One-electron Redox Chemistry of Amsacrine, mAMSA [9-(2-Methoxy-4-methylsulphonylaminoanilino)acridinium], its Quinone Di-imine, and an Analogue. A Radiolytic Study

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The redox chemistry of the clinical antileukemia drug mAMSA (amsacrine) and of its dimethylamino analogue (diAMSA) has been investigated by radiolytic methods. Steady-state and pulse radiolysis experiments show that upon one-electron oxidation both compounds give quinone di-imine radicals (mAQDI$^{\bullet}$ and diAQDI$^{\bullet}$) which disproportionate to the parent compounds and the corresponding quinone di-imines (mAQDI and diAQDI). One-electron reduction of the quinone di-imines give the same intermediate radicals which are not oxidized by oxygen. Nanosecond pulse radiolysis experiments show that the initial one-electron reduction of the quinone di-imines also occurs at the acridine ring, but is followed by rapid intramolecular electron transfer to the quinone di-imine side-chain. One-electron reduction of mAMSA occurs at the acridine ring and leads to acridan formation. The redox potential at pH 7.4 of the mAMSA–mAQDI$^{\bullet}$ couple is calculated as 915 mV (*versus* n.h.e.), while the redox potential of the mAMSA–mAQDI couple is 415 mV. The analogue diAMSA shows a similar reversible redox chemistry but is much more readily oxidizable, with a calculated redox potential diAMSA–diAQDI$^{\bullet}$ of 635 mV, and a redox potential diAMSA–diAQDI of 330 mV.

The 9-anilinoacridine class of compounds are noted for their anticancer properties, with amsacrine [9-(2-methoxy-4-methylsulphonylaminoanilino)acridinium] (mAMSA) (**1**) proving a valuable antileukemic drug,[1–3] an analogue (**2**) currently in clinical trial,[4] and several other members of the class now undergoing advanced preclinical evaluation.[5,6]

Amsacrine (**1**) is a DNA-intercalating agent,[3] and its biological activity has been postulated to be due to its ability to cause double-strand DNA breaks[7,8] by interfering with the normal function of the DNA nicking–closing enzyme topisomerase II,[9,10] although recent work[11] suggests that repair is inhibited at an earlier stage. Other studies[12] on the metabolism of amsacrine in rats have identified the glutathione conjugate (**3**) as the main biliary metabolite. This is postulated to be formed by nucleophilic, 1,4-addition to an intermediary quinone di-imine mAQDI (**4**). Other metabolic products detected include the quinone imine mAQI (**5**), presumably formed by competing hydrolysis of (**4**). Oxidation of amsacrine to mAQDI is reversible and facile, with a potential for the two-electron oxidation measured by cyclic voltammetry (in 40% acetonitrile; pH 4.5) of 280 mV,[13] and can be accomplished readily by liver microsomes[13] or by reaction with MnO_2.[14]

The role of this facile oxidation in the biological activity of amsacrine is not yet clear. Some studies have reported that mAQDI is more cytotoxic than amsacrine itself,[14] while rapid non-enzymatic cleavage of DNA is observed in the presence of amsacrine, oxygen, and copper salts, suggesting the possibility of redox cycling of the drug.[15,16] It is also possible that mAQDI could act as an alkylating agent towards macromolecules. Thus the redox chemistry of the 9-anilinoacridines is clearly important in terms of their biological activity. A preliminary study of the electrochemistry of the 9-anilinoacridines has been carried out[13] using cyclic voltammetry, exploring the redox process and the products of electrochemical and chemical oxidation of amsacrine (**1**) and selected derivatives. In this paper we use radiolytic methods to investigate in detail the redox chemistry of amsacrine and a related compound diAMSA (**6**) (one of the analogues under advanced biological evaluation) and demonstrate the involvement of radical intermediates in these processes.

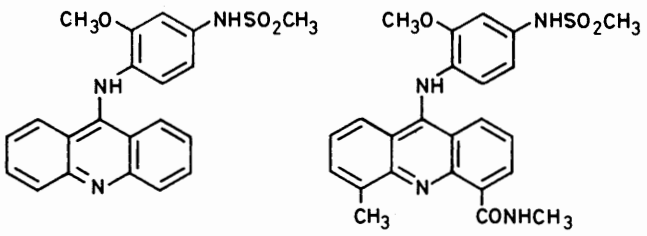
Experimental

Amsacrine (mAMSA)† (**1**), the 3′-dimethylamino derivative diAMSA (**6**), and their corresponding quinone di-imines mAQDI (**4**) and diAQDI (**7**) were synthesized by published methods.[3,5,13] mAMSA was used either as the HCl salt or as the methanesulphonate salt, and diAMSA was used as the HCl salt.

Steady-state irradiations were performed using a ^{60}Co γ-ray source (20 TBq) at a dose rate of *ca.* 0.5 Gy s^{-1}. Dosimetry was performed using the Fricke dosimeter. Spectra of irradiated solutions were recorded on a Varian DMA 100 spectrophotometer using a 1 cm cell. In experiments involving reductions of mAMSA the cell was an integral part of the irradiation vessel allowing spectra to be recorded without the solution coming in contact with the atmosphere.

Pulse radiolysis experiments were carried out using either a 1.8 MV Linac (1.5—4 Gy in 0.2 μs) or a 4 MV van de Graaff accelerator (2—10 Gy in 30 ns). The associated charge-monitoring devices and optical detection systems have been described.[17,18] Solutions were prepared in water purified by a Milli-Q system (Millipore Inc.). The dose delivered to the

† mAMSA, Amsacrine, 9-(2-methoxy-4-methylsulphonylaminoanilino)acridinium; diAMSA, 9-(2-dimethylamino-4-methylsulphonylaminoanilino)acridinium; mAQDI, N^1-(acridinyl)-N^4-methylsulphonyl-2-methoxycyclohexa-2,5-diene-1′,4′-di-imine; diAQDI, N^1-(acridinyl)-N^4-methylsulphonyl-2-dimethylaminocyclohexa-2,5-diene-1′,4′-di-imine; BQ, 1,4-benzoquinone; DMBQ, 2,5-dimethyl-1,4-benzoquinone.



