Pulse Radiolysis Studies on the Fragmentation of Arylmethyl Quaternary Nitrogen Mustards by One-Electron Reduction in Aqueous Solution

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Received: July 16, 1997; In Final Form: September 24, 1997[⊗]

The radical species formed on the reaction of e_{aq}^- with a series of aryl compounds, all containing a *N,N*-bis(2-chloroethyl)-*N*-methylammoniomethyl substituent, have been studied in neutral aqueous solutions by pulse radiolysis using optical absorption spectrophotometry. The benzene, **1**, and 4-methylsulfonylbenzene, **2**, derivatives fragmented immediately to yield different amounts of benzyl radicals, but the 2-nitrobenzene, **3**, and 4-nitrobenzene, **4**, derivatives were reduced to long-lived radical anions which decayed by bimolecular processes. These nitrobenzene derivatives differed from the corresponding benzyl halides in that they did not fragment to benzyl radicals. Similarly, no evidence was found for the formation of a benzyl-type radical from the radical anion of the 8-nitronaphthalene derivative, **5**. However, benzyl-type radicals were produced upon intramolecular electron transfer from the initially formed radical anions of the 4-nitro-5-imidazole, **6**, and the 5-nitro-2-pyrrole, **7**, derivatives at rate constants of $(8.0 \pm 1.0) \times 10^3$ and $(1.0 \pm 0.1) \times 10^4$ s^{-1}, respectively. The latter heterocyclic analogues provide an approach for release of cytotoxic tertiary amines via enzymatic or radiolytic reduction in hypoxic regions of tumors.

Introduction

The electron adducts (radical anions) of nitrobenzyl halides are known to undergo intramolecular electron transfer to eliminate the halide and form nitrobenzyl radicals.[1−3] The rate of this reaction is highly structure dependent being influenced by the nature of the leaving group (e.g. I > Br > Cl), the extent of stabilization of the resulting nitrobenzyl radical, and the dissociation energy of the bond being broken.[4] There has been little investigation of potential leaving groups other than halides, but some tosylate analogues also fragment via this mechanism.[2] It has been suggested that nitrobenzyl quaternary ammonium compounds might also fragment via intramolecular electron transfer from the radical anion to release a tertiary amine.[5] Such a mechanism would be of particular interest for enzyme-mediated reductive activation of drugs to release cytotoxic amines in hypoxic regions of tumors.

$$NO_2ArCH_2N^+R_1R_2R_3 + e_{aq}^- \rightarrow {}^{-\bullet}NO_2ArCH_2N^+R_1R_2R_3 \quad (1)$$

$${}^{-\bullet}NO_2ArCH_2N^+R_1R_2R_3 \rightarrow NO_2ArCH_2^\bullet + NR_1R_2R_3 \quad (2)$$

We have reported the preparation of a series of nitrobenzyl quaternary compounds in which the amine leaving group is the cytotoxic nitrogen mustard mechlorethamine (HN_2) and have demonstrated that some of these compounds are selectively toxic to mammalian cells under hypoxic conditions as a result of formation of mechlorethamine.[5−7] However, it is not known whether the proposed fragmentation occurs at the one-electron (nitro radical anion) reduction level as proposed above, or whether further reduction is required. Another possible mechanism of fragmentation also requires consideration since reduction of the non-nitro benzyltrimethylammonium cations by e_{aq}^- is known to cause fragmentation to the benzyl radical, with release of trimethylamine, but without the formation of a detectable precursor radical.[8] This fragmentation is presumably due to direct dissociative electron attachment to the quaternary nitrogen analogous to the reductive cleavage of low-potential aryl halides.[9] With the higher potential nitroarylmethyl quaternary cations it is expected that electron attachment to the nitroaryl moiety will successfully compete with this direct process. In the present study, radiation chemistry techniques are used to investigate the mechanisms of reduction of examples of both nitro and nonnitro arylmethyl quaternary salts in which the potential leaving group is mechlorethamine (compounds **1**−**7**). In particular, the possibility of and rate of fragmentation of the nitro-substituted compounds via intramolecular electron transfer is examined by pulse radiolysis.

Experimental Section

Fast reaction chemistry experiments were performed on The University of Auckland's 4MeV Dynaray linear accelerator using a PC-controlled custom-built optical absorption detection system. Pulses of electrons (typically 3−4 Gy (J kg^{-1}) absorbed dose in 200 ns) were used to initiate radical reactions in quartz cells of various optical path lengths (0.5−2.0 cm). Dosimetry was carried out using aerated KSCN solution assuming the $(SCN)_2^{\bullet-}$ radical produced has a radiation chemical yield, *G*, of 0.29 μmol J^{-1} (where a *G* of 1 μmol J^{-1} is equivalent to 10.3 species per 100 eV) and an extinction coefficient of 7580 L mol^{-1} cm^{-1}.[9] All compounds were synthesized in the Cancer Research Laboratory of The University of Auckland by reported methods.[7] Perchlorate salts of **1** and **2** were prepared by addition of dilute perchloric acid to aqueous solutions of the corresponding chlorides. Water was purified by a Millipore Milli-Q system.

