Iminoxy1 radicals and stable products from the one-electron oxidation of 1-methylindole-3-carbaldehyde oximes

Steven A. Everett,* Matthew A. Naylor,* Michael R. L. Stratford,* Kantilal B. Patel,* Eleonora Ford,* Alan Mortensen,* Amanda C. Ferguson,* Borivoj Vojvovic* and Peter Wardman*

* Gray Cancer Institute, PO Box 100, Mount Vernon Hospital, Northwood, Middlesex, UK HA6 2JR. E-mail: everett@graylab.ac.uk
** Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

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The radical intermediates and the stable products formed on one-electron oxidation of 1-methylindole-3-carbaldehyde oxime 2a were compared with those of 1-methylindole-3-carboxamidine oxime 4a in aqueous solution. The dibromide radical anion generated radiolytically by pulse radiolysis reacted with both 2a and 4a ≥C=NOH to yield the radical cations [≥C=NOH] +, which exist in prototrophic equilibria with the neutral iminoxy1 radicals [≥C=NO] (pKₐ = 3.53 ± 0.03 and 5.01 ± 0.01 at ionic strength 0.05 M, respectively). This was confirmed by the observed primary salt-effect which accelerated the decay of the radical cations but not the iminoxy1 radicals. Methylation of the N-hydroximino function in both 2a and 4a precluded deprotonation of the corresponding radical cations [≥C=NOCH] +. At low concentrations of 2a and high dose rates the 2a radicals [≥C=NO] decayed bimolecularly via unstable dimers to the aldehyde ≥C=O, with higher concentrations and lower dose rates favouring the chain-catalysed isomerisation of the N-hydroximino moiety. Radicals from 4a decay bimolecularly to form unstable dimers which degrade to produce an amide, nitrile and carboxylic acid. The observed differences in the oxidation chemistry of 2a and 4a probably reflect the enhanced stabilisation of iminoxy1 radicals through α-amino substitution.

Introduction

Iminoxy1 radicals (≥C=NO) were first characterised by Thomas in 1964 using electron paramagnetic resonance (EPR) spectroscopy. Since then a number of EPR studies have been reported utilising a range of chemical1–4 and enzymatic systems,5–8 for the oxidation of alkylaryl oximes to iminoxy1 radicals. Other approaches rely on radical-addition to aromatic nitrile oxides.9 The iminoxy1 radical is also formed when nitric oxide interacts with the tyrosine radical of photosystem II10 and the tyrosyl radical of prostaglandin H synthase-2.11 In all cases, EPR spectroscopy has been valuable in detecting iminoxy1 radicals which are characterised by a large splitting, aₙ ≈ 30 G, due to nitrogen. The electronic structure of iminoxy1 radicals, as deduced from EPR spectra, place the unpaired electron in a π-type orbital derived from a nitrogen sp³ orbital and an oxygen p orbital. This π-type orbital is believed to lie in the nodal plane of the C-N π bond, which requires it to be orthogonal to the molecular π system, so that iminoxy1 radicals have been described as σ radicals. Iminoxy1-type radicals (both O- and N-centred radicals) are possible candidates in the nitric oxide synthase (NOS)-catalysed oxidation of N⁰-hydroxy-l-arginine to nitric oxide and citrulline.12,13 They are also believed to be putative intermediates in the oxidation of aryl oximes by cytochrome P450 mono-oxygenases14,15 and NOS.16 In the present work the technique of pulse radiolysis has been utilised, in which a known concentration of free radicals is generated in less than a microsecond, to study the one-electron oxidation of 1-methylindole-3-carbaldehyde oxime derivatives (see Fig. 1) by the dibromide radical anion (Br₂⁻). Pulse radiolysis has been used extensively to study the spectral characteristics, acid–base, and redox properties of radicals from the oxidation of indole-based compounds. For example, the radical cations of tryptophan17 and indole-3-acetic acids can deprotonate to form indolyl radicals, the latter case in competition with oxidative decarboxylation to form the skatole radical.18–22 The radical cation of N-methylindole is not believed to deprotonate over the pH range of interest in this study23–24 so the indole oximes (Fig. 1) have been N¹-methylated in order to study their iminoxy1 radicals in isolation. Steady-state γ-radiolysis has been used to generate free radicals at a known, constant rate and, in combination with liquid chromatography, to study the stable products of free radical reactions and determine their yields relative to the precursor free radicals. α-Amino substitution in 1-methylindole-3-carbaldehyde oxime 2a (to generate 1-methylindole-3-carboxamidine oxime 4a) has a significant impact on both the physicochemical properties of the iminoxy1 radicals and the subsequent product profiles.

Fig. 1 Structures of 1-methylindole-3-carbaldehyde oxime derivatives.

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Results and discussion

Synthetic chemistry

The synthetic pathways to 1-methylindole-3-carbaldehyde oxime derivatives and their oxidation products are outlined in Scheme 1. Methylation and Vilsmeier formylation were followed by reaction with hydroxylamine to furnish the required aldehyde oxime 2a. The N-hydroxymino group exhibits (Z)-geometry relative to the indole core as determined by X-ray crystallography.²⁵ Photolysis of 2a resulted in a stoichiometric conversion to the corresponding geometric (E)-isomer 2b.²⁶ The sodium salt of 2a was O-methylated with iodomethane to give 6. Aldehyde oxime 2a was dehydrated to give the nitrile 3 with refluxing acetic anhydride-sodium acetate and this material was treated with hydroxylamine to give the required carboxamidine oxime 4a which was then converted into 4b by photolysis. This oxime could not be O-methylated with iodomethane via its sodium salt but the O-methyl derivative 5 was synthesised in low yield using trimethyloxonium tetrafluoroborate. The putative oxidation product, amide 8, was prepared from methyl indole-3-carboxylate by methylation and hydrolysis to the free acid followed by treatment with carbonyldimidazole and then ammonia.