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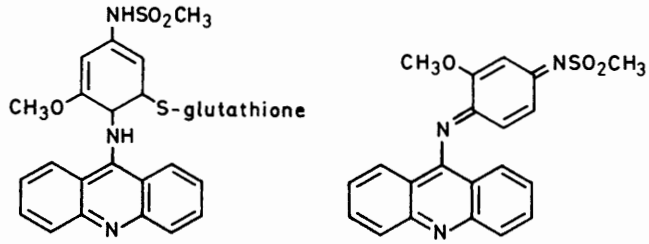

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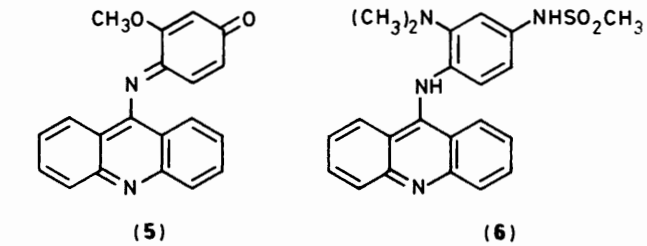

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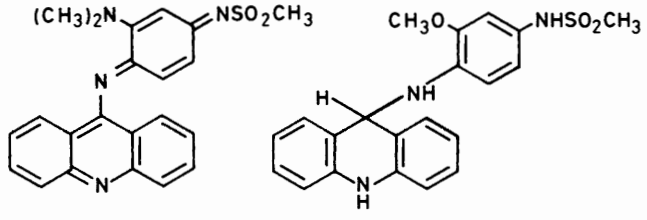


(7) (8)

solutions (21 °C) contained in a 2 cm pathlength cell was determined by the measurement of optical density at 472 nm in aerated KSCN[19] (10 mmol dm^{-3}) assuming an extinction coefficient ε of 758 m^2 mol^{-1} and a radiation chemical yield of 0.29 μmol J^{-1}. Transients were recorded on a Tektronic 7612D digitizer interfaced to a PDP 11/34 computer for data analysis. [Absorptions are presented as the product of the radiation chemical yield, G/mol J^{-1}, and the extinction coefficient, ε/m^2 mol^{-1}. The raw data are proportional to the differences in extinction coefficient between reactant(s) and product(s).]

The radical species formed upon the radiolysis (1) of water were used to study (i) the reduction or (ii) oxidation of substrates by (i) adding 2-methylpropan-2-ol to scavenge the oxidizing radicals or converting the oxidizing radicals into a reducing species by adding propan-2-ol; (ii) saturating solutions with N_2O to convert the e_{aq}^- into $^{\bullet}OH$ radicals which in turn were scavenged by added Br^- or N_3^- ions to form oxidizing inorganic radicals.

$$H_2O \wedge\!\!\!\rightarrow e_{aq}^-,\ {}^{\bullet}OH,\ H^{\bullet},\ H_2O_2,\ H_2,\ H_3O^+ \quad (1)$$

$$^{\bullet}OH(H^{\bullet}) + (CH_3)_3COH \rightarrow {}^{\bullet}CH_2(CH_3)_2COH + H_2O(H_2) \quad (2)$$

$$^{\bullet}OH(H^{\bullet}) + (CH_3)_2CHOH \rightarrow (CH_3)_2\dot{C}OH + H_2O(H_2) \quad (3)$$

$$e_{aq}^- + N_2O \rightarrow {}^{\bullet}OH + OH^- + N_2 \quad (4)$$

$$^{\bullet}OH + 2Br^-(N_3^-) \rightarrow Br_2^{\bullet -}(N_3^{\bullet}) + OH^- \quad (5)$$

Results and Discussion

(A) *Steady-state Radiolysis.—Amsacrine* (mAMSA)*–quinone di-imine* (mAQDI) *conversion.* When mAMSA was irradiated in an N_2O-saturated solution containing a high concentration of KBr (10 mmol dm^{-3}) it was converted into its quinone di-imine form mAQDI (Figure 1a). The observed isosbestic points indicate a clean conversion and the measured yield of mAQDI corresponded to half the yield of $Br_2^{\bullet -}$ formed under experimental conditions. This implies that $Br_2^{\bullet -}$ oxidizes

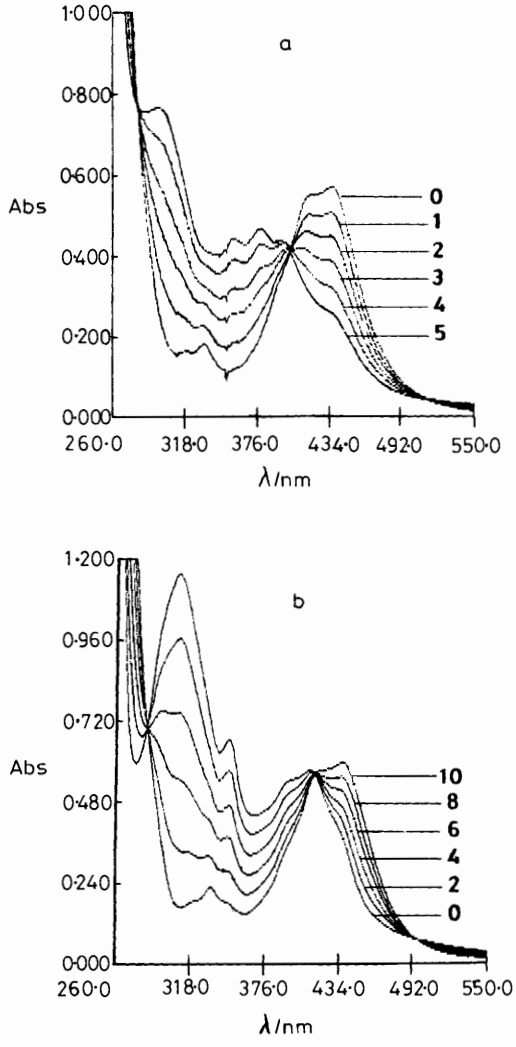


Figure 1. a, Dose-related spectra following the steady-state radiolysis (0.43 Gy s^{-1}) of an N_2O-saturated solution containing mAMSA methanesulphonate (50 μmol dm^{-3}), KBr (10 mmol dm^{-3}), and NaH_2PO_4 (4 mmol dm^{-3}) adjusted to pH 7.4 with NaOH. Times shown in min. b, Dose-related spectra following the steady-state radiolysis (0.45 Gy s^{-1}) of an N_2-saturated solution containing mAQDI hydrochloride (100 μmol dm^{-3}) in aqueous propan-2-ol (50% v/v) at pH 5. Times shown in min (solutions diluted 1:1 after irradiation)

mAMSA quantitatively to a radical intermediate which disproportionates to give mAMSA and mAQDI. This scenario is consistent with the formation and decay of quinone di-imine radical mAQDI• [reactions (6) and (7)].

