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Reduction Potentials in Relation to Physiological Activities of Benzenoid and Heterocyclic Nitroso Compounds: Comparison with the Nitro Precursors

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Reduction potentials were determined for various physiologically active benzenoid and heterocyclic nitroso compounds, namely, substituted nitrosobenzenes, 1-nitrosopyrene, and 1-methyl-2-nitrosoimidazole. The values, favorable for biological activity, ranging from 0.2 to −0.2 V, increased in acidic medium. These potentials were appreciably higher than those for the corresponding nitro parents. In most cases, the nitroso form was more biologically active than the nitro counterpart. Catalytic electron transfer processes may play a role *in vivo*, along with other actions, in the observed responses from the nitroso category. © 1990 Academic Press, Inc.

INTRODUCTION

Nitro derivatives of heteroaromatic compounds display physiological activity of various types (*1*), including antibacterial, amebicidal, anthelmintic, antiprotozoan, and carcinogenic (*2*). The mechanism of action for this general class has been reviewed recently (*3–9*). Involvement of redox processes via electron transfer (ET) is well documented, as evidenced by the generation of superoxide, hydrogen peroxide, and hydroxyl radicals. In an aerobic environment, an intermediate radical ion can transfer an electron to oxygen. The *in vivo* effects are inhibited by superoxide dismutase and catalase. In some cases, e.g., radiosensitization (*10*), activity is demonstrated under anaerobic conditions, indicating that the radical anion can function by other routes, such as interference with normal electron transport chains or direct cleavage of DNA (*11*). Various *in vivo* responses are also elicited by a number of nitrobenzenoid compounds of which the best known is chloramphenicol (*1*). Almost all of the nitro derivatives in the two categories possess favorable reduction potentials (*12–15*). In certain prior investigations a correlation existed between $E^{0\prime}$ or electron affinity and activity (antibacterial, mutagenic, radiosensitization, and toxicity) (*3–9, 16, 17*), lending credence to the proposed mode of action.

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Although substantial evidence supports a catalytic role for the nitro aromatic agents in redox cycling, questions have been raised concerning the possible participation of stable reduced metabolites (*18, 19*). When intermediates are considered, attention has usually been focused on the hydroxylamine stage (*20*). The same type of intermediates can be generated oxidatively from aromatic amines, such as dapsone (*20*); again, the hydroxylamine has been emphasized. Interconversion of nitroso and hydroxylamine appears to be facile; oxidation of the hydroxylamine can occur enzymatically (*21*).

In the nitroso category, 2-nitrosofluorene, which is neoplastic and highly mutagenic, is the principal oxidative product formed from *N*-hydroxy-*N*-2-fluorenylacetamide on exposure to lactoperoxidase. The precursor is commonly designated as the proximate carcinogenic metabolite of *N*-2-fluorenylacetamide (*22*). 2-Nitroso-1-naphthol, a suspected carcinogen, is apparently generated *in vivo* from tumor-producing 2-naphthylamine. From ESR studies on the nitroso derivative, free radicals were the primary metabolites formed during both reduction and oxidation (*23*). In the presence of hydrogen peroxide, enzymatic oxidation of arylamines, another carcinogenic class, to the corresponding nitroso compounds appears to be a fairly general process (*24*). Studies showed that oxidative metabolites, including nitroso, of procainamide (aromatic amine), are the active immunopharmacological agents (*25*). Activities of nitroso compounds investigated in the present work are presented under Results and Discussion.

Aromatic nitroso compounds are intense blue or green substances that equilibrate to various extents with colorless dimers. When the solid exists in the dimer form, dissociation may occur in solution as indicated by the development of color (*26*). *In vivo* it is likely that only the monomer is involved due to the low concentration. Earlier studies showed that nitroso on the benzenoid nucleus exerts a positive effect on the reduction potential. Thus, nitrosobenzene gave $E^{0\prime}$ of 0.25 V, much more positive than the values ($E_{1/2}$ of -0.68 to -0.91 V) for nitrobenzene (*27*).

