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# ELECTROCHEMICAL CHARACTERISTICS OF NITRO-HETEROCYCLIC COMPOUNDS OF BIOLOGICAL INTEREST. III. NITROSO DERIVATIVE FORMATION

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Upon electrolytic reduction of a range of nitro-aromatic complexes (including imidazoles, benzenoids, furans and pyrazoles) an associated oxidation-reduction process is observed at more positive potentials with respect to nitro group reduction when using repeat scan cyclic voltammetry. This new couple has been identified as the reversible first reduction of the nitroso derivative for chloramphenicol, by the addition of a genuine sample of nitrosochloramphenicol to the electrochemical cell. We have failed to observe formation of the new redox-active species for five 5-nitroimidazoles examined.

Possible reaction schemes for nitroso formation under electrolytic reduction conditions and the importance of the nitroso redox couple with respect to the cytotoxic action of the parent drug are discussed. The applicability of nitrosochloramphenicol as a model for the behaviour of nitroso-heterocycles in general is shown.

KEY WORDS: Nitro-heterocycles, nitroso-derivatives, reduction, free radicals, cyclic voltammetry.



## INTRODUCTION

The selective toxicity of nitroimidazoles and related nitro compounds under conditions of low oxygen concentration is dependent on the reductive activation of the $NO_2$ group.[1,2] Whilst it is accepted that the major target for reduced nitro group action is DNA,[3,4] the product responsible has not been identified.[5] Both the 2 and 4 electron addition products (the nitroso and hydroxylamine derivatives respectively) have been put forward as possible candidates. Unfortunately, attempts to prepare stable nitroso derivatives of biologically important nitro-heterocycles have not been successful.

Here we present evidence that in an electrochemical cell, we can detect a nitroso redox couple by cyclic voltammetry upon reduction of a range of nitro-aryls (including imidazoles, benzenoids and pyrazoles). This presents the opportunity to study the electrochemical properties of a range of nitroso derivatives of biologically important drugs, which cannot be isolated by synthetic procedures.

## MATERIALS AND METHODS

The nitro-aromatic compounds were supplied as follows and used without further

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purification. Misonidazole, benznidazole and ornidazole from Roche Products Ltd.; metronidazole and M&B 4998 from May and Baker Ltd.; chloramphenicol from Sigma Chemical Co.; nitrofurazone from Smith, Kline and French Laboratories Ltd.; tinidazole from Pfizer Ltd.; nimorazole from Carlo Erba Ltd, Rome; satranidazole from Ciba Geigy, India.

For general details concerning the electrochemical techniques employed and the equipment used, see the preceding article, this issue. Repeat scan cyclic voltammetry (CV) was the primary electrochemical method used. The working electrode was a hanging drop mercury electrode, with scan rates ranging from 10 mV to 10 $VS^{-1}$. For scans faster than 500 $mVs^{-1}$ a Telequipment DM64 storage oscilloscope was used in place of a Bausch and Lomb RE 0088 x–y recorder. All potentials are quoted against an aqueous Ag/AgCl reference electrode.

## RESULTS

Upon reduction of a wide range of nitro-aromatic compounds in aqueous solution, only a single reduction process was observed.[6] This was true for nitro-benzenoids, -imidazoles, -furans and -pyrazoles. The reduction step was irreversible in nature, with no indications of any return wave on the reverse sweep of the cyclic voltammogram at scan rates up to 10 $Vs^{-1}$. From coulometric studies,[7] and by comparison with nitrobenzene, the electrochemical reduction of nitro-aromatics was assigned to an irreversible 4-electron reduction, yielding the hydroxylamine derivative. This can be simplistically represented as in equation (1)

$$RNO_2 + 4e^- + 4H^+ \rightarrow RNHOH + H_2O \quad (1)$$

However, although a return wave for the nitro group reduction was completely absent (not surprising considering the gross changes involved), an associated oxidation wave was frequently observed on the return sweep of the CV. This was clearly due to the formation of a new redox-active species as a consequence of the irreversible 4-electron reduction, being completely absent if the scan was reversed prior to the nitro group reduction. By repeat cycling, we could also detect the reduction wave of the new redox-active species (illustrated in Figure 1 for misonidazole). Thus both oxidized and reduced forms of the reduction generated complex have significant lifetimes on the timescale of the electrochemical experiment. The voltammetric characteristics of the new complex are listed in Table I for the nitro compounds where we have electrochemically detected the formation of the associated species. For comparison the reduction potential for the corresponding $NO_2$ group is also listed. The return-to-forward peak current ratio ($ip_r/ip_f$) for the electrogenerated couple approached unity in all examples, except nitrofurazone, where repeat cycling showed no corresponding response on the negative sweep, irrespective of scan rate (up to 10 $Vs^{-1}$) or switching potential. As the scan rate was varied, $E_{1/2}$ and $\Delta E_p$ values remained essentially unchanged, and a linear relationship was found for ip (oxidative scan) *vs* $v^{1/2}$. The response was thus in line with a reversible, diffusion controlled charge-transfer process.

