Reactions of semiquinones in aqueous solution. A comparison of the one electron reduction of kalafungin and analogues with other semiquinones using pulse radiolysis

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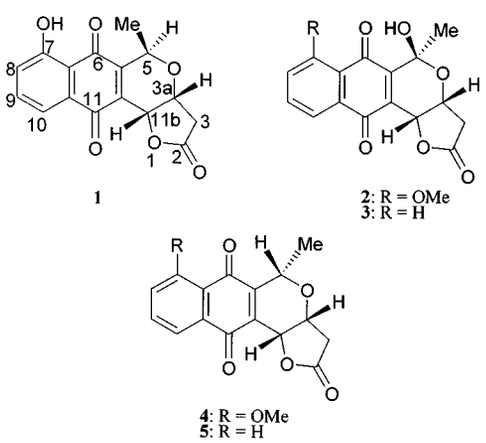
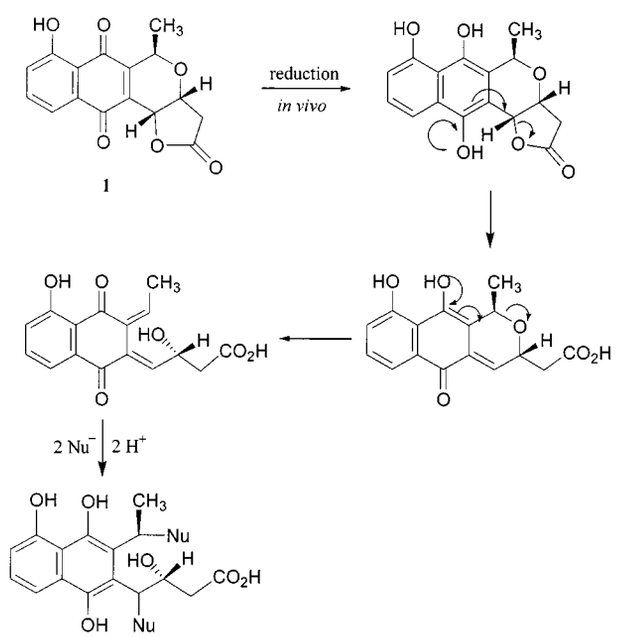
The radical anions of the pyranonaphthoquinone antibiotic kalafungin **1** and analogues have been studied in aqueous solution by pulse radiolysis using transient absorption spectrophotometry. Radical absorption spectra were similar regardless of the nature of the substituent at C-5 or C-7 and the decay followed second-order kinetics showing any potential first-order ring opening was too slow to compete with bimolecular disproportionation at the concentration of radicals produced by pulse radiolysis. Spectral studies using steady-state radiolysis confirmed the absence of ring opening. The pK_a of each of the semiquinones of compounds **1**–**5** were determined and whereas replacing a hydrogen at C-7 by a methoxy, **2** and **4**, raises the pK_a by 2.5–2.9 units demonstrating a significant substituent effect, no such increase occurs for OH, **1**. This and the fact that kalafungin, **1**, has the most positive one-electron reduction potential $E(1)$, -63 ± 10 mV in neutral solution, is attributed to H-bonding between the 7-OH and the oxygen of the semiquinone, with the H-bonding effectively nullifying the substituent effect. The presence of an OH on C-5 has a much less significant effect on the $E(1)$ and pK_a values. Comparison of kinetics and products in systems with and without *tert*-butanol brings into question a report that 2-(methoxymethyl)benzo-1,4-quinone and 2-(phenoxymethyl)benzo-1,4-quinone undergo dissociation on one-electron reduction.