During the past several years there has been a surge of interest involving the nitroso derivatives. Most of the investigations were concerned with the physiological characteristics. The present report addresses the electrochemical properties, mainly reduction potentials, of nitroso agents in the benzenoid, polynuclear, and heteroaromatic categories, which display various types of activity *in vivo*. Comparisons are made with the parent nitro compound, and some correlations are pointed out involving reduction potential, activity, and structure.

MATERIALS AND METHODS

1-Nitropyrene and 1-nitrosopyrene were obtained from Dr. Karam El-Bayoumy (American Health Foundation, Valhalla, NY). 1-Methyl-2-nitroimidazole, 1-methyl-2-nitrosoimidazole (*28*), nitrosochloramphenicol (*29*), and the substituted nitroso aromatics (*30*) were prepared by literature methods. The electrolyte used was tetraethylammonium perchlorate (0.1 M) (G. F. Smith Co., Columbus, OH). Absolute ethanol for solution preparation was purchased from U.S. Industrial

(Tuscola, IL). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All compounds were investigated at a concentration of 0.5 mM.

The cyclic voltammetric measurements were performed at ambient temperature with an IBM Corp. Model ED 225 voltammetric analyzer associated with a Houston Instrument Model 200 X-Y recorder. The operation of the instrument and the electrodes was checked against a benzil standard before each use. The scan rate generally ranged from 20 to 200 mV/s. Solutions were purged of oxygen for 15 min with prepurified nitrogen. The working electrode consisted of a hanging mercury drop electrode (HMDE). A platinum wire was used as the counter and saturated calomel (SCE) was the reference electrode. Observed potentials were converted to the normal hydrogen electrode (NHE) reference by adding 0.24 V to the SCE values. The reported data are an average of two or more measurements involving freshly made solutions.

The following equations were used for the half-wave potentials and current function calculations, respectively: $E^{0\prime} = [(E_{pc} + E_{pa})/2]$, and $CF = i_p/V^{1/2} \times C(A/V/s)^{1/2}M)$.

RESULTS AND DISCUSSION

Nitrosobenzenes

Chloramphenicol **1a** (Fig. 1), a powerful antibiotic, is the leading cause of drug-induced aplastic anemia (*29*). There is speculation that the side effect might be due to production of toxic metabolites which are intermediate reduction products of the nitro group. Support for this idea is provided by investigations with the analog, thiamphenicol, which lacks the nitro moiety, and has not been found to cause aplastic anemia. Examination of the nitroso analog revealed appreciably lower antibiotic activity. The nitroso derivative displays cytotoxic/genotoxic effects (*31*), and is more toxic than the parent drug. The single-strand breakage in DNA might be caused by the production of superoxide anion radicals resulting from redox cycling involving nitroso.

Nitrosochloramphenicol **1b** reduced in a two step process, with the first reaction (reversible, $i_{pa}/i_{pc} = 0.92$) occurring in a one-electron transfer with $E^{0\prime}$ of $+0.14$ V (CF ratio of 1.01). The larger second peak was irreversible with an $E_{p/2}$ value of -0.51 V. Recent studies found values of about $+0.2$ (reversible) and -0.4 (irreversible) V, respectively, for the peaks (*32*).

Nitrosobenzene and substituted derivatives cause hemolytic anemia (*33*). Of various p-substituted ones tested for mutagenic activity, the *p*-phenoxy derivative was found to be the most potent (*34*).