The primary radical species produced on the radiolysis of water with low LET radiation are e_{aq}^- ($G = 0.28$ μmol J^{-1}), $H^\bullet$ ($G = 0.06$ μmol J^{-1}), and $^\bullet OH$ ($G = 0.29$ μmol J^{-1}). Selective

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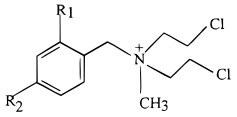
[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1997.

S1089-5639(97)02311-6 CCC: $14.00

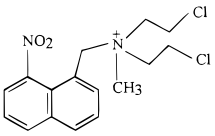


1. $R_1 = H$ $R_2 = H$

2. $R_1 = H$ $R_2 = CH_3SO_2$

3. $R_1 = H$ $R_2 = NO_2$

4. $R_1 = NO_2$ $R_2 = H$



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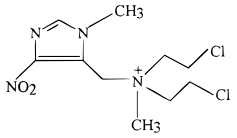

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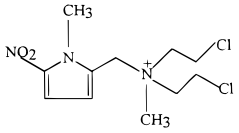

7.

one-electron reduction of substrates (0.1−1.0 mmol L^{-1}) was achieved upon their fast reaction with e_{aq}^- while scavenging the $^{\bullet}OH$ radicals with 2-methylpropan-2-ol (0.1−0.5 mol L^{-1}) to form a relatively inert radical. The H atoms can also react with substrates to form a transient adduct under conditions of high substrate concentrations (ca. 1 mmol L^{-1}) and low 2-methylpropan-2-ol concentration.

$$ArCH_2X + e_{aq}^- \rightarrow {}^{-\bullet}ArCH_2X \quad (1)$$

$$^{\bullet}OH\ (H^{\bullet}) + (CH_3)_3COH \rightarrow {}^{\bullet}CH_2(CH_3)_2COH + H_2O\ (H_2)$$

$$H^{\bullet} + ArCH_2X \rightarrow HArCH_2X$$

$$^{-\bullet}ArCH_2X + {}^{-\bullet}ArCH_2X(^{\bullet}HArCH_2X) \rightarrow \text{products} \quad (3)$$

The rate constants for the reaction of the e_{aq}^- with the compounds were determined from the pseudo-first-order decay rates observed at 650 nm. Time-resolved spectra of the radical species produced following one-electron reduction of the substrates are presented, $G\Delta\epsilon$, the product of the radiation chemical yield (G, μmol J^{-1}) and change in extinction coefficient relative to the absorbance of the unreduced parent compound ($\Delta\epsilon$, L mol^{-1} cm^{-1}). This is effectively the change in absorbance per unit dose per unit path length. All radical spectra are corrected for the minor absorbance of the 2-methyl-2-hydroxypropan-1-yl radical.[10] Kinetic parameters were obtained by curve fitting of transients over a range of radiation doses or, in the case of the second-order decay of the radical anions, reaction 3, from plots of the inverse of the observed half-life against the concentration of the radical species formed by the radiation dose.

The reaction of the radical anions with O_2, reaction 4, was investigated in solutions containing propan-2-ol (0.2 mol L^{-1}) to produce a higher yield of the electron adduct than from e_{aq}^- alone, and saturated with O_2 or O_2/N_2O gas mixtures. High radiation doses (10 Gy) were used to ensure sufficient radical anion formation for observation.

$$e_{aq}^- + N_2O \rightarrow {}^{\bullet}OH + OH^- + N_2$$

$$^{\bullet}OH(H^{\bullet}) + (CH_3)_2CHOH \rightarrow (CH_3)_2\dot{C}OH + H_2O(H_2)$$

$$(CH_3)_2\dot{C}OH + ArCH_2X \rightarrow {}^{-\bullet}ArCH_2X + (CH_3)_2CO + H_3O^+$$

$$(CH_3)_2\dot{C}OH + O_2 \rightarrow (CH_3)_2CO + O_2^{\bullet-} + H_3O^+$$

$$^{-\bullet}ArCH_2X + O_2 \rightarrow ArCH_2X + O_2^{\bullet-} \quad (4)$$

One-electron reduction potentials, $E(1)$, for the nitrocompounds were determined in aqueous solutions containing propan-2-ol (0.1 mol L^{-1}) buffered at pH 7.0 (10 mmol L^{-1}) by measuring the equilibrium constant[11] for electron transfer between the radical anions of the compounds and viologen reference standards. Steady-state radiolysis experiments were performed using a ^{60}Co γ-ray source at a dose rate of 0.74 Gy s^{-1}. Free chloride was analyzed by the ferrithiocyanate method[12] where immediately after irradiation ferric nitrate in 9 mol L^{-1} nitric acid was added, followed by saturated mercuric thiocyanate. The absorbance of the formed $FeSCN^{2+}$ species was measured at 460 nm. Loss of substrate and formation of mechlorethamine (following its derivatization as the diethyldithiocarbamate diadduct[13]) was investigated by HPLC[14] under the same radiolysis conditions.

