig. 4.11). From the drug interaction experiments, the darkening effect was observed only in the well containing the drug [112] but not in the well containing Nac (Fig. 4.7). Wilson and other workers reported that L-cysteine inhibited germ tube formation. Therefore, it can be inferred from the characteristic D-shape (Fig. 4.7) observed after drug interaction experiments using L-cysteine and the hydroxy drug [112] on C. albicans, that the hydroxy drug [112] is selective towards germ-tubes though the cause for the observed black coloration is not known. Niimi et al reported that germ-tube forming cells of C. albicans are more susceptible to clotrimazole (an imidazole derivative) than the yeast cells but did not observe any change in coloration. In the present study, 2-hydroxymethyl drug [112] interacted with C. albicans in the presence of imidazole, the effect was a synergistic one (Fig. 4.7), supporting Niimi's work and suggesting that, like clotrimazole, 2-hydroxymethyl drug [112] is more active against germ-tube cells than the yeast cells.

Chasker et al reported brown diffusible pigments on media that contained Fe and tryptophan as a sole source for nitrogen, suggesting pigmentation as a metabolic product. But the composition of nutrients in Neurospora medium (broth and agar), in the present study, is comparable to Sabourand yeast extracts used by Niimi et al. Although it cannot be completely ruled out that the pigmentation observed in the present work is due to a shift in metabolism, without the isolation and identification of such a metabolic product, the reason for the distinctive change in culture appearance (black pigmentation) remains unknown.
Various authors have shown that the development of germ-tubes from yeast (ovoid) cells is the result of active cell wall biosynthesis.\textsuperscript{187} The present study showed that the 2-hydroxymethyl drug [112] inhibits germ-tube formation, it is probable that it inhibits cell wall biosynthesis. On the basis of this hypothesis, the hydroxymethyl drug [112] may be acting as a structural analog of glucose in the cell wall biosynthesis. A target compound for future research [130] might be a useful 'probe' in elucidating or better understanding the cause(s) of the effect of this drug [112] on \textit{C. albicans}. The use of such compounds might also aid

\begin{center}
\includegraphics[width=0.5\textwidth]{structure.png}
\end{center}

in the understanding of why rupture of the cell wall of drug [112] treated \textit{C. albicans} was observed (Fig. 4.10) but none due to its derivatives e.g. 2-(halomethyl)imidazoles [59] and [60] (Fig. 4.11).

Surprisingly and interestingly, with the exception of 1-(p-nitrobenzyl)-4-nitroimidazole (MIC $> 250\mu g/ml$), the N-1 substituted-4-nitroimidazoles were active against the anaerobes and not the aerobes. (Table 4.1). The activity of some of these active 4-nitroimidazoles, e.g. 1-(p-nitrobenzyl)-2-methyl-4-nitroimidazole [66] (MIC $\approx 3\mu g/ml$) and 1-(1,2-dimethyl-5-nitroimidazol-2-yl)-4-nitroimidazole [67] (MIC $\approx 2\mu g/ml$) are similar to that of metronidazole [1] (MIC $\approx 0.2\mu g/ml$) against \textit{Cl. sporogenes} and therefore could be therapeutically useful. A plausible explanation for the activity of the 4-nitroimidazole is that the extra nitro and/or electron-withdrawing groups at the N-1 position enhance activity. This was confirmed when one of the nitro groups (e.g. the 4-nitro group) was removed [88]. The imidazole derivative [88] was inactive towards both anaerobes and aerobes screened, as were the other structural analogues of 2-substituted-2-nitropropanes— the diazoles. The problem in the latter in most instances, may be due to lack of water solubility making evaluation difficult.
l-(p-Nitrobenzyl)-4-nitroimidazole [65] was inactive to both anaerobes and aerobes tested. The only structural difference between this compound [65] and its analogue [66] is the methyl group present at the 2-position of the latter. It is possible that lipophilicity is enhanced in the latter [66] due to this methyl group assisting transport across the cell membrane. This could be compared with the activity of 1,2-dimethyl-5-nitroimidazole (dimetridazole) [2] and the non-active 1-methyl-5-nitroimidazole [111], where the only structural difference was also the methyl group at the 2-position of the nitroimidazole.

All the 2-nitroimidazole derivatives screened were active against the anaerobes but inactive to aerobes tested. The 2-Nitroimidazole [70] was more active than the 4-nitroimidazole analogue [67] perhaps because the effect of 2- and 5-nitro is additive in [70], a property which is lacking in the 4- and 5-nitroimidazole derivative [67]. Similarly, l-(p-nitrobenzyl)-2-nitroimidazole [69] is more active against Cl. sporogenes than its 4-nitro analogue [66]. The activity of the nitroimidazole [66] was more directly related to the activity of metronidazole (parallel slopes - Fig. 4.1) than that of nitroimidazole [70] against Cl. sporogenes. This could, however, merely be a consequence of solubility rather than a major difference in activity.