The reduction potentials of several simple substituted nitrosobenzenes and their parent nitro compounds were also determined (Tables 1 and 2 and Figs. 2 and 3). Electroreduction of nitrobenzenes produced reversible reductions in the range of -0.58 to -0.81 V. Upon addition of acid, the peak shifted in the positive direction about 200 mV in all cases. The reactions were diffusion controlled as evidenced by independence of CF versus scan rate. Prior literature on halogenated nitrobenzenes

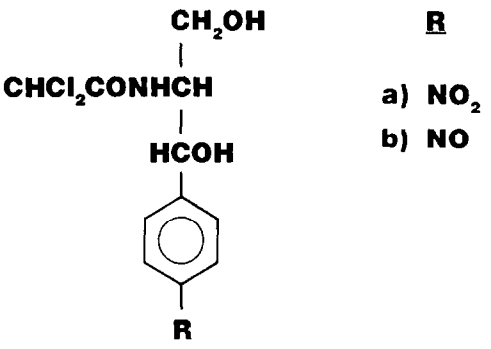

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FIGURE 1

reports $E_{1/2}$ values ranging from -0.70 to -0.40 V (*27*), with positive shifts at lower pH.

The nitrosobenzenes gave $E^{0\prime}$ values from $+0.04$ to $+0.25$ V. Presence of the nitroso monomer was indicated by a blue or green solution. At greater acidity (pH 5.0), the values shifted anodically by 0.15 to 0.21 V. All exhibited constant CF and showed linearity in the plot of peak current (i_p) versus the square root of the sweep rate. For alkyl and halo nitrosobenzenes, as well as the parent, literature values range from 0.0 to $+0.6$ V (*35, 36*).

TABLE 1

Reduction Potentials of Nitrobenzenes[a]

Compound	$E^{0\prime}$ (V)	i_{pa}/i_{pc}	CF ratio[b]
2a	-0.58	0.98	0.96
2a	-0.51[c]		
2b	-0.80	1.07	0.97
2b	-0.60[c]		
2c	-0.68	0.96	0.87
2c	-0.52[c]		
2d	-0.80	1.01	0.98
2d	-0.59[c]		
2e	-0.81	0.97	1.02
2e	-0.60[c]		

[a] 100 mV/s, HMDE, 50% EtOH, versus NHE.
[b] CF_{benzil} = 16.27 (0.5 mM, Pt); 0.185 (0.5 mM, HMDE).
[c] pH 5.0.

TABLE 2

Reduction Potentials of Nitrosobenzenes[a]

Compound	$E^{0\prime}$ (V)	i_{pa}/i_{pc}	CF ratio[b]
3a	0.20	0.96	1.81
3a	0.41[c]		
3b	0.15	1.02	1.72
3b	0.45[c]		
3c	0.23	0.96	1.75
3c	0.41[c]		
3d	0.18	0.87	1.82
3d	0.43[c]		
3e	0.15	0.97	1.83
3e	0.46[c]		
3f	0.10	0.95	1.64
3f	0.35[c]		
3g	0.04	0.94	1.60
3g	0.31[c]		
3h	0.25	0.94	1.57
3h	0.39[c]		

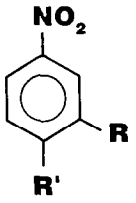
[a] 100 mV/s, HMDE, 50% EtOH, versus NHE.
[b] CF_{benzil} = 16.27 (0.5 mM, Pt); 0.185 (0.5 mM, HMDE).
[c] pH 5.0.

1-Nitrosopyrene

Many environmental sources, including diesel exhaust, contain polycyclic hydrocarbons, such as 1-nitropyrene **4a** (Fig. 4) which is mutagenic and carcinogenic (*37*). Reduction of the nitro function is required for the expression of mutagenicity (*38*). Since **4a** does not react with DNA directly, the genotoxicity must be mediated through metabolism to an intermediate which binds DNA. 1-Nitrosopyrene **4b,** which is the binding entity *in vitro*, is more mutagenic than **4a.** Depending upon the conditions, the nitroso form can be more (*39*) or less (*37*) carcinogenic than the nitro parent.