Free radical chemistry

The transient species formed by the one-electron oxidation of the oximes 2a, 4a and their corresponding O-methylated derivatives 6 and 5 by the dibromide radical anion \( \text{Br}_2^\text{-} \), \( E^\circ = 1.66 \text{ V vs NHE} \) were studied by pulse radiolysis. Spectral characteristics, prototropic equilibria and rate constants for the formation and decay of radicals are displayed in Table 1.

One-electron oxidation of 1-methylindole-3-carbaldehyde oxime 2a. The dibromide radical has a strong absorption in the wavelength range \( ca. 300 – 500 \text{ nm} \) \( (\lambda_{\text{max}} = 360 \text{ nm}) \).²⁷ In the absence of 2a, the \( \text{Br}_2^\text{-} \) radical decayed by a second-order process with a first half-life of ca. 0.2 ns, at an initial concentration of ca. 2.5 \( \mu\text{mol \text{ dm}^{-3}} \). In the presence of 25–100 \( \mu\text{mol dm}^{-3} \) 2a and at pH 7.4, the decay of the \( \text{Br}_2^\text{-} \) radical, monitored by the decrease in absorbance at 360 nm, followed pseudo-first-order kinetics with a rate constant proportional to the concentration of 2a. An increase in absorbance at 630 nm with the same rate constant (±10) was observed. These results indicated that the \( \text{Br}_2^\text{-} \) radical reacts with 2a; the rate constant for the reaction (1), obtained from the slope of the linear plot of pseudo-first-order rate constant of the build-up of absorption at 630 nm \( \text{versus concentration of 2a} \), was \( k_1 = (8.2 \pm 0.2) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) at ionic strength ca. 0.05 mol dm\(^{-3}\).

\[
\text{Br}_2^- + \text{C=NOH} \rightarrow [\text{C=NOH}]^+ + 2\text{Br}^- \tag{1}
\]

The absorption spectra of the radicals from the oxidation of 2a were determined at pH 2.5 and 7.4 and are shown in Fig. 2. At pH 2.5 two maxima are observed at 350 and 705 nm, with extinction coefficients 2868 and 604 \( \text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} \), respectively. This spectrum is attributed to the radical cation 2a \([\text{C=NOH}]^+\) formed \( \text{via} \) reaction (1). In neutral solution the radical cation deprotonates to the neutral iminoxyl radical 2a \([\text{C=NO}]^\cdot\) the spectrum of which is characterised by two absorption maxima at 350 and 620 nm, with extinction coefficients of 1810 and 420 \( \text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} \), respectively.

Confirmation that the absorption spectra in Fig. 2 were
Table 1  Spectroscopic characteristics, prototropic equilibria and rate constants for the formation and decay of radicals generated by the oxidation of 1-methyl-3-carbaldehyde oxime derivatives by the Br₂⁻ radical anion

<table>
<thead>
<tr>
<th>Radical species</th>
<th>pH</th>
<th>λmax/nm</th>
<th>εmax/M⁻¹ cm⁻¹</th>
<th>Radical pKₐ</th>
<th>kₑ/10⁶ dm³ mol⁻¹ s⁻¹</th>
<th>2kₑ/sec/10⁶ dm³ mol⁻¹ s⁻¹</th>
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<tbody>
<tr>
<td>2a⁺⁺</td>
<td>2.5</td>
<td>350</td>
<td>2688 ± 50</td>
<td></td>
<td>3.53 ± 0.03</td>
<td>8.2</td>
</tr>
<tr>
<td>2a⁻(−H)⁺⁻</td>
<td>5−9.5</td>
<td>705</td>
<td>604 ± 20</td>
<td>705</td>
<td>604 ± 20</td>
<td>8.2</td>
</tr>
<tr>
<td>2a⁻(−H)⁺⁻</td>
<td>5−9.5</td>
<td>350</td>
<td>1800 ± 50</td>
<td>620</td>
<td>420 ± 20</td>
<td>5.7</td>
</tr>
<tr>
<td>4a⁺⁺</td>
<td>3</td>
<td>335</td>
<td>2100 ± 100</td>
<td>670</td>
<td>350 ± 20</td>
<td>5.7</td>
</tr>
<tr>
<td>4a⁻(−H)⁺⁻</td>
<td>7−9.5</td>
<td>330</td>
<td>1700 ± 40</td>
<td>580</td>
<td>330 ± 170</td>
<td>5.01 ± 0.05</td>
</tr>
<tr>
<td>6⁺⁺</td>
<td>3−9</td>
<td>340</td>
<td>2575 ± 200</td>
<td>720</td>
<td>520 ± 180</td>
<td>4.1</td>
</tr>
<tr>
<td>5⁻⁻</td>
<td>3−9</td>
<td>350</td>
<td>2200 ± 200</td>
<td>750</td>
<td>410 ± 20</td>
<td>2.3</td>
</tr>
</tbody>
</table>

a Rate constants in H₂O are subject to a standard deviation of 10% and in D₂O of 15%. ¹[NaClO₄] = 500 mmol dm⁻³.

indeed due to the formation of the radical cation 2a [C=NOH]⁺ is ascribed to the kinetic isotope-effect in heavy water (D₂O) on the formation kinetics and the primary salt-effect on the decay kinetics of these radicals. At pH 2.5, the rate constant for the oxidation of 2a in D₂O was similar to that in H₂O within experimental error, kₑ = (8.2 ± 0.2) × 10⁶ dm³ mol⁻¹ s⁻¹ and (7.9 ± 0.2) × 10⁶ dm³ mol⁻¹ s⁻¹, respectively. The ratio of rate constants kₑ for the oxidation of 2a by the Br₂⁻ radical in H₂O and D₂O is 0.963, a value which is significantly higher than the ratio of viscosities of H₂O and D₂O, which is 0.8904 cP/1.0952 cP or 0.813.²⁴ The absence of a measurable kinetic isotope effect argues against a hydrogen atom transfer mechanism in favour of an electron-transfer mechanism to generate the radical cation 2a [C=NOH]⁺ as illustrated by reaction (1).