$$mAMSA + Br_2^{\bullet -} \longrightarrow mAMSA^{+\bullet} (\rightleftharpoons mAQDI^{\bullet} + H^+) + 2Br^- \quad (6)$$

$$2mAQDI^{\bullet} \longrightarrow mAMSA + mAQDI \quad (7)$$

The reaction also proceeds cleanly in the reverse direction when mAQDI undergoes reduction. Figure 1b shows dose-related spectra when mAQDI was irradiated in an N_2-saturated solution containing propan-2-ol. The yield of mAMSA corresponds to half the sum of the yields of e_{aq}^- and propan-2-oxyl radicals. In both systems the reactions go to at least 80% completion before other products, as shown by the departure from the isosbestic points, start to form. The spectra in Figure 1a and b do not match exactly as the spectrum of mAQDI changes significantly when the pH is increased from 5 to 7.5, the intensity of the 304 nm peak decreasing and with the formation of peaks at 356 and 376 nm. When the experiment of Figure 1a is carried out at pH 5 it matches Figure 1b.

Entirely similar results were obtained in corresponding experiments with diAMSA and its quinone di-imine diAQDI as shown in Figure 2a and b.

Effect of oxygen. We have studied the radiolysis of mAMSA (methanesulphonate) (50 μmol dm^{-3}) in the presence of KBr (10 mmol dm^{-3}) under atmospheres of N_2O, O_2, and N_2 up to pH 11.6 (above this pH mAMSA is unstable). Under N_2O mAMSA was converted into mAQDI as described above but in contrast atmospheres of O_2 and N_2 afforded complete protection. Under these gases the yield of $Br_2^{-\bullet}$ is half that formed under N_2O and the e_{aq}^- is scavenged by O_2 to give $O_2^{-\bullet}$ or by mAMSA under N_2 to give its electron adduct.[19] If the quinone di-imine radical mAQDI• formed by reaction (6) transferred an electron to O_2 the yield of mAQDI would be approximately the same under N_2O and O_2 as all mAQDI• formed under O_2 would be oxidized to mAQDI compared with only half when disproportionation occurs [reaction (7)]. The fact that almost complete protection is seen indicates that $O_2^{-\bullet}$ and the electron adduct of mAMSA both reduce the mAQDI back to mAMSA.

We have also compared the reduction of mAQDI in acidified aqueous propan-2-ol solutions (50% v/v) and up to pH 9.0 in the absence and presence of oxygen. When mAQDI (250 μmol dm^{-3}) was irradiated under N_2 or a 5% O_2 in N_2 gas mixture the yields of mAMSA were identical. Under these conditions O_2 scavenges *ca.* 40% of the e_{eq}^- and $(CH_3)_2\dot{C}OH$ radicals. The results indicate that not only does the quinone di-imine radical not transfer an electron to O_2 (this would give complete protection); but that $O_2^{-\bullet}$ itself reduced mAQDI. This was confirmed by irradiating mAQDI (100 μmol dm^{-3}) as above but under an atmosphere of O_2, where virtually all the primary radiolytic radicals give rise to $O_2^{-\bullet}$. The yields of mAMSA and the loss of mAQDI where the same as in the presence of N_2.

Reduction of mAMSA. In our previous paper[20] we reported pulse radiolysis experiments on the reduction of mAMSA by e_{aq}^- and $(CH_3)_2\dot{C}OH$. We have now made a brief study of this reduction under steady-state radiolysis conditions.

When a N_2-saturated acidified propan-2-ol solution (20% v/v) was irradiated at a dose of 285 Gy the yellow colour of the solution was destroyed and a small precipitate formed. On standing under N_2 this precipitate slowly disappeared and the yellow colour returned. Figure 3 shows the spectrum at various

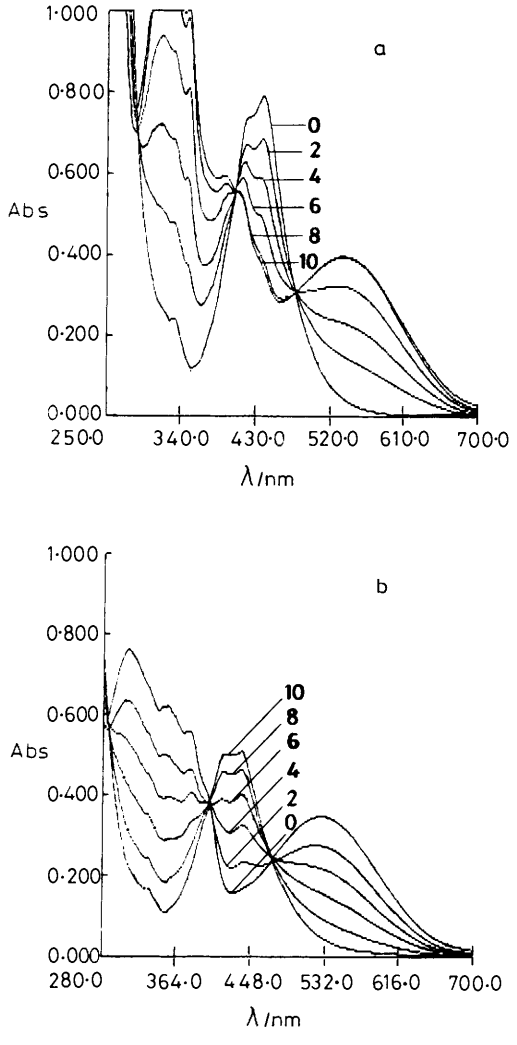


Figure 2. a, Dose-related spectra following the steady-state radiolysis (0.55 Gy s^{-1}) of an N_2O-saturated solution containing diAMSA·HCl (100 μmol dm^{-3}) and KBr (10 mmol dm^{-3}) unbuffered. Times shown in min (solutions diluted 1:1 after irradiation). b, Dose-related spectra following the steady-state radiolysis (0.55 Gy s^{-1}) of an N_2-saturated solution containing diAQDI (100 μmol dm^{-3}) in aqueous propan-2-ol (50% v/v) unbuffered. Times shown in min (solutions diluted 1:1 after irradiation)