Introduction

Kalafungin, **1**, is a member of the pyranonaphthoquinone family of antibiotics,[1] which are produced by various species of *Streptomyces* and have in common the benzoisochromanequinone skeleton. Kalafungin was shown to be inhibitory *in vivo* against a variety of pathogenic fungi, yeasts, protozoa and both gram-positive and gram-negative bacteria.[2] It was also found to inhibit platelet aggregation[3] and exhibited strong cytotoxicity against L5178Y mouse leukaemia cells *in vitro*.[4]



The activity of several classes of antitumor antibiotics (*e.g.* mitomycins,[5] anthracyclines[6] and the enediynes[7]) is based on the unmasking of specific functionality after reduction of a quinone nucleus. Thus the anthracycline daunomycin has an amino group positioned proximal to the quinone which is only jettisoned after quinone reduction whilst mitomycins undergo aziridine ring opening after a similar reduction. Moore[8,9] has suggested that *in vivo* reduction of pyranonaphthoquinones exemplified by kalafungin, causes a transformation to an active hydroquinone form which functions as a bis-alkylating agent in a similar manner to that observed for the mitomycins and anthracyclines (Scheme 1). A similar mechanism could be



Scheme 1

invoked using the one electron reduced species (the semiquinone) of kalafungin **1**. One objective of this work was to investigate this hypothesis which has not yet been proven.

O'Shea and Fox[10] have suggested from pulse radiolysis kinetic data that the protonated semiquinones of 2-(methoxymethyl)benzo-1,4-quinone and 2-(phenoxymethyl)benzo-1,4-

quinone dissociate by loss of the methoxide and phenoxide groups to give the *o*-quinone methide radical. In principle an equivalent cleavage at either O-1 or O-4 could occur on one-electron reduction of kalafungin, **1**. The corresponding methide radical, or both its disproportionation products would be potent alkylating agents.

Development of pyranonaphthoquinones as bioreductive alkylating agents hinges on a detailed study of the behaviour of these compounds under reducing conditions. In this paper we report our studies on the one-electron reduction of kalafungin, **1**, and analogues **2**–**5** using pulse radiolysis. We report pK_a values, redox potentials and kinetic behaviour of the semiquinone radicals, and discuss the implications of these results with respect to known semiquinone chemistry.

Experimental

Chemicals

Pyranonaphthoquinones **1**–**5** were prepared according to our previously published procedure.[11] Solutions were prepared in water purified by a Millipore "Milli-Q" system.

Methods

Rapid one-electron reduction of the compounds was carried out using pulse radiolysis. Pulsed electrons (4 Gy in 200 ns from the University of Auckland 4 MeV Dynaray linear accelerator) were delivered to deaerated aqueous solutions (10 mmol L^{-1} phosphate, pH 7) containing 0.1 mol L^{-1} sodium formate and 50–200 μmol L^{-1} kalafungin, **1** or analogues **2**–**5** [eqns. (1)–(5)].

$$H_2O \rightsquigarrow e^-_{aq} + {}^{\bullet}OH + H^{\bullet} + H_2O_2 + H_2 + H^+$$

$$e^-_{aq} + A \longrightarrow A^{\bullet -} \quad (1)$$

$$^{\bullet}OH(H^{\bullet}) + HCOO^- \longrightarrow CO^{\bullet -} + H_2O(H_2) \quad (2)$$

$$CO_2^{\bullet -} + A \longrightarrow A^{\bullet -} + CO_2 \quad (3)$$

$$HCO_2^{\bullet} \rightleftharpoons CO_2^{\bullet -} + H^+ \quad (4)$$

$$HCO_2^{\bullet} + A \longrightarrow A^{\bullet -} + CO_2 + H^+ \quad (5)$$

Under these conditions the one-electron reduced forms of **1**–**5**, $A^{\bullet -}$, are produced by direct scavenging of e^-_{aq} ($k_1 \geqslant 10^{10}$ L mol^{-1} s^{-1}) and through fast electron transfer from the $CO_2^{\bullet -}$ species. The absorbed dose in the 1.0 cm pathlength cell was determined using aerated potassium thiocyanate solution by monitoring the formed $(SCN)_2^{\bullet -}$ species at 475 nm.[12] Radical formation and transformations were followed by time-resolved UV/visible spectrophotometry. The pK_a values of the semiquinones were determined by following the changes in absorbance following one-electron reduction as a function of pH. For **2** and **4**, solutions contained 100 μmol L^{-1} compound, 0.1 mol L^{-1} sodium formate and were purged with N_2 gas. For **1**, **3**, **5** solutions contained 40 μmol L^{-1} compound, 1.0 mol L^{-1} sodium formate and were purged with N_2O gas. At low pH the e^-_{aq} react with H^+ to give hydrogen atoms which add to the quinones. Lower compound concentration, high formate concentration and N_2O saturation [to quantitatively convert e^-_{aq} into $^{\bullet}OH$ radicals (eqn. (6)] results in all primary radicals forming $CO_2^{\bullet -}$. Some