Results and Discussion

Table 1 summarizes much of the data measured in this study and discussed below. The e_{aq}^- reacted rapidly with all compounds with rate constants in the range (1−4) × 10^{10} L mol^{-1} s^{-1}.

Non-Nitro Benzene Derivatives. The spectrum of the transient species formed upon one-electron reduction of 1.0 and 0.5 mmol L^{-1} solutions of the benzyl quaternary ammonium mustard [*N*,*N*-bis(2-chloroethyl)-*N*-methyl-*N*-benzylammonium chloride], **1**, by e_{aq}^- (and reaction with H atoms) is displayed in Figure 1 and the spectral and rate constant data are summarized in Table 1. High concentrations of the substrate, which is of low electron affinity, were needed to ensure complete scavenging of the reducing radicals. The spectrum of the initially formed species, measured 25 μs after the electron pulse, exhibits two absorbance peaks, at 320 and 260 nm. To investigate whether the possible H atom adduct species contributes to the transient spectrum, a similar experiment was conducted at pH 1.0. Under these conditions the e_{aq}^- are converted to H atoms increasing the yield of the latter to 0.35 μmol J^{-1}.

$$e_{aq}^- + H_3O^+ \rightarrow H^{\bullet} + H_2O$$

Reaction of H atoms with **1** gives rise to a transient spectrum with a peak near 325 nm (ϵ = 4250 L mol^{-1} cm^{-1}) (Figure 1). This spectrum is similar to that reported for H atom addition to benzyltrimethylammonium cations[8] and can be assigned to the cyclohexadienyl radicals[15] formed upon H atom addition to the benzene ring. The cyclohexadienyl radical decayed slowly with second-order kinetics. Subtracting the contribution of the absorbance due to H atom addition from the spectrum obtained at pH 7.0 yields the corrected spectrum for the e_{aq}^- addition to **1** which is similar to the spectrum of the well-characterized benzyl radical.[16] Using the full yield of e_{aq}^- produced by the pulse (0.28 μmol J^{-1}) to calculate the maximum extinction coefficients of the corrected spectrum it is found, on comparison

TABLE 1: Values of One-Electron Reduction Potentials of Compounds, $E(1)$; $k(e_{aq}^- + \text{Compound})$ (k_1); Spectral Data on the Species Formed; Rate Constants for Conversion of Radical Anion to Benzyl-Type Radical (k_2), Bimolecular Decay ($2k_3$), and for Reaction with O_2 (k_4)

compd	$E(1)$/mV *vs* (reference compound)[a]	$10^{-10}k_1$/L mol^{-1}s^{-1}	radical anion λ_{max} ϵ/L mol^{-1} cm^{-1}	benzyl-type radical λ_{max} ϵ/L mol^{-1} cm^{-1}	$10^{-3}k_2$/s^{-1}	$10^{-8}2k_3$/ L mol^{-1} s^{-1}	$10^{-6}k_4$/ L mol^{-1} s^{-1}
1		1.0 ± 0.1	260/330 nm	*d*			
2		3.0 ± 0.3	*c*	275 nm 16800			
3	-380 ± 7[b]	2.5 ± 0.2	315 nm 16000	NF[e]		1.0 ± 0.2	3.8 ± 0.2
4	-358 ± 10[b]	4.0 ± 0.4	305 nm 9000	NF[e]		0.20 ± 0.02	1.6 ± 0.2
5	-371 ± 11[b]	3.5 ± 0.3	295/405 nm 3000/2850	NF[a]		0.44 ± 0.04	<0.2
6	-397 ± 8 (MV)	4.0 ± 0.2	<330 nm	415 nm 6150	8.0 ± 1.0		
7	-561 ± 8 (TQ)	4.0 ± 0.2	<300 nm	385 nm 16750	10 ± 1.0		

[a] MV, methylviologen $E(1) = -447 \pm 7$ mV, TQ, triquat $E(1) = -548 \pm 7$ mV (ref 22). [b] Reference 7. [c] Radical anion not observed, see text. [d] Benzyl-type radical minor product. [e] NF, not formed.

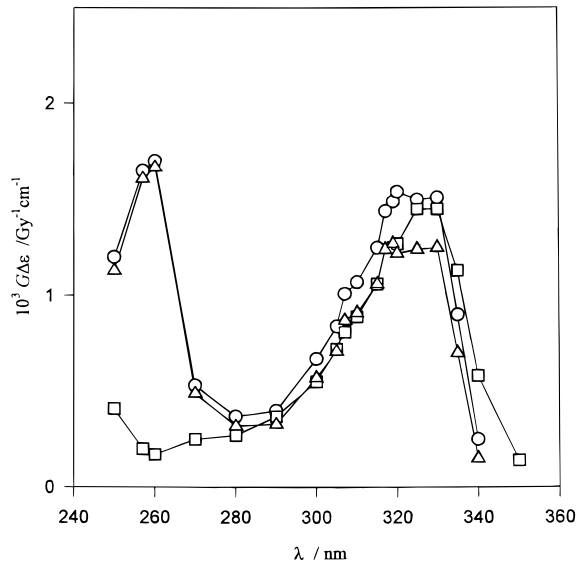

Figure 1. Pulse radiolysis of *N*,*N*-bis(2-chloroethyl)-*N*-methyl-*N*-benzylammonium chloride, **1**. Absorption spectra were determined after irradiating (4 Gy in 200 ns) of N_2-saturated aqueous solutions containing 0.5 mol L^{-1} 2-methylpropan-2-ol, 1.0 mmol L^{-1} **1** and (i) 5 mmol L^{-1} phosphate, pH 7.0, m: measured after 25 μs, and (ii) hydrochloric acid, pH 1.0, (□) measured after 25 μs. Spectrum of one-electron-reduced **1**, corrected for the contribution of the H atom adduct, △.