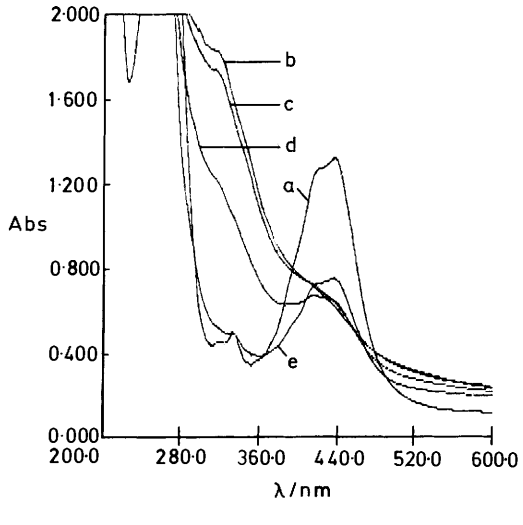


Figure 3. Spectra of acidified mAMSA (100 μmol dm^{-3}) in N_2-saturated propan-2-ol (20% v/v) before and at various times after a dose of 285 Gy. Unirradiated, (a); after 5 min, (b); 25 min, (c); 50 min, (d); and 110 min (e)

times after irradiation where 90% of the mAMSA had been regenerated after 22 h. When the reduction was carried out in 50% propan-2-ol no precipitation occurred and the regeneration of mAMSA was slower, 30% in 3 h. When the free base of mAMSA was reduced in 50% propan-2-ol the recovery of the starting material was much slower, 20% in 24 h. However if air was admitted to any of the above irradiated solutions regeneration of the colour appeared instantaneously with 85—95% of mAMSA being reformed.

We suggest that the radiation product is the substituted 9-aminoacridan (**8**), formed by the disproportionation of the electron adduct of mAMSA. 9-Aminoacridan is reported to reduce water.[21] The greater rate of regeneration of mAMSA with higher water concentration (20% *versus* 50% propan-2-ol) and with lower pH (acidified mAMSA *versus* the free base) is consistent with the reaction involving reduction of water to hydrogen by an acridan. The less than 100% regeneration may be due to the formation of a small amount of the biacridanyl by coupling of the electron adduct of mAMSA.

(B) *Pulse Radiolysis.—Sequential one-electron oxidation and reduction.* Oxidation of mAMSA by $Br_2^{-\bullet}$ [reaction (6)] was found to occur with a rate constant of *ca.* 2.5 × 10^8 mol^{-1} s^{-1}. The resultant spectrum was dose dependent which implies that under pulse radiolysis conditions some of the $Br_2^{-\bullet}$ radicals are lost through reaction with themselves. Oxidation by the $N_3^\bullet$ radical occurred much faster leading to a dose-independent (1.5—7 Gy) spectrum presented in Figure 4a. The rate constant of reaction (8) was determined by following the build-up in absorbance at 530 nm for three concentrations of mAMSA, k_8 2.5 ± 0.2 × 10^9 dm^3 mol^{-1} s^{-1}.

The oxidized radical form of mAMSA (mADQI$^\bullet$) decayed with second-order kinetics, $2k$ 1.1 ± 0.1 × 10^9 dm^3 mol^{-1} s^{-1} (varying [mAMSA] 1—1.5 × 10^{-4} mol dm^{-3} and dose 1.5—7 Gy) to a new spectrum presented in Figure 4a. Spectra corrected for the bleaching of mAMSA absorption for both the intermediate and product (assuming 50% restoration) are presented in Figure 4b. The decay rate of the intermediate (mAQDI$^\bullet$) was uninfluenced by O_2 (measurements made as above in an N_2O–O_2 4:1 saturating gas mixture).

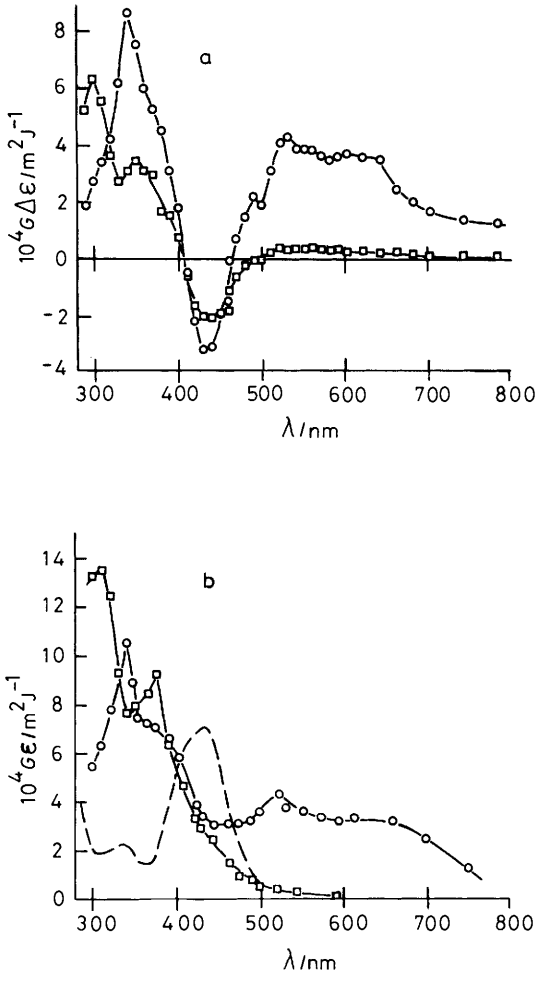


Figure 4. a, Changes in absorption (presented as the product of the yield of radicals, G/mol J^{-1}, and the change in extinction, $\Delta\varepsilon/m^2$ mol^{-1}) following pulse radiolysis (2 Gy in 0.1 μs) of an N_2-saturated solution containing mAMSA (100 μmol dm^{-3}) and sodium azide (10 mmol dm^{-3}): spectra measured at ○ 70 μs and □ 15 ms following the pulse. b, The absorption spectra of the intermediate, ○ and product, □ (G × 2) from Figure 4a corrected for the bleaching of mAMSA absorption, — — —