At both pH 2.7 and 7.4 and low concentrations of 2a ([2a] = 50 mmol dm⁻³), the radicals decayed on a millisecond timescale with second-order kinetics, where the rate of decay increased proportionally with the radical concentration (1−15 mmol dm⁻³). At pH 2.7, the rate constant 2kₑ₂a = (5.7 ± 0.2) × 10⁶ dm³ mol⁻¹ s⁻¹ for bimolecular decay of the radical cation 2a [C=NOH]⁺ via reaction (2a) was obtained from the slope of the linear plot of the inverse of the first half-life (= 2kₑ₂a × initial concentration) for the decay in absorbance at 720 nm versus the initial radical concentration.

\[
\frac{[\text{C=NOH}]^+} + \frac{[\text{C=NOH}]^+} → \text{product(s)} \quad (2a)
\]

\[
\frac{[\text{C=NO}]^-} + \frac{[\text{C=NO}]^-} → \text{product(s)} \quad (2b)
\]

The inclusion of 0.5 mol dm⁻³ NaClO₄ increased the rate of radical decay by a factor of 2.7 to 2kₑ₂a = (1.6 ± 0.2) × 10⁶ dm³ mol⁻¹ s⁻¹ and is broadly consistent with the acceleration of the rate of decay of a species with net charge ±1, namely the radical cation 2a [C=NOH]⁺. According to the Debye–Hückel–London–Braunsted–Davis equation,²⁵ the rate of second-order decay of a charged radical (either monopositive or mononegative) would have increased by a factor of 1.8, a value lower than determined experimentally, but the formula is not expected to be accurate at such a high ionic strength. As expected, the increased salt concentration had a negligible effect on the decay of the neutral iminoxyl radical 2a [C=NO]⁻ via reaction (2b): the values obtained in the absence and presence of 0.5 mol dm⁻³ NaClO₄ were determined to be 2kₑ₂a = (1.2 ± 0.4) × 10⁶ and (1.1 ± 0.2) × 10⁶ dm³ mol⁻¹ s⁻¹, respectively.

The decay kinetics of the radical cation 2a [C=NOH]⁺ was independent of the concentration of 2a from 0.05 to 1 mmol dm⁻³. However, at pH 7.4 the decay of the iminoxyl radicals 2a [C=NO]⁻ was slower and began to deviate from first-order and second-order kinetics, particularly at low initial radical concentrations and higher 2a concentration ([2a] = 1 mmol dm⁻³). The product analysis discussed later provides evidence that iminoxyl radical 2a [C=NO]⁻ can initiate and propagate the chain-catalysed isomerisation of the N-hydroxymino group from 2a ≥C=NOH₂ to 2b ≥C=NOH₂. Solubility constraints prevented a thorough study of the kinetics above at [2a] > 1 mmol dm⁻³ at which the interaction between the iminoxyl radical and the parent oxime would be more obvious. No spectral evidence was obtained for the formation of a possible three-electron bonded radical-adduct in reaction (3), which if formed, must have a very short half-life of <1 µs.

\[
\frac{[\text{C=NO}]^-} + \frac{[\text{C=NO}]} → \frac{[\text{C=NO}_3^-]} \quad \text{(3)}
\]

Alternatively, isomerisation of the N-hydroxymino group by the iminoxyl radical may be an outer-sphere process with no adduct involved.

One-electron oxidation of 1-methylindole-3-carboxamidine oxime 4a. The absorption spectra of the radicals generated by the oxidation of the amidoxime 4a by the Br₂⁻ radical are shown in Fig. 3. At pH 3.5 two absorption maxima are observed and

at 335 and 670 nm with extinction coefficients of 2180 and 580 
dm$^{-3}$mol$^{-1}$cm$^{-1}$, respectively, which have been attributed to the amidoxime radical cation $4a\ [\text{C}=-\text{NOH}]^{+}$. Once again at neutral 
pH the radical cation deprotonates to the neutral iminoxyl 
radical $4a\ [\text{C}=-\text{NO}]$, the spectrum of which is characterised by 
two absorption maxima at 330 and 580 nm with extinction 
coefficients of 2170 and 1160 dm$^{-3}$mol$^{-1}$cm$^{-1}$, respectively. As 
previously observed for the oxime $2a$, the rate of formation of 
either $4a\ [\text{C}=-\text{NOH}]^{+}$ or $4a\ [\text{C}=-\text{NO}]$ radicals were similar in 
$D_{2}O$ and water suggesting that, at pH 7.4, the Br$_{2}^{-}$ radical 
oxidises the amidoxime by electron transfer to the radical 
cation which rapidly deprotonates to the iminoxyl radical $4a\ 
[\text{C}=-\text{NO}]$ (see Table 1).

At pH 3.5 and 7.4 and low concentrations of amidoxime $4a$, ca. 0.05–0.1 mmol dm$^{-3}$, the amidoxime radicals decayed by 
second-order kinetics, where the rate of decay increased prop-
portionally with the radical concentration, 2–17 Gy. At pH 
3.5, the rate constant $k_{d_{a}} = (1.1 \pm 0.1) \times 10^{7}$ dm$^{-3}$mol$^{-1}$s$^{-1}$ for 
declay of the radical cation $4a\ [\text{C}=-\text{NOH}]^{+}$ was obtained from 
the slope of the linear plot of the pseudo-first-order rate 
constant for the decay of absorption at 720 nm versus initial 
radical concentration. Acceleration of this rate by a factor of 
2.3 to $k_{d_{a}} = (2.5 \pm 0.1) \times 10^{7}$ dm$^{-3}$mol$^{-1}$s$^{-1}$ in the presence of 
0.5 mol dm$^{-3}$ NaClO$_{4}$ was again consistent with our desig-
nation of the 720 nm absorption to the radical cation $4a\ 
[\text{C}=-\text{NOH}]^{+}$. No salt effect was observed on the rate of decay of the 
580 nm absorbing species at pH 7.4 ascribed to the neutral 
iminoxyl radical. The rate of decay of both $4a\ [\text{C}=-\text{NOH}]^{+}$ and 
$4a\ [\text{C}=-\text{NO}]^{-}$ radicals was independent of $[4a] \approx 0.05–2$ mmol 
dm$^{-3}$.