with published spectra of the cyclohexadienyl radical[8,15] and benzyl radical, that only ca. 30% of the e_{aq}^- lead to the fragmentation of **1** to the benzyl radical. This implies that there is an additional site of attack for the e_{aq}^-. Possible sites are the two alkyl chloride moieties of the mustard as dechlorination and formation of an alkyl radical is known to occur upon e_{aq}^- attack on such substrates with rate constants less than an order of magnitude smaller than attack at a benzyl quaternary ammonium center.[17] Substantial production of chloride was found when the perchlorate salt of **1** was irradiated under deaerated conditions in the presence of 2-methyl-2-propanol (0.1 mol L^{-1}) at pH 7, Table 2. The determined *G* value of 0.24 ± 0.01 for the production of chloride is marginally higher than that expected on the basis of the pulse radiolysis data, where higher substrate concentrations were employed, but substantiates the alkyl halide moiety as the major site of e_{aq}^- attack. A satisfactory mass balance for loss of substrate and the production of mechlorethamine and chloride was obtained (Table 2).

TABLE 2: G Values (μmol J^{-1}) for the Loss of Compounds 1 and 2 and the Production of Mechlorethamine and Chloride Following ^{60}Co γ-Irradiation of Aqueous Solutions Containing 50 μmol L^{-1} of the Compounds 2-methylpropan-2-ol (0.05 mol L^{-1}) at pH 7.0

compd	*G* (compound)	*G* (mechlorethamine)	*G* (chloride)
1	0.28 ± 0.03	0.06 ± 0.01	0.24 ± 0.01
2	0.29 ± 0.01	0.21 ± 0.02	0
3			0
6			0

The spectrum of the transient species measured immediately (at 25 μs) following the one-electron reduction of **2**, the 4-methylsulfonyl analogue of **1,** is displayed in Figure 2. The spectrum displays a strong absorbance peak at 275 nm and a minor peak at 310 nm. To determine whether the observed transient could arise from a benzyl-type radical or some other radical, one-electron reduction of both 4-(methylsulfonyl)benzyl chloride, and 4-(methylsulfonyl)toluene, were carried out. Reduction of 4-(methylsulfonyl)benzyl chloride produced the same transient species as observed for **2** (with a slightly higher absorbance at 275 nm, $\epsilon = 19\,000 \pm 1000$ L mol^{-1} cm^{-1}), suggesting the formation of the benzyl-type radical in both cases through the rapid loss (<1 μs) of the chloride ion or the tertiary amine respectively, as leaving groups. One-electron reduction of 4-(methylsulfonyl)toluene produced a weakly absorbing featureless transient which is unlikely to arise from the formation of a radical anion as electron transfer to a wide range of characterized electron-accepting viologen compounds[18] could not be demonstrated (data not shown). In contrast to **1**, no production of chloride was observed upon the steady-state radiolysis of the perchorate salt of **2** (Table 2), implying that the electron deficient sulfonyl-substituted aromatic ring in **2** is attacked in preference to the alkyl halide moiety of the mustard. Our studies do not give any information on the possibility that a short-lived electron adduct of **2** could be initially formed followed by fast intramolecular electron transfer to the quaternary ammonium center. The measured high yield of mechlorethamine produced on reaction of e_{aq}^- with **2** (Table 1) indicates that the reducing equivalent transfers to the quaternary ammonium center.

Nitrobenzene Derivatives. Rapid reduction of the 4-nitrobenzene derivative, **3,** by e_{aq}^- gave an initial spectrum (at 2 μs) with a major absorbance peak at 315 nm and a minor peak at 395 nm, Figure 3. The absorbance at 395 nm decreased quickly with first-order kinetics ($k = 4 \times 10^4$ s^{-1}, independent of substrate concentration and radiation dose) with a concurrent