$$mAMSA + N_3^\bullet \rightarrow mAMSA^{+\bullet} (mAQDI^\bullet + H^+) + N_3^- \quad (8)$$

$$e_{aq}^- + mAQDI + H^+ \longrightarrow mAQDI^\bullet \quad (9)$$

$$(CH_3)_2\dot{C}OH + mAQDI \longrightarrow mAQDI^\bullet + (CH_3)_2CO \quad (10)$$

$$2mAQDI^\bullet \longrightarrow mAMSA + mAQDI \quad (11)$$

$$diAMSA + N_3^\bullet \rightarrow diAMSA^{+\bullet} (\rightleftharpoons diAQDI^\bullet H^+) + N_3^- \quad (12)$$

$$e_{aq}^- + diAQDI + H^+ \longrightarrow diAQDI^\bullet \quad (13)$$

$$(CH_3)_2\dot{C}OH + diAQDI \longrightarrow diAQDI^\bullet + (CH_3)_2CO \quad (14)$$

$$2\ diAQDI^\bullet \longrightarrow diAMSA + diAQDI^\bullet \quad (15)$$

The one-electron reduction of mAQDI was achieved by the simultaneous scavenging of e_{aq}^- ($k_9 \geqq 2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) and electron transfer from propan-2-oxyl radicals (k_{10} *ca.* 3 × 10^9 mol^{-1} s^{-1}) in N_2-saturated solutions containing propan-2-ol (Figure 5a).

The corrected spectrum measured 150 μs after the pulse (Figure 5b) corresponds to that measured upon the oxidation of mAMSA by $N_3^\bullet$ [reaction (8)] (Figure 4b). The radical intermediate formed by reactions (9) and (10) decayed with second-order kinetics ($2k$ *ca.* 10^9 dm^3 mol^{-1} s^{-1} at pH ≦7 decreasing to 4 × 10^8 dm^3 mol^{-1} s^{-1} at pH 10) to a final spectrum presented in Figure 5a. The final spectrum corrected for the bleaching of mAQDI absorption (assuming 50% restoration) is presented in Figure 5b. The corrected spectrum measured 9 ms after the pulse corresponds to that of mAMSA (Figure 4b).

These results show that the intermediate mAQDI$^\bullet$, produced upon either the one-electron oxidation of mAMSA or the reduction of mAQDI, dismutates to yield mAMSA and mAQDI [reaction (11)].

Entirely analogous results were obtained with diAMSA and diAQDI where oxidation of diAMSA by $N_3^\bullet$ [reaction (12)] (k *ca.* 2 × 10^9 dm^3 mol^{-1} s^{-1}) and reduction of diAQDI by e_{aq}^- [reaction (13)] ($k \geqq 2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) or by propan-2-oxyl radicals [reaction (14)] (k 4 ± 0.2 × 10^9 dm^3 mol^{-1} s^{-1}) gave rise to the same intermediate diAQDI$^\bullet$ (Figures 6 and 7).

The diAQDI$^\bullet$ intermediate, produced by both methods, decayed with second-order kinetics ($2k$ *ca.* 10^9 dm^3 mol^{-1} s^{-1} for pH ≦ 8, 5 × 10^8 dm^3 mol^{-1} s^{-1} at pH 10) to yield diAMSA and diAQDI [reaction (15)] (Figures 6 and 7).

Intramolecular electron transfer. One-electron reduction of

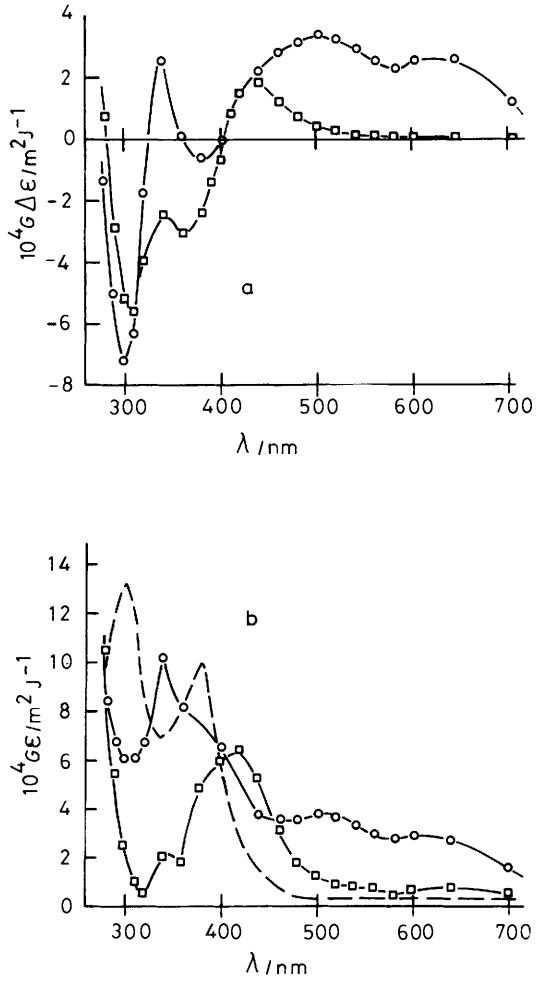


Figure 5. a, Changes in absorption (presented as the product of the yield of radicals, G/mol J^{-1}, and the change in extinction, $\Delta\varepsilon/m^2$ mol^{-1}) following pulse radiolysis (4 Gy in 0.1 μs) of an N_2-saturated solution containing mAQDI (40 μmol dm^{-3}) in 20% propan-2-ol: spectra measured at ○ 150 μχ and □ 9 ms following the pulse. b, The absorption spectra of the intermediate, ○ and product, □ ($G \times 2$) from Figure 5a corrected for the bleaching of mAQDI absorption — — —