$$N_2O + e^-_{aq} + H_2O \longrightarrow N_2 + {}^{\bullet}OH + OH^- \quad (6)$$

decay of the semiquinones occurred before reduction with $CO_2^{\bullet -}$ was complete and the decay curve was extrapolated back to zero time after the pulse to obtain the true absorbance of the semiquinone.

Semiquinone spectra were measured at pH 7 and low pH as the change in extinction coefficient at different wavelengths derived from changes in absorbance per unit dose assuming published radical yields for solutions containing high concentrations of formate.[13] Redox equilibria involving reference quinone compounds[14] were measured in deaerated solutions containing 0.2 mol L^{-1} propan-2-ol to ensure the dissolution of all compounds and to convert $^{\bullet}OH$ radicals into reducing 2-hydroxy-2-propyl radicals [eqns. (7) and (8)].

$$^{\bullet}OH + (CH_3)_2CHOH \longrightarrow (CH_3)_2C^{\bullet}OH \quad (7)$$

$$(CH_3)_2C^{\bullet}OH + Q \longrightarrow (CH_3)_2CO + Q^{\bullet -} + H^+ \quad (8)$$

In steady-state radiolysis experiments, also at pH 7, 5 mL samples were irradiated in a cobalt-60 source using a glass vessel with a spectrophotometer cell attached on a side-arm. Fricke dosimetry was used to measure the dose-rate and spectra were run between 200 and 600 nm wavelength for various doses up to 2 mol equivalents of reducing radicals.

Results and discussion

Much of the data obtained in this study are summarised in Table 1.

Absorption spectra and decay kinetics of the semiquinones

The spectra of transient species formed upon electron transfer from the $CO_2^{\bullet -}$/$HCOO^{\bullet}$ radicals to **1**, relative to the absorption of the parent compound, are presented in Fig. 1 for both pH 7.0 and low pH. The bimolecular rate constants for the reduction of all compounds (Table 1) were obtained by observing the formation kinetics at several wavelengths over the 300 to 640 nm region. At pH 7 all electron adducts of **1**–**5** exhibit a major peak in the 385–395 nm region which shift by 5–15 nm to shorter wavelength at low pH (Table 1). All electron adducts decayed by pure second-order kinetics (shown by the fact that plots of (half-life)$^{-1}$ against radiation dose (radical concentration) had no intercept) showing that any potential first-order ring opening was too slow to compete with bimolecular disproportionation. In all cases the observed second-order rate constants increased with decreasing pH, implying that the neutral protonated electron adducts decayed faster [eqn. (10)] than the

$$AH^{\bullet} \rightleftharpoons A^{\bullet -} + H^+ \quad (9)$$

$$2AH^{\bullet} \longrightarrow AH_2 + A \quad (10)$$

singly negatively charged semiquinones themselves [eqn. (11)].

$$2A^{\bullet -} (+2H) \longrightarrow AH_2 + A \quad (11)$$

At pH 7 it is most likely that the observed radical decay involves a mixture of protonated and deprotonated species [eqn. (12)].

$$AH^{\bullet} + A^{\bullet -} (+H^+) \longrightarrow AH_2 + A \quad (12)$$

This is to be expected and has been found for the most simple model case, the decay of benzosemiquinone where values of 1.7×10^8 and 1.1×10^9 L mol^{-1} s^{-1} have been found at pH 7 and 2 respectively.[15]

Semiquinone pK_a values

The prototropic equilibria of the electron adducts [eqn. (9)], were determined by following the initial absorption changes for each compound in the 380–395 nm region over the pH range 0.5 to 7 (Fig. 1 Insert, data for **1**). As none of the unreduced parent compounds undergo prototropic equilibria in this pH region, plots of the absorption of each of the electron adducts as a function of pH, yield semiquinone pK_a data (Table 1). It is of interest to compare the pK_a values for the semiquinones of compounds **1**–**5** (Table 1) with other published values of semiquinones of other compounds: benzo-1,4-quinone, 4.0;[15]