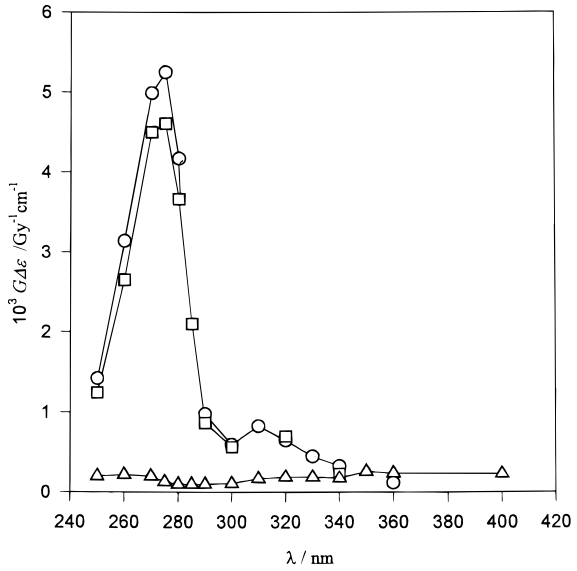

Figure 2. Absorption spectra measured after irradiating (4 Gy in 200 ns) N_2-saturated aqueous solutions containing 0.5 mol L^{-1} 2-methylpropan-2-ol, 5 mmol L^{-1} phosphate, pH 7.0, and (i) 1.0 mmol L^{-1} methanesulfonylbenzene derivative **2**. o: measured after 25 μs; (ii) 0.2 mmol L^{-1} 4-(methanesulfonyl)benzyl chloride. (○) measured after 5 μs; (iii) 0.2 mmol L^{-1} 4-(methanesulfonyl)toluene, (△) measured after 5 μs.

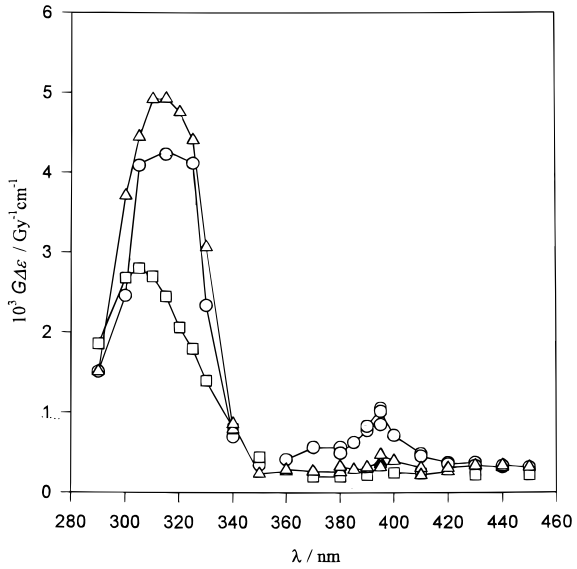

Figure 3. Pulse radiolysis of the nitrobenzene derivatives **3** and **4**. Absorption spectra measured after irradiating (4 Gy in 200 ns) N_2-saturated aqueous solutions containing 0.5 mol L^{-1} 2-methylpropan-2-ol, 5 mmol L^{-1} phosphate, pH 7.0, and 0.2 mmol L^{-1} substrate. (○) **3** measured after 2 μs, (△) **3** after 40 μs. (□) **4** after 2 μs.

increase in absorbance at 315 nm. It is possible that the e_{aq}^- could attack other sites on the substrate apart from direct electron attachment to the nitroaryl center, followed by intramolecular electron transfer to the electron affinic nitroaryl center. No chloride ion was found to be released upon reaction of e_{aq}^- with **3**. The absorption maximum and extinction coefficient of the latter species (Table 1) are similar to that reported for the radical anion of 4-nitrobenzyl chloride,[1] suggesting it to be the radical anion of **3**. However, the radical anion of **3**, unlike the radical anion of 4-nitrobenzyl chloride which decays rapidly to form the benzyl radical,[2] decayed with second-order kinetics, presumably through radical−radical reactions, reaction 3, which may also involve interaction with the formed 2-methyl-2-hydroxypropan-1-yl radical. Similar experiments were carried out with the 2-nitrobenzyl quaternary ammonium mustard, **4**. The spectrum measured 2 μs after the electron pulse exhibits only one peak at 305 nm and is similar to that reported for the radical anion of the corresponding 2-nitrobenzyl chloride.[1] The radical anion of **4** decayed with second-order kinetics (Table 1) in contrast to rapid first-order decay kinetics ($k = 1 \times 10^4$ s^{-1}) reported for the radical anion of 4-nitrobenzyl chloride.[1] A steady-state radiolysis experiment was performed to check whether the radical anion of **4** could undergo fragmentation more slowly than is observable on the pulse radiolysis time-scale (i.e. ca. <10 s^{-1}). Cumulative radiation doses were given to a deaerated solution of **4** (100 μmol L^{-1}) containing 2-methylpropan-2-ol (0.5 mol L^{-1}) and the UV/vis spectra recorded. Isosbestic points were maintained up to almost two reducing equivalents for the e_{aq}^- (at 690 Gy), consistent with disproportionation of the electron adduct to starting material and the nitrosobenzene. The loss of the isosbestic points at higher doses is presumably caused by reduction of the formed electron-affinic nitrosobenzene. Hence evidence from both pulse radiolysis and steady-state radiolysis support the conclusion that the nitrobenzyl quaternary ammonium mustards, unlike the nitrobenzyl halides compounds, do not undergo side chain scission to form the nitrobenzyl radical upon one-electron reduction. This conclusion is also supported by a recent HPLC study[14] which investigated product formation after the steady-state radiolysis of **3** and **4** in the presence of formate ions. The study demonstrated a multielectron stoichiometry for loss of the parent compounds, which was accompanied by release of mechlorethamine but without formation of the bibenzyl dimers which are characteristic stable products from nitrobenzyl radicals.