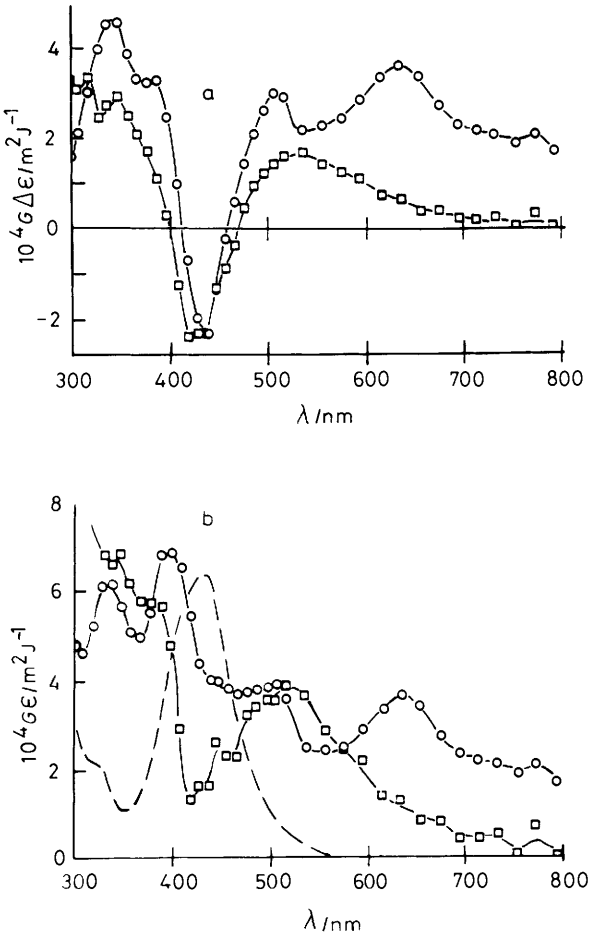


Figure 6. a, Changes in absorption (presented as the product of the yield of radicals, G/mol J^{-1}, and the change in extinction, $\Delta\varepsilon/m^2$ mol^{-1}) following pulse radiolysis (1.5 Gy in 0.1 μs) of an N_2-saturated solution containing diAMSA (50 μmol dm^{-3}) and sodium azide (10 mmol dm^{-3}): spectra measured at ○ 50 μs and □ 8 ms following the pulse. b, The absorption spectra of the intermediate, ○ and product, □ ($G \times 2$) from Figure 6a corrected for the bleaching of diAMSA absorption — — —

both mAQDI and diAQDI by the e_{aq}^- was further studied in N_2-saturated solutions containing 2-methylpropan-2-ol. The absorption due to the e_{eq}^- quickly decayed to a transient species m(di)AQDI$^{-\bullet}$ which in turn converted to the spectra observed for the quinone di-imine radicals [Figure 8, insert Figure 9].

The observed rates of conversion [reactions (17) and (18)]

$$e_{aq}^- + \text{m(di)AQDI} \longrightarrow \text{m(di)AQDI}^{\bullet -} \quad (16)$$

$$\text{mAQDI}^{\bullet -} \xrightarrow{H^+} \text{mAQDI}^{\bullet} \quad (17)$$

$$\text{diAQDI}^{\bullet -} \xrightarrow{H^+} \text{diAQDI}^{\bullet} \quad (18)$$

were independent of substrate concentrations and dose (2—10 Gy). These observations indicate that the conversions are intramolecular electron-transfer reactions. The e_{aq}^- reacts very rapidly with the acridine ring[20,22] and by analogy with quinones[23] is expected to react equally rapidly with the quinone di-imine side-chain. We suggest that intramolecular electron transfer occurs from the one-electron reduced low-potential acridine ring [E(1) at pH 7 -803 ± 10 mV[20]] to the higher-potential quinone di-imine side-chain (see later). The rate constant for electron transfer for diAQDI [reaction (18)] is markedly dependent on pH (Figure 9) and might be related to a protonation of the 3-dimethylamino substituent on the side-chain which would create a positive centre. Both quinone di-imine radicals exhibit spectral changes at different pH. In basic solution both radicals exhibit pK *ca.* 8.5 which might arise from the deprotonation of the side-chain (see Conclusions) and spectral changes in acidic solution might be related to protonation on the acridine ring.

Redox potentials. The pulse radiolysis method of establishing reversible equilibria[24,25] was used to determine the redox couples mAQDI–mAQDI$^{-\bullet}$ and diAQDI–diAQDI$^{-\bullet}$ at pH 7.4 against reference quinones Q. The equilibrium constant K can

$$\text{m(di)AQDI}^{\bullet} + Q \rightleftharpoons \text{m(di)AQDI} + Q^{\bullet -} + H^+ \quad (19)$$

be estimated from the absorption of the equilibrium concentration of mAQDI$^{\bullet}$ (at 540 nm) or diAQDI$^{\bullet}$ (at 650 nm) before significant decay occurs *via* reactions (11) and (15). Since [m(di)AQDI$^{\bullet}$] + [$Q^{-\bullet}$] is a constant at a fixed radiation dose, the ratio [m(di)AQDI$^{\bullet}$]/[$Q^{-\bullet}$] can be calculated and hence K for various substrate concentrations. Since the redox potentials