In our electrochemical studies (Table 3), **4a** underwent a one-electron reduction which exhibited reversibility by the presence of a reoxidation wave and an i_{pa}/i_{pc} value of 0.97. The peak was sharp, with $E^{0\prime}$ of -0.73 V. When the electrochemistry was performed at pH 5.0, the peak shifted +150 mV, $E^{0\prime} = -0.58$ V. Compound **4a** is reported to have an $E_{1/2}$ of -0.51 V (*40*). Compound **4b** produced a reversible ($i_{pa}/i_{pc} = 0.88$) wave with $E^{0\prime} = -0.21$ V, and a smaller, irreversible peak at $E_{p/2} = -0.47$ V. At pH 5.0, the peak broadened slightly, with a positive shift to $E^{0\prime} = -0.11$ V for the reversible peak, and $E_{p/2} = -0.36$ V for the irreversible one.

In prior work with nitro derivatives of polynuclear aromatic hydrocarbons, including **4a,** a comparison of reduction potential with activity showed that the strongest mutagens possessed the most positive $E_{1/2}$ values (*40, 41*).



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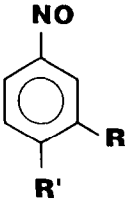
	R	R'
a)	H	F
b)	H	Cl
c)	Cl	Cl
d)	H	Br
e)	H	I

FIGURE 2

1-Methyl-2-nitrosoimidazole

Nitroimidazoles see use against a variety of anaerobic bacterial and protozoal infections (*42*) and are currently in clinical trials as radiation sensitizers of hypoxic tumor cells (*43*). Extensive examinations have shown a number of effects which correlate with reductive metabolism (*44*), including a preferential toxicity toward hypoxic cells as compared to aerobic cells (*45*), mutagenicity (*46*), chemosensitization (*47*), DNA binding (*48*), and depletion of cellular thiols (*49*). The model for these various effects involves metabolic reduction, with some species produced by that reduction being biologically active. The selective toxicity toward hypoxic cells arises since high concentrations of oxygen inhibit reduction. Nitroimidazoles are generally less electron affinic than oxygen, so that the product of one-electron reduction of the former transfers its extra electron to the latter reverting to parent nitroimidazole (*50*).

The implication that reduction plays a role in the mechanism of action has led to an interest in the chemistry involved, and a search for the biologically active species. The six-electron reduction products, aminoimidazoles, are known compounds, but are biologically inactive (*51*). 2-Hydroxylaminoimidazoles, arising



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	R	R'
a)	H	F
b)	H	Cl
c)	Cl	Cl
d)	H	Br
e)	H	I
f)	H	CH_3
g)	H	C_6H_5
h)	H	NO_2

FIGURE 3

TABLE 3
Cyclic Voltammetry of 1-Nitro- and 1-Nitrosopyrene[a]

Compound	$E^{0\prime}$ (V)	i_{pa}/i_{pc}
4a	−0.73	0.97
4a[b]	−0.58	—
4b	−0.21	0.88
	−0.47[c]	
4b[b]	−0.11	—
	−0.36[c]	

[a] 100 mV/s, HMDE, 50% EtOH, versus NHE.
[b] pH 5.0.
[c] $E_{p/2}$.

from four-electron reduction, have also been prepared (*52*). These are unstable under physiological conditions, undergoing chemical reactions which account for some of the biological effects of nitroimidazoles, in particular, DNA and thiol binding (*52*). However, neither the hydroxylamine nor its major decomposition product shows enhanced toxicity toward cells (*28, 53*).

Recently, the preparation of the first member of the 2-nitrosoimidazole class, 1-methyl-2-nitrosoimidazole **5b** (Fig. 5), has been accomplished (*28*). The compound exists in the monomer form both in the solution (*54*) and in the crystal (*55*). Like the hydroxylamine, compound **5b** is unstable at physiological pH, undergoing rapid reactions with thiols and ascorbic acid (*55*), and even decomposing in phosphate-buffered solutions (*28*). Compound **5b** is reported to be more toxic than the parent compound, 1-methyl-2-nitroimidazole **5a** (*56*).