Although all the N-1 substituted nitroimidazoles synthesised were 4-nitro derivatives, none (except where assay was prevented due to lack of solubility) showed $E^2$ values typical of 4-nitroimidazoles, but resemble the redox potential ($E^2$) of 5-nitroimidazoles (Table 4.5). The reason for this is not clear but it could be that the bulky group (1-methyl-1-nitroethyl-) does modify the nitro group in these nitroimidazoles such that it accepted an electron more readily than the 4-nitroimidazole analog. In view of the $E^2$ values, it is not surprising that 1-substituted-4-nitroimidazoles showed activity against the anaerobes. This is supported by reports that a necessary condition for the activity of nitro-drugs is that they be reduced at redox potential present in cultures of the target organisms.

The e.s.r. studies of 1-(p-nitrobenzyl)-2-methyl-4-nitroimidazole [66] and its analog [65] showed that the added electron was located on the benzyl nitro group and
not on the 4-nitroimidazole. Polarographic studies of these nitroimidazoles, particularly [66], showed two $E_1^*$ values, one of which closely agreed with $E_1^*$ for metronidazole ($E_1^* = -475 \text{mV}$) and the other is lower ($E_1^* = -328 \text{mV}$ and $-448 \text{mV}$). Thus, the observed activity may be a direct consequence of reduction of either or both of the nitro groups.

Metronidazole [1] and 1-(1,2-dimethyl-5-nitroimidazol-2-yl)-2-nitroimidazole [70] have been shown to decrease the viscosity (flow time) of DNA as a consequence of reduction of the nitro group (Figs. 4.13 and 4.14). For metronidazole a decrease in flow time from 134 to 118s was reported by Edward et al. [82] as the difference in flow times between double- and single-stranded DNA. Although the flow times obtained from the present study for metronidazole is lower (127 - 115s), many factors such as the extent of dialysis before viscometric assay and the rate of reduction of the drug in the presence of DNA are important. The former factor would result in a sample for viscometry which consists of a mixture of double and single stranded DNA, which could explain the lower (i.e. faster) flow times obtained. A decrease in flow time from 107 to 92s was observed for DNA reduced in the presence of 1-(1,2-dimethyl-5-nitroimidazol-2-yl)-2-nitroimidazole. The electrolytic reduction of 1-(p-nitrobenzyl)-2-nitroimidazole [69] in the presence of calf-thymus DNA produced no change in flow time of DNA. This might be due to lack of solubility of this drug [69] at the concentration required for the assay. The drug was shown to be active against the anaerobes (Tables 4.1 and 4.2).

**Conclusion**

The observed MIC values for the $\alpha$-substituted 1,2-dimethyl-5-nitroimidazoles, particularly for the 2-(halomethyl)- and 2-(hydroxymethyl)-1-methyl-5-nitroimidazole Cl. sporogenes are within the accepted range of values for potential therapeutic use. The order of activity of 5-nitroimidazoles against Cl. sporogenes was found to be:

$$\text{Cl} > \text{Br} > \text{SPh} > \text{SPy} > \text{SPym.} > \text{N}-(\text{CH}_2)_7\text{Me} > \text{NMe}_3$$
More work is required to explain the darkening effects observed for 2-(hydroxymethyl)-1-methyl-5-nitroimidazole [112] against \( \text{C. albicans} \).

The N-1 alkylated-4-nitroimidazoles except 1-(p-nitrobenzyl)-4-nitroimidazole [65] were shown to possess activity against the anaerobes \( \text{Cl. sporogenes} \) and \( \text{Cl. histolyticum} \). The MIC of some of these nitroimidazoles, e.g. [66] and [67] (2.49 and 1.96\( \mu \)g/ml respectively), as well as the N-1 alkylated 2-nitroimidazole derivatives e.g. [70] (0.44\( \mu \)g/ml), are within the accepted range for potential therapeutically useful compounds.

\( \text{Cl. sporogenes} \) is more susceptible to these nitroimidazoles than \( \text{Cl. histolyticum} \). The order of activity observed for the N-1 alkylated-2-and 4-nitroimidazoles is: [1] > [70] > [69] > [67] > [66] > [64] > [63] > [62]

Structure

[1] 
\[
\begin{array}{c}
\text{O}_2\text{N}\\
\text{N}\\
\text{X}\\
\text{R}
\end{array}
\]

[70] 
\[
\begin{array}{c}
\text{O}_2\text{N}\\
\text{N}\\
\text{Me}\\
\text{CH}_2\\
\text{CH}_2\text{OH}
\end{array}
\]

[69] 
\[
\begin{array}{c}
\text{O}_2\text{N}\\
\text{N}\\
\text{Me}\\
\text{CH}_2\\
\end{array}
\]

[64] 
\[
\begin{array}{c}
\text{O}_2\text{N}\\
\text{N}\\
\text{Me}
\end{array}
\]

[66] 
\[
\begin{array}{c}
\text{O}_2\text{N}\\
\text{N}\\
\text{Me}
\end{array}
\]

[67] 
\[
\begin{array}{c}
\text{O}_2\text{N}\\
\text{N}\\
\text{Me}
\end{array}
\]
The diazoles were found to be inactive against both anaerobes and aerobes.