It will be noted from Table I that the new complex is formed for a wide range of nitro-aromatic ring complexes, from 2-nitroimidazoles to -furans, -pyrazoles and -benzenoids. However, although we have examined a number of 5-nitroimidazoles (including metronidazole, tinidazole, satranidazole, ornidazole and nimorazole) we

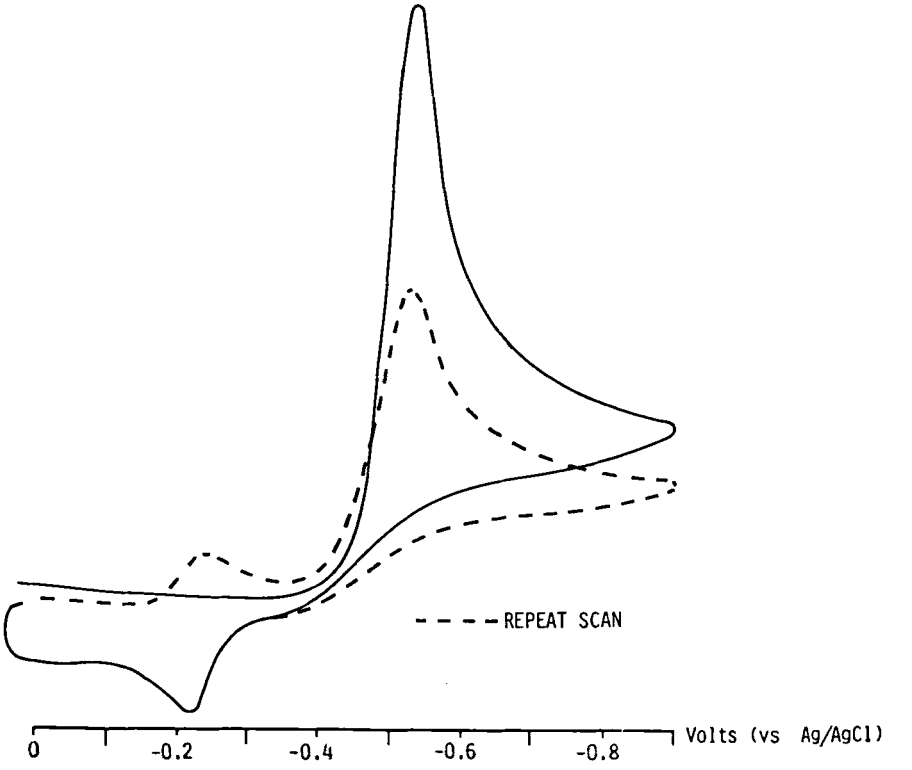


FIGURE 1 The cyclic voltammogramm of misonidazole, showing the effect of repeat scans.

TABLE I
Electrochemical characteristics of the nitroso couple[a]

| drug | $RNO_2/RNHOH$ $Ep_c(V)$ | redox-active product | | |
|---|---|---|---|---|
| | | $E_{1/2}(V)$ | Ep(mV) | $ip_r/ip_f$[b] |
| nitrofurazone | −0.410 | −0.070[c] | - | - |
| M&B 4998 | −0.742 | −0.245 | 40 | 1.0 |
| CAP | −0.615 | −0.130 | 40 | 0.95 |
| misonidazole | −0.540 | −0.230 | 35 | 0.95 |
| benznidazole | −0.495 | +0.025 | [d] | - |

[a] measured in 0.1 ssc buffer
[b] $ip_r/ip_f$ ratio determined at $v = 100\,mVs^{-1}$
[c] peak potential of forward "oxidation" wave
[d] return wave present but marred by proximity of Hg oxidation front

have found no evidence for the chemical reaction following the irreversible 4-electron reduction of the nitro group to yield the new redox-active product.

We noted the close similarity of the $E_{1/2}$ value and the general electrochemical behaviour of the new species produced on the reduction of chloramphenicol with that found from our independent studies on nitrosochloramphenicol (NO-CAP), see

preceding article, this issue. We showed conclusively that the electrode response appearing as a consequence of reduction of the nitro function was due to a nitroso couple by the addition of a genuine sample of NO-CAP to CAP in the electrochemical cell. We then observed a NO-CAP reduction at $E_{1/2} = -0.45\,V$, corresponding exactly with that for the electrochemically produced species, but now present on the *first* negative scan. On the reverse sweep, the oxidation wave of the nitroso couple has a greater response if the sweep range includes the reduction of CAP, than if $E_\lambda > Ep_c$ (CAP).