In electrochemical studies (Table 4), compound **5a** underwent one-electron reduction, as evidenced by a CF ratio of 0.94 (benzil as reference). The reversible reaction (i_{pa}/i_{pc} = 1.0) occurred at $E^{0\prime}$ of −0.71 V. At reduced pH (5.0) the value shifted about 100 mV to −0.61 V. Compound **5b** reacted in a reversible manner with $E^{0\prime}$ = −0.18 V in neutral aqueous solution. The constant CF of 16.3 and

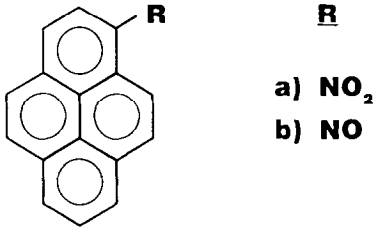

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FIGURE 4

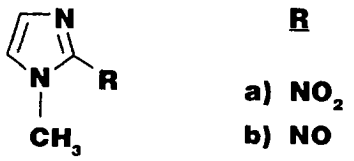

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FIGURE 5

linear plot with intercept of zero for the peak current versus the square root of the sweep rate indicated a diffusion controlled process. At pH 5.0, one-electron reduction took place at $E^{0\prime}$ of -0.04 V, with 60 mV separating the cathodic and anodic peaks.

In vitro nitrosoimidazole **5b** is cytotoxic in micromolar quantities (*28, 56*), while much higher concentrations are required for toxicity with the parent nitro compound, as well as with hydroxylamino and amino reduction products. A further key observation is that the toxicity of **5b** is the same with aerobic and hypoxic cells (*28, 56*), unlike the situation with the parent nitro compound. Thus, the nitrosoimidazole is beyond the stage of the oxygen inhibition which results in nitroimidazoles having an aerobic–hypoxic differential. A further observation is that allowing **5b** to decompose prior to addition to the cells results in complete loss of activity (*28*). At concentrations where the toxicity of **5b** first appears, an assay of the intracellular thiol glutathione reveals that the latter has been depleted, down to 10–20% of the original level (*56, 57*). There also appears to be significant removal of protein thiol groups (*58*). The nitrosoimidazole has also been demonstrated to cause strand breaks in DNA (*59*). At the present time the hypothesis which is most consistent with the experimental results is that the nitrosoimidazole is the toxic species arising on reduction of nitroimidazoles. The favorable reduction potential, more positive than for **5a**, is consistent with a catalytic ET mechanism involving **5b**, conceivably stabilized by site binding.

TABLE 4

Electrochemical Characteristics of 2-Nitro- and 2-Nitroso-1-methylimidazole[a]

Substrate	$E^{0\prime}$ (V)	i_{pa}/i_{pc}	CF ratio[b]
5a	−0.71	1.0	0.94
5a	−0.61[c]	0.97	0.93
5b	−0.18	0.97	1.57
5b	−0.04[c]	0.95	1.57

[a] 100 mV/s, HMDE, 50% EtOH versus NHE.
[b] CF_{benzil} = 16.27 (0.5 mM, Pt); 0.185 (0.5 mM, HMDE).
[c] pH 5.0.

Electrolytic reduction of various heteroaromatic nitro drugs in the imidazole, furan, and pyrazole classes was recently examined (*60*). Reduced redox-active products, apparently the nitroso counterparts, were formed, which displayed reduction potentials in the favorable range of 0.2 to −0.05 V. In contrast with the 2-nitroimidazoles, the 5-nitro derivatives gave no evidence for generation of redox-active materials (*60, 61*).

OTHER CONSIDERATIONS

How might electrochemical events translate into drug action? After site fixation, ET could produce adverse effects on vital cell reactions, leading in turn to the observed physiological activity. Application of the ET concept to the area of nitro compounds is not novel. Previous articles have shown the possibility of ET through redox cycling to be a viable mechanism of action (*12–15*). In this study, we have focused on the nitroso product, which can be one of the intermediates in the reduction of nitro compounds, and in most cases is more active than the nitro counterpart (*31, 38, 39, 56*). The nitroso form may effect catalytic ET, which would be favored by hydrogen bonding, resulting in the biological properties exhibited by these compounds. Other modes of action are also probably involved.

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