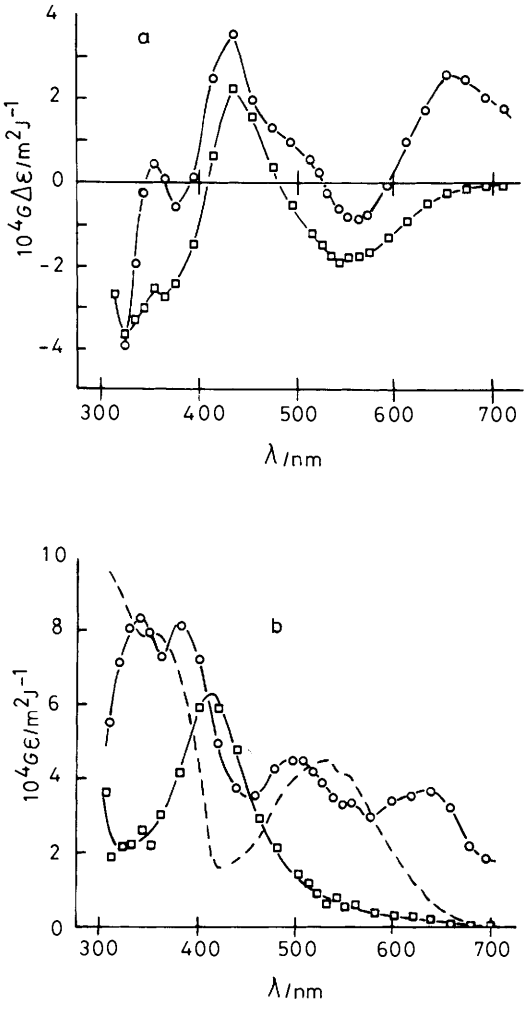


Figure 7. a, Changes in absorption (presented as the product of the yield of radicals, G/mol J^{-1} and the change in extinction, $\Delta\varepsilon/m^2$ mol^{-1}) following pulse radiolysis (4 Gy in 0.1 μs) of an N_2-saturated solution containing diAQDI (40 μmol dm^{-3}) in 10% propan-2-ol: spectra measured at ○ 80 μs and □ 8 ms following the pulse. b, The absorption spectra of the intermediate, ○ and product, □ ($G \times 2$) from Figure 7a corrected for the bleaching of diAQDI absorption — — —

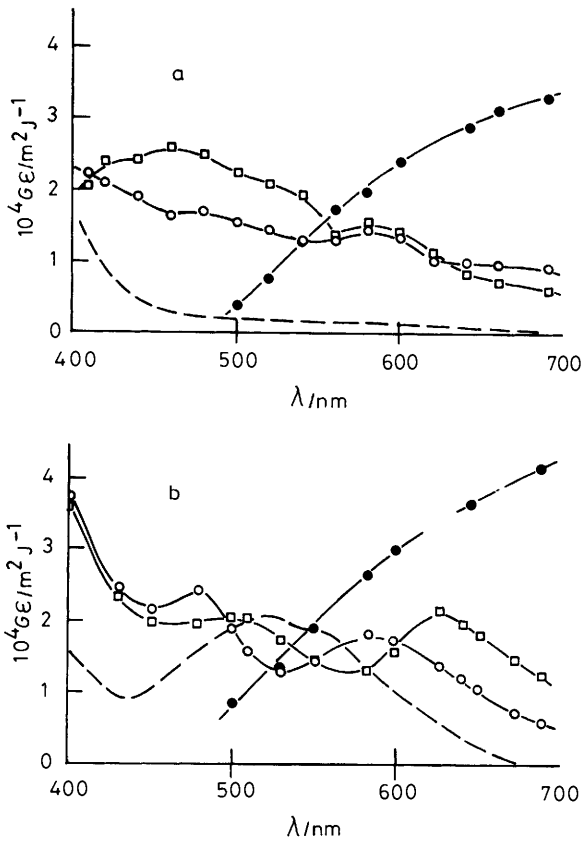


Figure 8. Absorption spectra of the initial intermediates observed following pulse radiolysis (10 Gy in 30 ns) of N_2-saturated solutions containing 2-methylpropan-2-ol (20%) and either a, mAQDI (100 μmol dm^{-3}) or b, dAQDI (100 μmol dm^{-3}) measured at ● 100 ns, ○ 1.5 μs, and □ 30 μs following the pulse. The absorption spectra are corrected for substrate absorption — — —

E[m(di)AQDI/m(di)AQDI$^{\bullet}$] = E(Q/$Q^{-\bullet}$) − 59 log K it is possible to calculate E[m(di)AQDI/m(di)AQDI$^{\bullet}$] using, for 1,4-benzoquinone E(BQ/$BQ^{-\bullet}$) 99 mV and for 2,5-dimethyl-1,4-benzoquinone E(DMBQ/$DMBQ^{-\bullet}$) −66 mV [26] (*versus* n.h.e.). From four solutions, varying [mAQDI] (50—70 μmol dm^{-3}) and [BQ] (50—200 μmol dm^{-3}) in 20% propan-2-ol N_2-saturated solution, K 1.75 ± 0.66. Hence ΔE 14 ± 10 mV and E(mAQDI/mAQDI$^{\bullet}$) 85 ± 10 mV. The mAQDI$^{\bullet}$ species was also produced upon the oxidation of mAMSA by $N_3^{\bullet}$ radicals and equilibrium (19) established using DMBQ. The solution contained [mAMSA] (100 μmol dm^{-3}), [DMBQ] (1 mmol dm^{-3}), and [NaN_3] (20 mmol dm^{-3}) in N_2O-saturated solution. K was calculated from measurements made *ca.* 1 ms after the pulse (for both 1.6 and 2.7 Gy) assuming $G(N_3^{\bullet})$ 0.6 μmol J^{-1}; K 0.0028 ± 0.0004. Hence ΔE −151 ± 5 mV and E(mAQDI/mAQDI$^{\bullet}$) 85 ± 5 mV in agreement with above.