1-(1,2-Dimethyl-5-nitroimidazol-2-yl)-2-nitroimidazole [70] was shown to reduce the flow time of DNA indicating damage. Although the assay was not carried out for all the nitroimidazoles with interesting MIC values, and a similar activity may be assumed, factors such as drug solubility may be very crucial in such activity as was the case for 1-(p-nitrobenzyl)-2-nitroimidazole.

The half-wave potential ($E_1$) and e.s.r. spectroscopy confirmed the ability of the synthesised nitroimidazoles to accept an electron to form the reduced radical-anion. Unlike in e.s.r. studies, two $E_1$ readings were observed for each nitro group present in the molecule, this made it easy to compare $E_1$ values with reference 4- and 5-nitroimidazoles and hence predict their activity.
CHAPTER 5
GENERAL CONCLUSION

Although each section of the discussion bears its own conclusion, an overall summary was thought appropriate to correlate the results from different studies - the chemical in vitro studies, the biological screening studies, e.s.r. spectroscopy and measurement of redox potentials - for the synthesised nitroimidazoles.

$S_{RN^1}$ substitution and oxidative addition reactions proved useful for the synthesis of N-alkylated nitroimidazoles but yielded only the 4-nitroisomers when 4(5)-nitroimidazoles were used. The synthetic procedure was also successful for the preparation of a range of other nitroimidazole derivatives (see Chapter 2).

$\alpha$-Substituted 1,2-dimethyl-5-nitroimidazoles (except the quaternary ammonium derivatives) were shown to be more active against the anaerobes than the aerobes. MIC values for these nitroimidazoles against Cl. sporogenes range from 0.03µg/ml for the hydroxymethyl drug [112] to 62.5µg/ml for the thio derivatives.

The half-wave potential ($E_{1/2}$) measurements of these nitroimidazoles supported their antimicrobial properties. The $E_{1/2}$ values presented in Table 4.5 are all within the range observed for 5-nitroimidazole derivatives which have established activity e.g. metronidazole [1] (MIC = 0.2µg/ml), thereby indicating correlation between reduction potential and activity towards the anaerobes. Consequently, the $E_{1/2}$ measurements could possibly be used to distinguish between 4- and 5-nitroimidazoles, and also to predict the potential activity of a novel nitroimidazole.

The studies using e.s.r. spectroscopy showed that $\alpha$-substituted 1,2-dimethyl-5-nitroimidazoles formed stable radical-anions. Dissociation of these nitroimidazole radical-anions to radicals and anions was only observed in 2-(bromomethyl)-1-methyl-5-nitroimidazole [59] at 77K. It was not possible to detect dissociation of the other derivatives e.g. chloro [60], 2-pyridylthio derivatives (pirimidazole) [120], metronidazole and ronidazole. The e.s.r. spectroscopic studies did not show any significant difference in the structure between the radical-anions of the 4-
and 5-nitroimidazole derivatives. However, the $E_{1/2}$ measurements for both derivatives were significantly different, suggesting that the low activity of 4-nitroimidazoles as opposed to 5-nitroimidazoles can be explained by lack of reduction in biological systems and not difference in the structure of the radical-anion.

The 2-chloro derivative [60] was successfully reacted with thiolates in mode of action studies, either via an $S_N^2$ or $S_{ET}^2$ mechanism (see Chapter 3, Part B). Further research is required to determine the mechanism of the reactions. The antimicrobial activity of these $\alpha$-substituted 1,2-dimethyl-5-nitroimidazoles is similar; supporting the proposal that activity may not be totally dependent on the strength of the $C_2-CH_2-X$ bond but possibly also on the formation of radical-anions and whether the reactive species can survive dissociation before reaching the proposed site of action - DNA.17

4-Nitroimidazoles were shown to possess little or no antimicrobial activity compared with their 5-nitro analogs.13,24,44 The 2-nitroimidazoles are most active but are not significantly different from the 5-nitro derivatives.13,24,44 The e.s.r. spectroscopic studies of $N$-alkyl-4-nitroimidazoles synthesised showed that the extra electron resides on the nitro group on the $N$-1 alkyl group. Thus, the differences in activity (e.g. [70] and [66], a 2-nitro and 4-nitroimidazoles respectively - see Table 4.2) cannot be readily explained by differences in radical-anion structure, and other factors should be considered.

The $E_{1/2}$ measurements of the $N$-1 alkyl nitroimidazoles gave two readings for the two reducible nitro groups in each compound, neither of which was typical of the 4-nitroimidazole derivatives, but closer to the 5-nitro derivatives (Table 4.5). Consequently, it was inferred that the observed antimicrobial activity of these $N$-1 alkyl-4-nitroimidazoles against the clostridium species was due to the fact that they have a similar redox potential to that present in the anaerobic organisms. The MIC values for these nitroimidazoles, e.g. against Cl. sporogenes are within the accepted therapeutic range for potentially useful compounds (Table 4.2).
The $E_\frac{1}{2}$ measurements are also significantly different for the 2,4- and 5-nitroimidazoles, therefore providing a means of predicting the different antimicrobial activities.
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