**Acid–base properties of iminoxyl radicals.** The different 
absorption spectra obtained for the oxidation of $2a$ and $4a$ by 
the Br$_{2}^{-}$ radical at low and neutral pH (see Figs. 2 and 3) 
suggest that their corresponding radical cations exist in proto-
tropic equilibrium (reaction (4)) with the iminoxyl radicals

$$[\text{C}=-\text{NOH}]^{+} \rightleftharpoons [\text{C}=-\text{NO}]^{-} + \text{H}^{+} \quad (4)$$

By measuring the change in absorption at 800 nm as a function 
of pH 2–9 it was possible to determine the $pK_{a}$ of the 
radical cation $2a\ [\text{C}=-\text{NOH}]^{+}$. The change of absorption when 
fitted to the appropriate function gave a $pK_{a} = 3.53 \pm 0.03$ for 
radical cation $2a\ [\text{C}=-\text{NOH}]^{+}$ (see the insert in Fig. 2). The 
insert in Fig. 3 shows the increase in absorbance of the in-
imoxyl radical $4a\ [\text{C}=-\text{NO}]$ with increasing pH 2.5–10, which is 
paralleled by a decline in absorbance of the radical cation $4a\ 
[\text{C}=-\text{NOH}]^{+}$ at 750 nm giving a $pK_{a} = 5.01 \pm 0.05$. Additional 
support for the existence of the acid–base equilibrium reaction 
(4) was obtained by pulse radiolysis of the O-methylated ana-
logues of $2a$ and $4a$, namely $6$ and $5$, respectively. The spectrum 
for the oxidation of $6$ by the Br$_{2}^{-}$ radical is characterised by 
two absorption maxima at 340 and 720 nm with corresponding 
extinction coefficients of 3350 and 1450 dm$^{-3}$mol$^{-1}$cm$^{-1}$, 
respectively. The spectrum was identical over a broad pH range, 
2.5–9.4, reflecting the inability of the radical cation $6\ [\text{C}=\ 
\text{NOCH}_{3}]^{+}$ to deprotonate. Similarly, the absorption spectra 
obtained on oxidation of $5$ by the Br$_{2}^{-}$ radical was also iden-
tical at pH 2.5–9 also indicating that $O$-methylation prevented 
deprotonation of the radical cation $5\ [\text{C}=-\text{NOCH}_{3}]^{+}$ (see Table 
1).

**Product profiling and quantification**

Stable products generated by the one-electron oxidation of 
1-methylindole–3-carbaldehyde oxime $2a$. Fig. 4 shows typical 
HPLC traces obtained on the oxidation of the oxime $2a$ by the 
Br$_{2}^{-}$ radical. Table 2 contains the radiochemical yields for 
the loss of the parent molecule and the formation of products 
which were dependent on the concentration of the parent $2a$,
Table 2  Concentration, pH and dose rate effects on the oxidation of the oxime 2a by the dibromide radical anion

<table>
<thead>
<tr>
<th>[2a]/mmol dm⁻³</th>
<th>pH</th>
<th>Dose rate/Gy min⁻¹</th>
<th>G(−2a)/µmol J⁻¹</th>
<th>G(2b)/µmol J⁻¹</th>
<th>G(1)/µmol J⁻¹</th>
<th>G(NO²⁻/NO₃⁻)/µmol J⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>7.41</td>
<td>25.46</td>
<td>1.05 ± 0.05</td>
<td>0.58 ± 0.03</td>
<td>0.124 ± 0.002</td>
<td>—</td>
</tr>
<tr>
<td>0.2</td>
<td>7.41</td>
<td>5.1</td>
<td>1.25 ± 0.11</td>
<td>1.23 ± 0.07</td>
<td>0.093 ± 0.003</td>
<td>—</td>
</tr>
<tr>
<td>0.2</td>
<td>7.41</td>
<td>2.25</td>
<td>1.38 ± 0.09</td>
<td>1.39 ± 0.15</td>
<td>0.083 ± 0.004</td>
<td>—</td>
</tr>
<tr>
<td>0.1</td>
<td>7.38</td>
<td>5.1</td>
<td>0.94 ± 0.09</td>
<td>0.52 ± 0.05</td>
<td>0.121 ± 0.001</td>
<td>0.011 ± 0.002</td>
</tr>
<tr>
<td>0.4</td>
<td>7.38</td>
<td>5.1</td>
<td>1.59 ± 0.24</td>
<td>1.457 ± 0.14</td>
<td>0.077 ± 0.006</td>
<td>0.004 ± 0.003</td>
</tr>
<tr>
<td>0.8</td>
<td>7.38</td>
<td>5.1</td>
<td>3.48 ± 0.12</td>
<td>2.780 ± 0.14</td>
<td>0.081 ± 0.001</td>
<td>0.012 ± 0.002</td>
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<td>0.1</td>
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<td>5.1</td>
<td>0.76 ± 0.04</td>
<td>0.62 ± 0.13</td>
<td>0.182 ± 0.001</td>
<td>—</td>
</tr>
<tr>
<td>0.1</td>
<td>7.40</td>
<td>5.1</td>
<td>1.16 ± 0.06</td>
<td>0.43 ± 0.01</td>
<td>0.116 ± 0.005</td>
<td>—</td>
</tr>
<tr>
<td>0.1</td>
<td>9.46</td>
<td>5.1</td>
<td>1.94 ± 0.31</td>
<td>2.70 ± 0.59</td>
<td>0.061 ± 0.005</td>
<td>—</td>
</tr>
</tbody>
</table>