Table 1 Spectral properties, reaction kinetics[a] and equilibrium data of the semiquinones of kalafungin, **1**, and analogues

	Compound				
	1	**2**	**3**	**4**	**5**
$k_3(CO_2^{\bullet -} + A)/10^9$	2.4	1.6	2.1	2.9	1.0
$k_5(HCO_2^{\bullet -} + A)/10^9$	4.0	1.6	2.5	2.9	1.1
$A^{\bullet -}$, λ_{max}/nm	385	395	395	385	385
ε/L mol^{-1} cm^{-1}	9075	10000	13470	10000	9160
$AH^{\bullet}$, λ_{max}/nm	380	385	380	375	375
ε/L mol^{-1} cm^{-1}	10260	7090	7970	8090	5750
pK_r	1.89 ± 0.21	4.58 ± 0.10	1.96 ± 0.06	4.86 ± 0.18	2.26 ± 0.17
$2k_{12}(A^{\bullet -})/10^8$ (pH 7)	10.9	1.2	1.5	6.5	4.8
$2k_{10}(A)/10^8$ (pH 2)	14.7	8.3	8.2	26.7	(10.2)
K_{13} (equilib. *vs.* quinones)[b]	0.95 ± 0.11[c]	7.00 ± 0.47[d]	0.36 ± 0.11[c]	4.48 ± 1.66[d]	0.19 ± 0.01[c]
$E(1)$/mV	−68 ± 10	−150 ± 10	−93 ± 10	−162 ± 10	−109 ± 10
K_{14} (equilib. *vs.* O_2)[b]	0.031 ± 0.005	0.483 ± 0.035	0.130 ± 0.041	1.71 ± 0.05	0.69 ± 0.07
$E(1)$/mV	−66 ± 9	−136 ± 7	−103 ± 9	−169 ± 7	−140 ± 8
$k_{14}(A^{\bullet -} + O_2)/10^8$	0.12	0.87	0.4	1.6	0.5
$k_{-14}(O_2^{\bullet -} + A)/10^8$	6.2	1.6	2.0	0.8	1.4
K_{14} (k_{14}/k_{-14})	0.019	0.542	0.20	2.00	0.357
$E(1)$/mV	−54	−139	−114	−173	−129
$E(1)$/mV (average) (pH 7)	−63 ± 8	−142 ± 7	−103 ± 11	−168 ± 6	−126 ± 16

[a] Units of rate constants are L mol^{-1} s^{-1}. [b] Calculated using eqn. (15). [c] Equilibrium using 2,5-dimethylbenzoquinone. [d] Equilibrium using menadione.

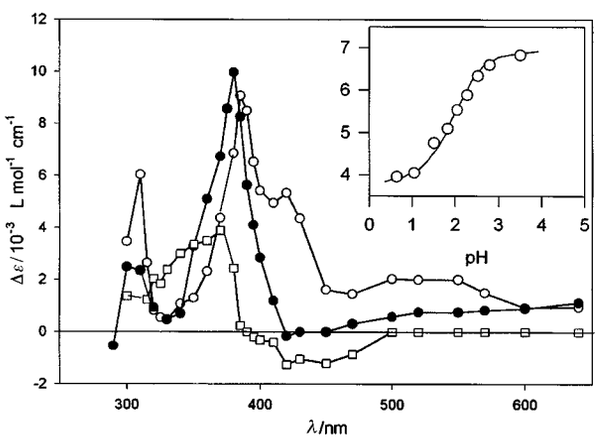


Fig. 1 Transient absorption spectra (radicals and products minus the absorption of unirradiated parent compound) following pulse radiolysis (4 Gy in 200 ns) of kalafungin, **1** (40 μmol L^{-1}) in N_2O-saturated aqueous solution at; (i) pH 7, containing 0.1 mol L^{-1} sodium formate, (●) 20 μs, (○) 5 ms after pulse, and (ii) pH 1.0, containing 1 mol L^{-1} sodium formate, □ 20 μs after pulse. **Insert:** Effect of pH on absorption changes at 385 nm for the radical of **1**.