Pulse radiolysis of the 8-nitronaphthalene derivative, **5** (spectrum not shown), gave an initial spectrum with absorbance peaks at 295 and 405 nm which was similar to that reported for the initial reduction products from the 8-nitronaphthylmethyl halides[2] and is therefore assigned as the radical anion. Minor spectral changes subsequently occurred with first-order kinetics, but the resulting spectrum was different from that of the benzyl-type radical formed from 8-nitronaphthylmethyl bromide.[2] We conclude that **5**, like the simpler nitrobenzyl analogues, does not fragment by intramolecular electron transfer.

Nitroimidazole Derivative. Pulse radiolysis of the 4-nitro-5-imidazole derivative, **6**, gave a species upon reaction with e_{aq}^-, which absorbed weakly in the UV/visible region, Figure 4. This initial species decayed quickly with first-order kinetics to a form a spectrum with an absorbance maximum at 415 nm measured 400 μs after the electron pulse (corrected for subsequent second-order decay of this species). To assist with the assignment of the observed radical species, comparative experiments on the reduction of 5-(chloromethyl)-1-methyl-4-nitroimidazole by e_{aq}^- were carried out. The initial species measured 2 μs after the electron pulse quickly converted ($k =$ ca. 2.5×10^5 s^{-1}) to a species absorbing at 415 nm, measured after 20 μs. The spectrum of the latter is similar to that from **6**, when the differences in the absorption spectra of the unirradiated compounds are taken into account, and is assigned as the benzyl-type radical of the 4-nitroimidazole formed upon the rapid elimination of chloride. This assignment is confirmed by a recent HPLC study[14] of the steady-state radiolysis of **6** and 5-chloromethyl-1-methyl-4-nitroimidazole which demonstrated that both give a high yield of 1-methyl-5-[2-(1-methyl-4-nitro-5-imidazole)ethyl]-4-nitroimidazole, the expected product from dimerization of the 1-methyl-4-nitroimidazole-5-methyl radical. The spectrum of the benzyl-type radical of **6** exhibits an apparent extinction coefficient of 6150 L mol^{-1} cm^{-1} at 415

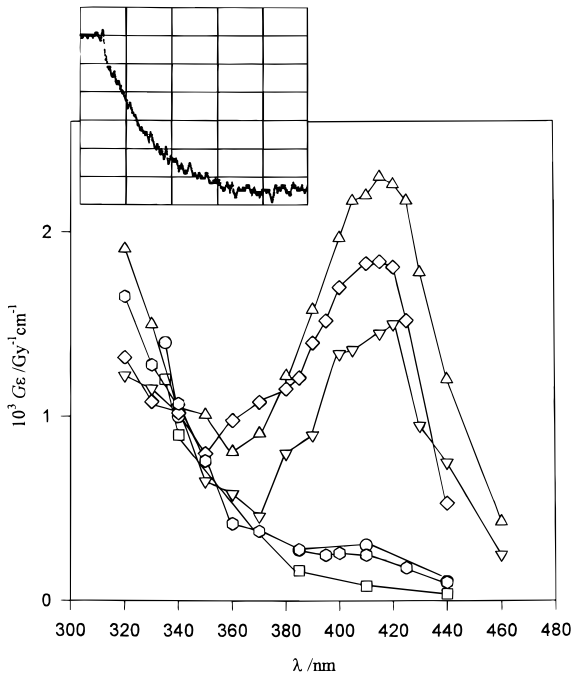

Figure 4. Absorption spectra following pulse radiolysis (4 Gy in 200ns) of N_2-saturated aqueous solutions containing 0.5 mol L^{-1} 2-methylpropan-2-ol, 5 mmol L^{-1} phosphate, pH 7.0, and 0.1 mmol L^{-1} of (i) the 4-nitroimidazole derivative **6**; measured after 5 μs, (⬡) after 400 μs; and (ii) 5-(chloromethyl)-1-methyl-4-nitroimidazole; (▽) measured after 2 μs, (△) after 20 μs. Absorption spectra following pulse radiolysis (10 Gy in 200 ns) of an N_2O/O_2 mixture saturated solution ($[O_2] = 0.3$ mmol L^{-1}) containing 0.5 mol L^{-1} propan-2-ol and 0.75 mmol L^{-1} of **6**; (○) measured after 5 μs, (□) after 400 μs. All spectra corrected for the absorbance of the unirradiated parent compound. Insert. The logarithm of transmittance (0.001 per division, *Y*-axis) against time (100 μs per division, *X*-axis) for **6** at 409 nm.