* Radiation chemical yield of the dibromide radical anion G(Br₂⁺) = 0.69 µmol J⁻¹.  

| [C=NO⁺] + >C=NOH₂ ↔ [C=NO⁺] + >C=NOH₂ | (5) |

which became stoichiometric at 2.3 Gy min⁻¹. A greater yield of the aldehyde 1 >C=O was obtained at 25.46 Gy min⁻¹ but still only represented ca. 10% of the loss of 2a. Increasing the concentration of 2a from 0.1 to 0.8 mmol dm⁻³ increased the loss of parent significantly from G(−2a) = 1.25 µmol J⁻¹ to 3.48 µmol J⁻¹ at a dose rate of 5.1 Gy min⁻¹. The results suggest that the Br₂⁺ radical initiates a chain-propagated turnover of 2a and greater chain lengths occur by prolonging the lifetime of the 2a >C=NO⁻ radical.

The chemical kinetics have demonstrated that under certain experimental conditions both the radical-cation 2a [C=NOH]⁺ and the iminoxyl radical 2a [C=NO⁻] will decay bimolecularly to products via reactions (2a) and (2b), respectively. Although the oxidation of alkyl, dialkyl and aryl oximes can generate products from 2a and 4a by the Br₂⁺ radical which is discussed below. In both cases, the observations are consistent with the formation of unstable iminoxyl radical dimers, e.g. 2a and high dose rate the yield of 1 >C=O only accounted for ca. 12% of the products formed. No evidence was obtained for the generation of the nitroxy anion (NO⁻) in reaction (7) by standard assays including trapping by thiol to generate the disulfide and hydroxylamine and by the formation of ferrous-nitrosyl complexes with methaemoglobin.³⁰ The low yields (ca. 1%) of nitrite and nitrate measured in the presence of oxygen were below the limits of experimental error. The inability to detect nitrogen oxides in general and the fact that reaction (7) cannot account for the observed balance in radiation chemical yields between the oxidation of 2a and the products formed suggest that reaction (8) may be the preferred pathway for the formation of the aldehyde 1 >C=O.

Stable products generated by the one-electron oxidation of 1-methylindole-3-carboxamidine oxime 4a. Oxidation of the amidoxime 4a by the Br₂⁺ radical results in the formation of the nitrite 3, the amide 8, the carboxylic acid 7 (generated exclusively at low pH 3.5) plus stable dimers. As with 2a, there was no evidence for nitrogen oxide production from 4a. The radiation chemical yields for the oxidation of the amidoxime 4a and formation of these products at different parent concentrations, pH and dose rate are given in Table 3. In marked contrast to the oxidation of the oxime 2a by the Br₂⁺ radical, geometric isomerisation of 4a to 4b represented only a minor contribution to the loss of the parent under the different experimental conditions employed. At pH 7.4 and a dose rate 5 Gy min⁻¹, an increase in the concentration of 4a from 0.2 to 1 mmol dm⁻³ resulted in a small increase in the radiation chemical yield for the loss of the amidoxime G(−4a) = 0.33 to 0.42 µmol J⁻¹ and an increase in the yield of the isomer G(4b) = 0.013 to 0.050 µmol J⁻¹. However, this represents ca. 2% isomerisation of the N-hydroxyimino group in 4a relative to 2a (see Table 2) under similar experimental conditions. At 0.2 mmol dm⁻³ 4a, pH 7.4, lowering the dose rate from 25.5 to 2.3 Gy min⁻¹ actually decreased the loss of 4a from G(−4a) = 0.35 to 0.31 µmol J⁻¹. In fact, in the majority of the experiments performed the radiation chemical yields for the loss of the amidoxime 4a were usually less (ca. 50%) than that expected from G(Br₂⁺) = 0.69 µmol J⁻¹ and clearly rules out any significant contribution from a chain reaction. Fig. 5 (lower panel) shows the loss of 4a and the formation of products which, in marked contrast to 2a (upper panel), is linear up to ca. 150 Gy. At pH 7.4 and 5 Gy min⁻¹ the loss of the 4a was not stoichiometric with the quantifiable products, e.g., G(−4a) = 0.35 µmol J⁻¹ whereas G(4b + 3 + 8) = 0.24 µmol J⁻¹ with ca. 30% products unaccounted for. At lower concentrations of 4a (0.014) = 0.9 mmol dm⁻³) the material balance was better, e.g., G(−4a) =
Table 3  Concentration, pH and dose rate effects on the oxidation of the amidoxime 4a by the dibromide radical anion$^*$$^*$

<table>
<thead>
<tr>
<th>[4a]$^{\text{m mol dm}^{-3}}$</th>
<th>pH</th>
<th>Dose rate/ G(−4a)/ μmol J$^{-1}$</th>
<th>G(4b)/ μmol J$^{-1}$</th>
<th>G(3)/ μmol J$^{-1}$</th>
<th>G(8)/ μmol J$^{-1}$</th>
<th>G(7)/ μmol J$^{-1}$</th>
<th>G(NO$_3^−$)/ μmol J$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>7.36</td>
<td>25.46</td>
<td>0.35 ± 0.008</td>
<td>−0.012</td>
<td>0.20 ± 0.004</td>
<td>0.032 ± 0.004</td>
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<tr>
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<td>5.03</td>
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<td>−0.013</td>
<td>0.17 ± 0.006</td>
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<td>0.021 ± 0.017</td>
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<tr>
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<td>5.02</td>
<td>0.217 ± 0.007</td>
<td>−0.007</td>
<td>0.022 ± 0.001</td>
<td>0.050 ± 0.002</td>
<td>0.092 ± 0.004</td>
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<tr>
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<td>5.02</td>
<td>0.305 ± 0.007</td>
<td>−0.010</td>
<td>0.168 ± 0.006</td>
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<td>0.255 ± 0.005</td>
<td>−0.034</td>
<td>0.243 ± 0.003</td>
<td>0.005 ± 0.001</td>
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</table>

$^*$ Radiation chemical yield of the dibromide radical anion $G$(Br$_2^−$) = 0.69 μmol J$^{-1}$. Not detected = nd. $^*$ NO$_3^−$. $^*$ NO$_2^−$.