From four solutions varying [diAQDI] (420—580 μmol dm^{-3}) and [BQ] (30—40 μmol dm^{-3}) in 10% propan-2-ol of N_2-saturated solution, K = 70 ± 23. Hence ΔE 109 ± 9 mV and E(diAQDI/diAQDI$^{\bullet}$) −10 ± 9 mV. From two solutions of [diAQDI] (100—200 μmol dm^{-3}) and [DMBQ] (275 μmol

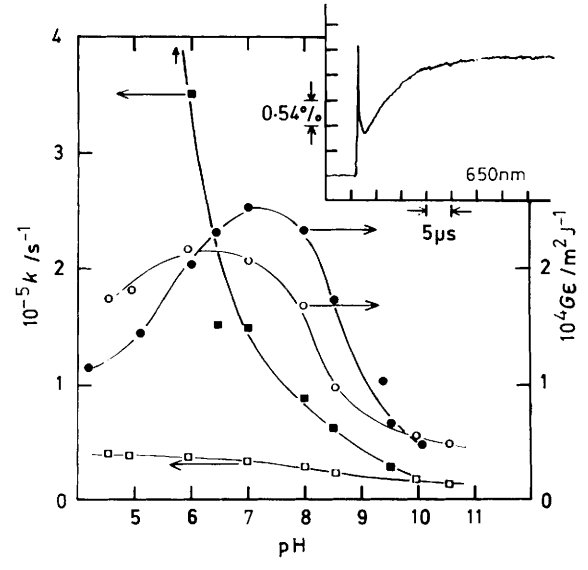
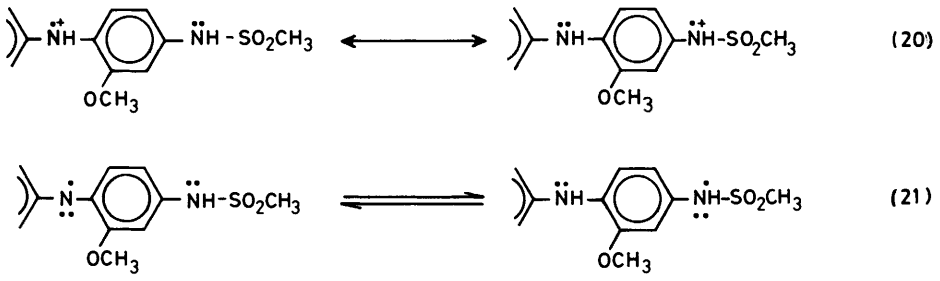


Figure 9. The dependence of first order rate constant (k) and absorption increase ($G\varepsilon$) on pH for the appearance of the second transient following pulse radiolysis (2 Gy in 30 ns) of N_2-saturated solutions containing 2-methylpropan-2-ol (20%) and (i) mAQDI (100 μmol dm^{-3}), open symbols measured at 540 nm; (ii) diAQDI (100 μmol dm^{-3}), closed symbols measured at 650 nm. Inset: Oscilloscope trace displaying the change in percentage absorption (ordinate) against time (abscissa) for diAQDI at pH 7

dm^{-3}) in 10% propan-2-ol N_2-saturated solution, K 0.36 ± 0.09. Hence ΔE −26 ± 6 and E(diAQDI/diAQDI$^{\bullet}$) −40 ± 10 mV. Averaging both series of experiments gives E(diAQDI/diAQDI$^{\bullet}$) −25 ± 15 mV.

Conclusions

These studies show conclusively that the reduction of mAMSA is associated with the acridine ring moiety and oxidation with the anilino side-chain. Reduction of mAQDI occurs dominantly at the relatively high-potential quinone di-imine moiety and the pulse radiolysis studies show that the facile reversible interconversion of mAMSA and mAQDI occurs through the same radical intermediate, mAQDI$^{\bullet}$. The first electron loss from mAMSA could give the radical mAMSA$^{+\bullet}$ [process (20)] and loss of a proton would give in principle the tautomer pair [process (21)]. In view of the negative inductive effect of the methylsulphonyl group, the equilibrium may well lie to the right. The same radical would also be expected to be formed upon one-electron reduction of mAQDI followed by protonation and we assign this structure of mAQDI$^{\bullet}$. The fact that the radical is not oxidized by molecular oxygen up to pH 11.6 suggests its pK_a is greater than this figure as its conjugate base should be a much stronger reducing agent.



The recent cyclic voltammetry study[13] has determined the two-electron oxidation potential for the couple E-(mAMSA/mAQDI) to be 280 mV *versus* s.c.e. at pH 4.5, *i.e.* 524 mV *versus* n.h.e. Correcting this value to pH 7.4 on the basis of 29 mV per pH unit and making a further correction of *ca.* 25 mV by comparison with other solvents of similar composition and dielectric constant (*e.g.* methanol)[27] gives an estimate for E(mAMSA/mAQDI) (at pH 7.4) 415 mV. From our pulse radiolysis studies the one-electron redox potential for E(mAQDI$^{\bullet}$/mAQDI) −85 mV and hence as the two-electron redox potential is equal to half the sum of the two one-electron redox potentials E(mAMSA/mAQDI$^{\bullet}$) 915 mV. Performing similar calculations for diAMSA gives E(diAMSA/diAQDI) 330 mV (calculated from cyclic voltammetry data[13]), E(diAQDI$^{\bullet}$/diAQDI) 25 mV and E(diAMSA/diAQDI$^{\bullet}$) 635 mV. (Although $E_{\frac{1}{2}}$ values measured by cyclic voltammetry are not exactly the same as the thermodynamic redox potentials, the difference will be small.)

The measured and calculated redox potentials indicate that diAMSA can undergo both one- and two-electron oxidations more readily than mAMSA. While the ΔE between the compounds for two-electron oxidations is *ca.* 85 mV a much larger ΔE for their one-electron oxidations of *ca.* 280 mV exists. Such differences in potential indicate that the oxidation of diAMSA would be much more facile through the two one-electron steps than a single two-electron process compared to mAMSA.

Previous work[28] has shown that a close relationship exists between *in vivo* antitumour potency (D_{40}, the dose of drug needed to produce an increase in lifespan of 40%) and DNA association constants for mAMSA and derivatives, with tight binding related to high potency. However, although mAMSA and diAMSA show similar binding levels, diAMSA is *ca.* 10-fold less potent.[29] The much more facile cellular oxidation of diAMSA could provide an explanation for this anomaly, if oxidation to the quinone di-imine diAQDI followed by conjugation with thiols such as glutathione is a detoxification pathway, as suggested for mAMSA.[12]

The broader spectrum of action of diAMSA, especially against remotely sited solid tumours,[29] makes it of great interest as a possible second-generation drug. The detailed information about its redox chemistry gained here is of value in planning treatment protocols (for example, the co-administration of ascorbate, which cleanly re-reduced diAQDI to diAMSA, to minimize oxidation metabolism).

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