naphtho-1,4-quinone, 4.1;[14] 2-(methoxymethyl)benzo-1,4-quinone, 3.8;[10] 2-(phenoxymethyl)benzo-1,4-quinone, 3.9;[10] 2-(bromomethyl)benzo-1,4-quinone, 4.1;[10] 2-(chloromethyl)-benzo-1,4-quinone, 4.0;[10] juglone, 5-hydroxynaphtho-1,4-quinone, 3.65;[16] naphthazarin, 5,8-dihydroxynaphtho-1,4-quinone, 2.7.[17]

pK_a values can be sensitive to the effects of substituents and of hydrogen bonding. The almost identical values for benzo-1,4-semiquinone and naphtho-1,4-semiquinone suggests that the additional fused ring has little effect on the semiquinone pK_a. Mukherjee[16] attributes the lower value for juglone relative to naphthoquinone ($\Delta pK_a = 0.45$) to hydrogen-bonding between the 5-OH and the semiquinone oxygen which stabilises the base form of the semiquinone of juglone. However, the 5-OH electron-donating properties might also have a significant effect on the pK_a, but in the opposite direction. Our kalafungin analogues allow us to partially separate the hydrogen-bonding and electron-donating substituent effects. Comparison of **4/5** and **2/3** show that the replacement of H by CH_3O raises the pK_a by 2.6 units. Assuming the electron donation power of the HO and CH_3O groups are similar, comparison of **1/4** show that the H-bonding effect alone lowers the pK_a by 3.0 (assuming the inversion of chirality on the α-carbon has no effect). As the pK_a of **1** is 0.37 less than that of **5** in this system the H-bonding is slightly greater but in the opposite direction to the substituent effect. Thus the hydrogen bonding effect alone in juglone would lead to a ΔpK_a greater than 0.45 recorded.

As the 7-OH group stabilises the base form of the semiquinone it might be anticipated that the OH group on the carbon at the 5 position would also have a significant effect as in both cases the H involved is part of a six membered ring. Comparisons **2/4** (4.58 and 4.86) and **3/5** (1.96 and 2.26) suggest that there might be an effect, but it is very much less. This can be rationalised in terms of the hydrogen of the phenoxy 7-OH being more positive than that of the alkoxy 5-OH (phenols being more acidic than alcohols). The difference in planarity of the two types of ring may also be significant. The pK_a value of the semiquinone of compound **5** is 1.84 units lower than that of naphtho-1,4-quinone. It is not clear why the 2,3-ring disubstitution should lower the pK_a to such an extent, but entropy changes due to solvation can be very significant.

O'Shea and Fox[10] suggest that hydrogen bonding occurs between the 2-(methoxymethyl) oxygen and the hydrogen of the protonated semiquinone of 2-(methoxymethyl)benzo-1,4-quinone. If this were the case the protonated semiquinone would be stabilised by this hydrogen bonding and its pK_a would be greater than that of semibenzoquinone itself. But their own measurements show it to be 0.2 units lower. Thus any H-bonding effect is insufficient to cancel out any substituent effect of the methoxymethyl group.

One-electron reduction potentials and reaction with oxygen

The one-electron reduction potentials of all compounds at pH 7 [$E(1)$] were determined against two reference quinones, Q [menadione, 2-methylnaphtho-1,4-quinone ($E(1) = -203 \pm 5$ mV[18]) and 2,5-dimethylbenzoquinone ($E(1) = -67 \pm 10$ mV[19])] and oxygen by quickly establishing redox equilibrium[14] [eqns. (13) and (14)] before significant loss of the semiquinones through radical–radical reactions.

$$A^{\bullet -} + Q \rightleftharpoons A + Q^{\bullet -} \quad (13)$$

$$A^{\bullet -} + O_2 \rightleftharpoons A + O_2^{\bullet -} \quad (14)$$

Equilibrium constants, K_{13} were determined from separate measurements of the absorptions of both the reference quinones, Q, and each compound, A, monitoring the A_{max} of both our semiquinones and those of the reference compounds at the