0.305 μmol J$^{-1}$ whereas $G$(4b + 3 + 8) = 0.26 μmol J$^{-1}$ (ca. 15% products unaccounted for).

Pulse radiolysis of 4a clearly demonstrated that both the radical radical reactions (2a) and (2b) produce the same products: the carboxylic acid $\text{C}−\text{N}−\text{O}$ and benzoic acid, a product profile which is mirrored by the oxidation of the amidoxime 4a to amide 8, the nitrile 3 and the carboxylic acid 7.

Fig. 3 shows that the decay of both the radical cation and the iminoxyl radical results in an increase in absorbance at ca. 300–600 nm, which is likely formed ca. 16 ms after the electron pulse. It is likely that the absorbance increase is caused by the formation of unstable iminoxyl radical dimers. Between 300 and 450 nm this species decays in 250 ms to another product which is stable up to 10 s later. However, at slightly longer wavelengths, ca. 480 nm (where the stable product does not absorb), the rate of decay of this species was found to depend both on the concentration of 4a and the initial radical concentration. At low doses per pulse (ca. 2 μmol dm$^{-3}$ radicals) the decay is first-order in [4a]$^+$ = 0.1–1 μmol dm$^{-3}$ giving a rate constant for the reaction between iminoxyl radical dimers and 4a of $k ≈ 2 \times 10^7$ dm$^3$ mol$^{-1}$ s$^{-1}$. However, at higher doses per pulse (ca. 25 μmol dm$^{-3}$ radicals) the change in absorbance at 480 nm is independent of the concentration of 4a up to 1 mmol dm$^{-3}$ where dimer-dimer reactions appear to predominate.

Changing the pH had a significant effect on the products formed in the one-electron oxidation of the amidoxime. When [4a] = 0.2 mmol dm$^{-3}$ and the dose rate was 5 Gy min$^{-1}$ an increase in pH from 7.4 to 9.5 increased the radiation chemical yield of the nitrile G(3) = 0.168 ± 0.024 μmol J$^{-1}$ and decreased that of the amide G(8) = 0.08 ± 0.005 μmol J$^{-1}$. The combined yield of these products became stoichiometric with the oxidation of 4a. However, at low pH = 3.55 an additional product, the carboxylic acid 7, was formed in higher yield G(7) = 0.092 μmol J$^{-1}$ than the combined yield of the other quantifiable products G(3 + 4b + 8) = 0.079 μmol J$^{-1}$.

Scheme 2 shows a possible mechanism which may account for observed products at different pHs. The self-reaction of iminoxyl radicals 4a generates a mixture of stable and unstable iminoxyl dimers. The combined radiation chemical yields of the quantifiable products indicate that at low concentrations (4a) = 0.2 mmol dm$^{-3}$) the unstable iminoxyl dimers predominate. Degradation of the dimer (e.g. C−N−O − (−O)=C) yields the parent 4a plus a putative intermediate which forms the observed products: the carboxylic acid 7 at low pH, the amide 8 at neutral pH, and the nitrile 3 at higher pH. Although the NO$^−$ anion is likely to be a by-product of these reactions, the assays employed for its direct measurement have to out-compete the rapid decay of two NO$^−$ anions to N$_2$O$_3$ Schemes can be drawn for the decay of azine bis-N-oxides to the observed products at different pHs without the formation of NO$^−$ although these pathways cannot account for the regeneration of the parent amide 4a. The reason why iminoxyl radicals should generate a mixture of stable and unstable dimers is unclear. However, when [4a] = 1 mmol dm$^{-3}$ the combined radiation chemical yield G(3 + 4b + 8) = 0.13 μmol J$^{-1}$ is far less than G(−4a) = 0.42 μmol J$^{-1}$ suggesting that the unstable dimers formed initially can react with 4a to form the more stable dimers detectable by HPLC.

Conclusions

One-electron oxidation of the oxime 2a and the amidoxime 4a by the dibromide radical anion in aqueous solution yields radical cations which deprotonate to give iminoxyl-type radicals at neutral pH. The ratios of the rates of reaction of the dibromide radical anion with the oximes in H$_2$O and D$_2$O solutions are...
Scheme 2 Possible mechanism for the formation of products from the one-electron oxidation of the amidoxime 4a.

Experimental

Materials

Potassium bromide, sodium or potassium phosphate salts, and potassium thiocyanate were from Merck and were of analytical quality. Deuterium oxide (heavy water, 99.9% D) was obtained from Sigma–Aldrich (Dorset, UK). Solutions were prepared with water purified by a Milli-Q system (Millipore). Before irradiation, solutions were bubbled with zero-grade nitrous oxide (N₂O, oxygen content <10 ppm) or with N₂O–O₂ (80 : 20%) mixtures (British Oxygen Company). 1-Methylindole-3-carbaldehyde derivatives and putative oxidation products were prepared by derivation of commercially available indoles (Sigma–Aldrich, Dorset, UK). All experiments were performed at room temperature (20 ± 2 °C).

Radiation chemistry

The pulse radiolysis experiments were performed with a 6 MeV linear accelerator as described previously. A pulse of 0.5 µs delivered doses of 1–35 Gy, as determined by thiocyanate dosimetry. Steady-state irradiations were performed with a 60Co γ-source with a nominal activity of 2000 Ci. The solutions were irradiated in air-tight vials at dose rates in the range 2–25 Gy min⁻¹, as determined by Fricke dosimetry. Radiation chemical yields (G) in µmol J⁻¹ for the loss of the parent molecule and formation of products were calculated from the slope of plots of change in concentration versus radiation dose.