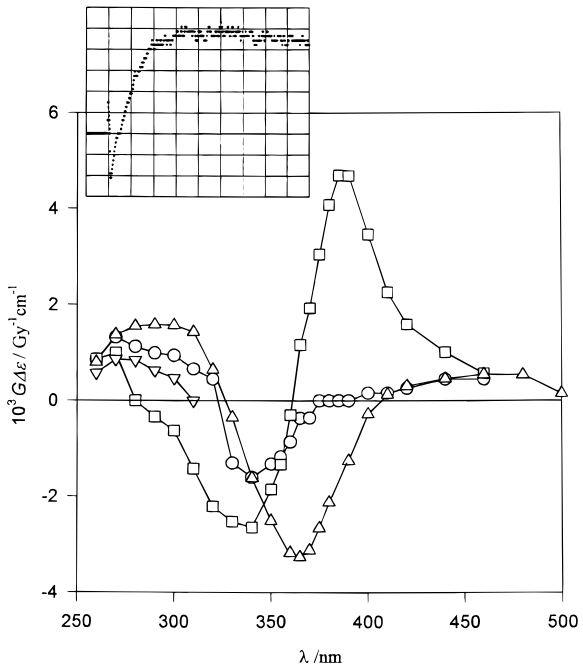

Figure 5. Absorption spectra following pulse radiolysis (4 Gy in 200 ns) of N_2-saturated aqueous solutions containing 0.5 mol L^{-1} 2-methylpropan-2-ol, 5 mmol L^{-1} phosphate, pH 7.0, and 0.1 mmol L^{-1} of (i) the 5-nitropyrrole derivative **7**; (○) measured after 5 μs, (□) after 400 μs; and (ii) 0.1 mmol L^{-1} 2-chloromethyl-1-methyl-5-nitropyrrole; (△) measured after 5 μs. Absorption spectra following pulse radiolysis (10 Gy in 200 ns) of an N_2O/O_2 mixture saturated solution ($[O_2] = 0.3$ mmol L^{-1}) containing 0.5 mol L^{-1} propan-2-ol and 0.5 mmol L^{-1} of **7**; (▽) measured after 400 μs. Insert. The logarithm of transmittance (0.001 per division, *Y*-axis) against time (100 μs per division, *X*-axis) for **7** at 310 nm.

nm compared to 8000 L mol^{-1} cm^{-1} determined from the 5-chloromethyl derivative, indicating that not all of the e_{aq}^- lead to the elimination of mechlorethamine from **6**. Again, as in the case of the nitroaryl compound **3**, no release of chloride from the alkyl chloride side chain occurred. However, it is clear from the spectral data presented here that intramolecular electron transfer from the nitro group to the methylquaternary moiety, with fragmentation to release the amine, does occur in the 4-nitroimidazole analogue (although with slower kinetics than for the corresponding chloromethyl compound).

Nitropyrrole Derivative. Pulse radiolysis of the 5-nitro-2-pyrrole derivative, **7**, was also consistent with intramolecular electron transfer to form a benzyl-type radical. The initially formed transient, measured 2 μs after the electron pulse, absorbed less strongly than the parent compound (λ_{max}, 332 nm, $\epsilon = 9440$ L mol^{-1} cm^{-1}) in the 330−380 nm region, resulting in bleaching, and decayed with first-order kinetics to form a spectrum with absorbance maximum at 385 nm, measured 400 μs after the electron pulse, Figure 5, Table 1. Comparative experiments were carried out with 2-chloromethyl-1-methyl-5-nitropyrrole ($\lambda_{max} = 360$ nm, $\epsilon = 11\,900$ L mol^{-1} cm^{-1}). The species formed 2 μs after the electron pulse again absorbs less than the parent compound, but in the 330−410 nm region reflecting the spectral shift of the compound compared to **7** (Figure 5). The initial species formed by both compounds on reaction with e_{aq}^- have similar absorption spectra when corrected for loss of their respective parent compounds (corrected spectra not shown), and are assigned as the radical anions. Unexpectedly, the radical anion of 2-chloromethyl-1-methyl-5-nitropyrrole proved to be quite stable over the pulse radiolysis observation time (ca. 1 s) and did not yield evidence for a benzyl-type radical of the 1-methyl-5-nitro-2-pyrrolyl species. However, the first-order kinetic formation of a species absorbing maximally near 400 nm, where most benzyl-type radicals of nitroaryl compounds have been reported to absorb, compared to the radical anion of **7**, supports the conclusion that the benzyl-type radical of 1-methyl-5-nitro-2-pyrrolyl is formed by the rapid elimination of mechlorethamine.

Effect of Added Oxygen. It has been generally considered that the hypoxia-selectivity of nitroaromatic bioreductive drugs, which are activated via a one-electron-reduced intermediate either directly or following subsequent bimolecular reactions, is a consequence of redox cycling. In the presence of O_2 the one-electron intermediate transfers its electron to O_2 to regenerate the parent compound and form $O_2^{\bullet -}$.[19]

$$^{-\bullet}ArCH_2X + O_2 \rightarrow ArCH_2X + O_2^{\bullet -} \qquad (4)$$

We have used pulse radiolysis to ascertain whether such a reaction is rapid enough to compete with the fragmentation of nitroaryl quaternary salts, reaction 2. Both the 4-nitrobenzene derivative, **3**, and 2-nitrobenzene derivative, **4**, form observable radical anion intermediates upon one-electron reduction and are oxidized by O_2 (determined using four concentrations of O_2) with rate constants reported in Table 1. The measured rate constants are of comparable magnitude to those for the reaction of O_2 with the radical anions of other nitroaryl compounds[20] of similar one-electron reduction potential.[7] In contrast, the decay rates of the radical anions of the 4-nitroimidazole derivative **6** and the 5-nitropyrrole derivative **7** were unaffected by O_2, even using O_2 saturation. Neither radical anion, however, gave the earlier observed benzyl-type radicals in the presence of O_2 presumably because they react quickly with O_2 to form peroxy

radicals. Spectral data for the formation of the presumed peroxyl radicals of **6** and **7** are given in Figures 4 and 5.