## DISCUSSION

The formation of a new redox-active species as a consequence of the electrochemical reduction of the nitro group has been observed for a number of nitro-aromatic compounds, ranging from imidazoles to furans, pyrazoles and benzenoids. The response has been conclusively identified for chloramphenicol as being due to the first redox couple for the nitroso derivative by the addition of a genuine sample of NO-CAP to the electrochemical cell. A similar assignment for the other nitro compounds where an associated redox couple has been found would seem appropriate. Information can therefore be gathered on the electrochemical properties of nitroso compounds which are not available by bulk preparation techniques.

There are a number of explanations as to how the nitroso couple arises from nitro group reduction. Assume initially that the nitroso couple involves a 1-electron transfer, a $RNO/RNO^{\cdot-}$ couple, this was the more likely mechanism as discussed in detail for the electrochemistry of NO-CAP, (see preceding article, scheme 1). Although the electrochemistry of the parent nitro-compounds suggests a straightforward 4-electron conversion to the hydroxylamine,[5] we know very little concerning the efficiency of this process, and it is possible to envisage the formation of intermediate reduction products. RNO and $RNO^{\cdot-}$ involve the addition of 2 and 3 electrons respectively. Nitroso is also the product of the disproportionation pathway of the nitro radical anion[8] (equation 2)

$$2RNO_2^{\cdot-} + 2H^+ \Leftrightarrow RNO_2 + RNO + H_2O \qquad (2)$$

We have noted that when mixed solvents have been employed to permit identification and stabilization of the electrochemically generated $RNO_2^{\cdot-}$,[6,9] the electrode response for the nitroso couple was significantly decreased when the scan range included *only* the $RNO_2/RNO_2^{\cdot-}$ couple. If the following irreversible 3-electron addition to form the hydroxylamine was included in the potential sweep, the nitroso couple was similar to that in purely aqueous media. In this instance therefore, equation (2) appears to represent a relatively minor route to nitroso formation.

The nitroso derivative may also be generated by reaction of the hydroxylamine with unreduced $RNO_2$ in the bulk solution (equation 3). This mechanism would be favoured by mixed-solvent data where significant nitroso couple response was only observed after formation of the hydroxylamine derivative.

$$RNO \xrightarrow{+4e^-,+4H^+} RNHOH + RNO_2 \rightarrow 2RNO + H_2O \qquad (3)$$
$$\text{(in bulk)}$$

Obviously at the potentials required to reduce $RNO_2$, the RNO formed by any of the above reactions would immediately undergo reduction to $RNO^{\cdot-}$.

If the more unlikely alternative of the nitroso couple involving a 2-electron step occurred, then the cyclic voltammetry is detecting the hydroxylamine derivative formed upon the 4-electron reduction of the $NO_2$ group (equation 4)

$$RNO_2 \xrightarrow{+4e^-,\ +4H^+} RNHOH \underset{}{\overset{+2e^-,\ +2H^+}{\rightleftharpoons}} RNO \qquad (4)$$

Although we have made repeated attempts, we have failed to detect a nitroso couple for any of the five 5-nitroimidazoles examined. The reason for the difference in behaviour of the 5-nitroimidazoles is unclear. It is possible that the 5-nitrosoimidazole couple may be shifted to positive potentials outside the range of the Hg working electrode. Alternatively either RNO or $RNO^{\cdot-}$ may be too reactive to observe on the electrochemical time-scale (*e.g.* at a scan rate of $10\,Vs^{-1}$, lifetime less than 50 to 100 msec.), or a different reaction pathway entirely is followed. All are feasible explanations. It should also be noted that the 5-nitroimidazoles are the only nitro-aromatics to produce significant quantities of nitrite under bulk reduction conditions.[7] It would appear that some differences in the reduction mechanism of the 5-nitroimidazoles exist as compared to the other nitro-aromatics. The importance of this with repsect to their biological activity is unknown, but is receiving attention.

The identification using repeat scan cyclic voltammetry of a nitroso couple upon reduction of a range of nitro-aromatic compounds, has allowed us to determine some of the electrochemical characteristics of nitroso derivatives of possible biological importance, which are not available via bulk synthetic techniques. Electrochemistry thus allows us to generate and record the behaviour of the nitroso couple *in situ* at the working electrode surface, without the need to isolate the reactive nitroso species.

Our studies have demonstrated that in terms of the electrochemical characteristics, nitrosochloramphenicol (NO-CAP), represents a good model system for investigating the biological activity of related nitroso derivatives of heterocyclic compounds.

In the extensive examinations which have been carried out on the complete range of nitro-aromatic compounds, no data has been obtained to suggest that the damaging species produced on reductive activation, or that the mechanism of cytotoxicity is different for any particular heterocycle.[10,11,12] From the present work we can detect *no* evidence for the formation of a nitroso couple for the 5-nitroimidazoles, which would suggest that the nitroso derivative does not play a major role in the selective hypoxic cytotoxicity of the nitro-heterocycles. The apparent differences in the overall reduction mechanism of the 5-nitroimidazoles does not appear to affect its DNA damaging ability, but may influence the side-effects produced.



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