The dibromide radical anion (Br₂⁻) was generated by radiolysis of N₂O-saturated solutions of KBr (50 mmol dm⁻³) in phosphate buffer (4 mmol dm⁻³). Under these conditions, the radiolysis of water generates radical, ions, and molecular products [reaction (9)]. (The numbers in parentheses are the radiation chemical yields in µmol J⁻¹.) The hydrated electron (eaq⁻) reacts with N₂O and is converted into the hydroxyl radical (•OH), which becomes the main primary product of water radiolysis [reaction (10)]. The Br₂⁻ radical anion is then rapidly formed (<0.5 µs) by the sequence of reactions [reactions (11)–(13)].

\[
\text{H}_2\text{O} \rightarrow \text{•OH (0.28), } \text{e}_{\text{aq}}^- (0.27), \text{H}^+ (0.06), \text{H}^+ (0.34), \text{H}_2 (0.05), \text{H}_2\text{O}_2 (0.07) \quad (9)
\]

\[
\text{e}_{\text{aq}}^- + \text{N}_2\text{O} + \text{OH}^- \rightarrow \text{OH} + \text{OH}^- + \text{N}_2 \quad (10)
\]

\[
\text{OH} + \text{Br}^- \rightarrow \text{HOBr}^- \quad (11)
\]

\[
\text{HOBr}^- \rightarrow \text{HO}^- + \text{Br}^- \quad (12)
\]

\[
\text{Br}^- + \text{Br}^- \rightarrow \text{Br}_2^- \quad (13)
\]

In pulse radiolysis experiments the reaction of the Br₂⁻ radical anion with the oxime derivative was monitored by absorption spectroscopy and the extinction coefficients of the transient species were calculated using the value 0.69 µmol J⁻¹ for the radiation chemical yield of the Br₂⁻ radical anion.
Synthetic chemistry

NMR spectra were obtained at 60 MHz with a JEOL MY60 spectrometer and SiMe$_3$ as internal standard. Elemental analyses were determined by MEDAC Ltd., Brunei Science Centre, Egham, Surrey, UK TW20 0ZJ, and all compounds were chromatographically homogeneous by TLC and HPLC–MS (Waters, Watford, UK). Solutions in organic solvents were obtained by standard procedures and dried with Merck Kieselgel 60 H grade (230–400 mesh). Silica gel for flash column chromatography was Merck Kieselgel 60 H grade (230–400 mesh). Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected.

1-Methylindole-3-carbaldehyde oxime 2a

Indole (3.0 g, 0.026 mol) was dissolved in DMF (30 cm$^3$) and sodium hydride (0.88 g of a 60% dispersion, 22 mmol) was gradually added with stirring under a nitrogen atmosphere.

After 1 h at ambient temperature methyl iodide (30 cm$^3$, 92.6 mmol) was added and the solution stirred for 2 h, then poured onto a solution of sodium hydrogen sulfate (10%, 500 cm$^3$) and saturated sodium bicarbonate solution (250 cm$^3$), dried and evaporated. The residue was added to a previously prepared solution of DMF (11.4 g, 15.5 mmol) which had been cooled to 0°C, phosphorus oxychloride (5.2 g, 33.4 mmol) was added dropwise, and the mixture was stirred for 0.5 h. The reaction mixture was then stirred for 18 h at ambient temperature, poured onto ice (250 g) and sodium hydroxide (37%, 100 cm$^3$) added. The product was extracted with ethyl acetate and purified on silica, eluting with ethyl acetate–hexane, 1:1, to give 1-methylindole-3-carboxaldehyde (1.0 g, 24%) as a white solid, mp 68–70°C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 9.94 (1 H, s), 8.3 (1 H, m), 7.58 (1 H, s), 7.32 (3 H, m), 3.81 (3 H, s).

Hydroxylamine hydrochloride (10.2 g, 147 mmol) was dissolved in methanol (150 cm$^3$) and potassium hydroxide (1.21 g, 21.7 mmol) was added. The solution was heated at 50°C for 0.5 h, cooled and methyl iodide (1 cm$^3$, 16.1 mmol) added. The solution was then heated at 60°C for 0.5 h, cooled and sodium hydrogen sulfate (10%, 15 cm$^3$) added. The solution was extracted with ethyl acetate, washed with sodium bicarbonate and brine, dried and evaporated. The residue was purified on silica gel, eluting with ethyl acetate–hexane 2:1 to give 5 (60 mg, 27%) as a pale yellow oil. $^1$H NMR (CDCl$_3$) δ 8.03 (1 H, s), 7.83 (1 H, m), 7.29 (4 H, m), 4.08 (3 H, s), 3.80 (3 H, s). MS (EI) m/z (relative intensity) 188 (M$^+$, 100%), 157 (43), 142 (54), 130 (28), 115 (14), 103 (14).

1-Methylindole-3-carboxylic acid

Methyl indole-3-carboxylate (3.0 g, 17.15 mmol) was dissolved in THF (50 cm$^3$) and sodium hydride (1.1 g of a 60% dispersion, 24.4 mmol) added with stirring under nitrogen. Methyl iodide (10 cm$^3$, 161 mmol) was then added and the solution heated at 60°C for 1 h, cooled and poured onto sodium hydrogen sulfate (10%, 100 cm$^3$). The solution was extracted with ethyl acetate and washed with sodium bicarbonate and brine, dried and evaporated. The residue was triturated with ether and the solid collected and washed with ether to give 2.6 g (85%) of methyl 1-methylindole-3-carboxylate, mp 88°C.