$$^{-\bullet}ArCH_2X \rightarrow ArCH_2^{\bullet} + X^- \quad (2)$$

$$ArCH_2^{\bullet} + O_2 \rightarrow ArCH_2OO^{\bullet}$$

Limits on the second-order rate constants for the electron-transfer reaction of O_2 with the radical anions of **6** and **7** can be set as $<6.0 \times 10^6$ and $<8.0 \times 10^6$ L mol^{-1} s^{-1}, respectively.

The failure of oxygen to reoxidize the initial radical intermediate, in competition with its first-order fragmentation, does not preclude hypoxia-selective toxicity since **6** is 100-fold more potent to EMT6 cells under anoxic than aerobic conditions (unpublished data). This suggests that oxygen can inhibit metabolic activation of the nitroarylmethyl quaternary compounds by mechanisms other than redox cycling. Another bioreductive drug which undergoes pseudo-first-order fragmentation to release a cytotoxin on one-electron reduction, the cobalt(III)−nitrogen mustard complex SN 24771, also shows significant selective toxicity toward hypoxic cells despite the fact that its initial one-electron reduction product (the Co(II) intermediate) is not significantly reoxidized by O_2.[21] The latter study suggested that competition between O_2 and the bioreductive drug for metabolic reductants might be an alternative mechanism by which O_2 suppresses metabolic activation. This mechanism may also contribute to the hypoxic selectivity of nitroarylmethyl quaternary ammonium salts as bioreductive drugs.

Conclusions

This study has shown that there are multiple mechanisms by which arylmethyl quaternary ammonium compounds fragment on reduction. Unlike the nitrobenzyl halides, the nitrobenzyl quaternary ammonium mustards show no evidence of intramolecular electron transfer from their radical anions to form benzyl radicals. Steady-state radiolysis studies[14] show reductive release of the tertiary amine (mechlorethamine) from such compounds occurs from a product in which the nitro group is reduced further by more than one electron. Fragmentation of the nonnitro benzyl quaternary compounds does occur on one-electron reduction, as demonstrated by the prompt formation of the corresponding benzyl radicals in the present study. With the low-potential benzene derivative **1**, this appears to be predominantly due to direct electron attachment to the benzyl quaternary moiety, while for the more electron-deficient 4-SO_2CH_3 derivative there is indirect evidence for initial electron attachment to the aromatic system followed by very rapid transfer to the benzylic position. In the case of the nitroheterocyclic analogues, **6** and **7**, dissociative intramolecular electron-transfer kinetics are observed. The faster electron-transfer kinetics for the nitroheterocycles than the nitrobenzene or nitronaphthalene derivatives might well be related to their electron-rich π-systems where six electrons are spread over five atoms and the addition of a further electron aids the expulsion of the leaving group. On the basis of results for the nitrobenzyl and 4-nitroimidazole ring systems, the kinetics of intramolecular electron transfer are slower for a tertiary amine rather for a halide leaving group, but the 5-nitropyrrole system is an exception to this with the nitro radical anion of the chloromethyl compound being surprisingly stable.

This investigation has several implications for the use of arylmethyl quaternary salts as reductively-activated drugs for destroying hypoxic cells in tumors. It indicates that particular attention should be directed to nitroheterocyclic analogues. Fragmentation of these compounds at the one-electron reduction level has the advantage of providing a more favorable stoichiometry for the desired release of a cytotoxin from the compounds and also avoids interception of subsequent reduction products through other reactions. Although rapid unimolecular fragmentation prevents reoxidation of the intermediate nitro radicals by oxygen, this does not preclude selectivity for hypoxic cells as there are possibly other mechanisms by which oxygen inhibits the release of the cytotoxin from the compounds.[21] Although benzyl radicals are formed promptly on one-electron reduction of the non-nitro benzyl quaternary compounds **1** and **2**, these low-potential compounds will be difficult to reduce enzymatically. Their activation in tumors might be achievable using ionizing radiation, although the efficiency would be expected to be low because competing electron acceptors would be unlikely to subsequently reduce these compounds. In light of these findings we are currently investigating further nitroheterocyclic arylmethyl quaternary salts, incorporating much more potent tertiary amine leaving groups such as amino *seco*-cyclopropylbenz[*e*]indolines,[22] as drugs for enzymatic or radiolytic activation in hypoxic regions of tumors.

Acknowledgment. This study was supported by the National Cancer Institute (contract no. NO1-CM47019) and The Health Research Council of New Zealand.

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