Hydroxylamine hydrochloride (10.2 g, 147 mmol) was dissolved in methanol (150 cm$^3$) and potassium hydroxide (1.21 g, 21.7 mmol) was added. The solution was heated at 50°C for 0.5 h, cooled and methyl iodide (1 cm$^3$, 16.1 mmol) added. The solution was then heated at 60°C for 0.5 h, cooled and sodium hydrogen sulfate (10%, 15 cm$^3$) added. The solution was extracted with ethyl acetate, washed with sodium bicarbonate and brine, dried and evaporated. The residue was purified on silica gel, eluting with ethyl acetate–hexane 2:1 to give 6 (50 mg, 27%) as a pale yellow oil. $^1$H NMR (CDCl$_3$) δ 8.03 (1 H, s), 7.83 (1 H, m), 7.29 (4 H, m), 4.08 (3 H, s), 3.80 (3 H, s). MS (EI) m/z (relative intensity) 188 (M$^+$, 100%), 157 (43), 142 (54), 130 (28), 115 (14), 103 (14).

1-Methylindole-3-carboxamide

1-Methylindole-3-carboxaldehyde oxime 2a (10.0 g, 57.5 mmol) was dissolved in acetic anhydride (100 cm$^3$) together with sodium acetate (10 g, 122 mmol). The solution was then heated at 100°C for 4 h, cooled and evaporated. The residue was purified on silica, eluting with ethyl acetate–hexane (1:3) to give 3-cyano-1-methylindole 3 (5.2 g, 58%) as a yellow oil. $^1$H NMR (CDCl$_3$) δ 7.09–7.43 (5 H, m), 3.60 (3 H, s). Hydroxylamine hydrochloride (1.51 g, 21.7 mmol) was dissolved in methanol (100 cm$^3$) and potassium hydroxide (1.21 g, 21.7 mmol) was added with stirring. After 1.5 h the solution was filtered and the filtrate added to a solution of 3 (0.6 g, 3.9 mmol) in ethanol (30 cm$^3$). The solution was heated at 100°C for 18 h, cooled and evaporated. The residue was purified on silica, eluting with ethyl acetate to give 0.26 g (35%) of 1-methylindole-3-carboxamide oxime 4a as a white solid, mp 149–150°C (lit.,18 189–190°C). $^1$H NMR ([CD$_3$]$_2$SO) δ 9.16 (1 H, s), 8.05 (1 H, d), 7.43 (2 H, s), 6.94–7.16 (3 H, m), 5.51 (1 H, br s), 3.75 (3 H, s). MS (EI) m/z (relative intensity) 189 (M$^+$, 100%), 172 (72), 157 (60), 142 (20), 131 (33), 103 (12) (Calc. for C$_3$H$_7$N$_2$O$_2$H$_2$O: C, 59.7; H, 6.1; N, 20.9). Found: C, 59.7; H, 5.6; N, 20.9%.

O.1-Dimethylindole-3-carboxamidine oxime 5

Carboxamidine oxime 4a (0.2 g, 1.1 mmol) and trimethyl-oxonium tetrafluoroborate (1.0 g, 6.67 mmol) were dissolved in dichloromethane (10 cm$^3$) and tetrahydrofuran (2 cm$^3$) and the solution stirred for 24 h at ambient temperature. Ethyl acetate (50 cm$^3$) was then added and the solution washed with saturated sodium bicarbonate solution (50 mM) and brine, dried and evaporated. The residue was purified by flash column chromatography eluting with ethyl acetate followed by radial chromatography (4 mm plate eluting with hexane–ethyl acetate, 2:1) to give 5 (13 mg, 5.8%) as a pale yellow oil. LC–MS, 96.4%, MS (EI) m/z (relative intensity) 203 (M$^+$, 100%), 172 (37), 157 (40), 131 (37).

O.1-Dimethylindole-3-carboxaldehyde oxime 6

Compound 2a (174 mg, 1 mmol) was dissolved in DMF (5 cm$^3$) and sodium hydride (50 mg of a 60% dispersion, 1.2 mmol) was added with stirring and under nitrogen. The solution was heated at 50°C for 0.5 h, cooled and methyl iodide (1 cm$^3$, 16.1 mmol) added. The solution was then heated at 60°C for 0.5 h, cooled and sodium hydrogen sulfate (10%, 15 cm$^3$) added. The solution was extracted with ethyl acetate, washed with sodium bicarbonate and brine, dried and evaporated. The residue was purified on silica gel, eluting with ethyl acetate–hexane 2:1 to give 6 (50 mg, 27%) as a pale yellow oil. $^1$H NMR (CDCl$_3$) δ 8.03 (1 H, s), 7.83 (1 H, m), 7.29 (4 H, m), 4.08 (3 H, s), 3.80 (3 H, s). MS (EI) m/z (relative intensity) 188 (M$^+$, 100%), 157 (43), 142 (54), 130 (28), 115 (14), 103 (14).
HPLC detection of products

Product analysis following γ-irradiation of 2a and 4a was performed by separation on a 100 mm × 3.2 mm base-deactivated reversed-phase column (Hichrom RP18, Hichrom, Reading, UK) at a flow rate of 1 cm min⁻¹. The products from the oxidation of the oxime 2a were eluted isocratically with 35% methanol, 7.5% acetonitrile, 57.5% water. The products of the amidoxime 4a were eluted with mixtures of (A) heptanesulfonic acid (5 mmol dm⁻³), KH₂PO₄ (5 mmol dm⁻³), H₃PO₄ (5 mmol dm⁻³) and (B) 75% acetonitrile, 25% water with a linear gradient of 25–65% B, in 5 min. Eluted peaks were detected by absorption at 265 and 292 nm using a Waters 996 diode array detector (Watford, UK) and concentrations were determined from peak areas using Waters Millennium Software.

Ground state pKₐ values were determined by chromatographing either 2a or 4a in citrate buffers of different pH, and measuring the retention time. Protonation of the hydroxamino group in 2a and 4a occurred at low pH with pKₐ(RR,C=NOH + H⁺) = 23 ± 0.1 and 3.5 ± 0.1, respectively. No further changes in retention time for either 2a or 4a were observed between pH 4 and 9 implying that pKₐ(RR,C=NO + H⁺) > 9.

Nitrite and nitrate were determined by reversed-phase HPLC with ion-pairing using tetrabutylammonium hydroxide, with UV detection at 214 nm as described previously.³⁸

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References