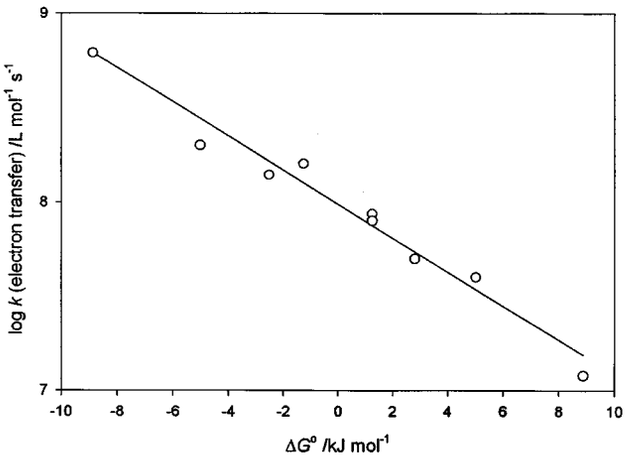


Fig. 2 Dependence of the forward or back rate constants of eqn. (14) on ΔG° between the compounds and oxygen.

corresponding wavelengths, $A_{Q}\cdot^-$, $A_{A}\cdot^-$, and at equilibrium A_{obs} for at least three mixtures of Q (80–160 μmol L^{-1}) and A (50–100 μmol L^{-1}). Averaged values for K_{13}, determined from expression (15), are presented in Table 1.

$$K_{13} = \{[A](A_{obs} - A_{A}\cdot^-)\}/\{[Q](A_{Q}\cdot^- - A_{obs})\} \quad (15)$$

Similarly, K_{14} was determined by saturating the solutions with different concentrations of oxygen (100–500 μmol L^{-1}), obtained on gas mixing with N_2, where superoxide, $O_2{}^{\bullet -}$, absorbs negligibly in the observed 380–395 nm region. The kinetics of the approach to equilibrium, k_{obs} using O_2, k_{11}, k_{-11} gave a linear relationship [eqn. (16)] from which fits to k_{obs}/

$$K_{obs}/[A] = k_{-11} + k_{11}([O_2]/[A]) \quad (16)$$

[A] *vs.* $[O_2]/[A]$ yield rate constants for the forward and back reactions between O_2 and $A^{\bullet -}$, k_{14}, k_{-14} as well as providing additional measurement of K_{14} (k_{14}/k_{-14}) (Table 1).

The $E(1)$ of **1** is the most positive of all five compounds and is most probably due to intramolecular hydrogen bonding of the 7-hydroxy group stabilising the anionic semiquinone. The 7-methoxy group lowers the redox potential in the pairs of compounds **3/2** and **5/4**. As seen for the pK_a values, the hydroxy group on the carbon at the 5-position of the saturated six membered ring, only has a small effect on the redox potentials of **2/4** and **3/5**.

The observed rate constants of equilibrium (14), k_{14}, k_{-14}, are activation controlled and can be related to the standard Gibbs free energy change, ΔG° of the reactions using electron transfer theory.[20] Over the limited range in ΔE studied, the predicted relationship between log k and ΔG° is nearly linear (Fig. 2) and has been observed by several groups.[21] A change in k of an order of magnitude for a ΔE of 0.1 V is observed, which is within the reported range in values.

Steady-state radiolysis

Evacuated solutions of compounds **1**–**5** in 0.5 mol L^{-1} propan-2-ol were irradiated with successive doses up to 2 mol equivalents of reducing radicals, and spectra run after each irradiation. For all compounds isosbestic points were obtained showing these semiquinone radicals were undergoing disproportionation to quinone and hydroquinone (Fig 3). The spectral changes matched those of the pulse radiolysis experiments at long times when radical–radical reactions were complete. On admission of air oxygen slowly oxidised the hydroquinones back to the parent quinone providing further evidence that the semiquinones had not undergone first-order ring-opening on a timescale longer than that of the pulse-

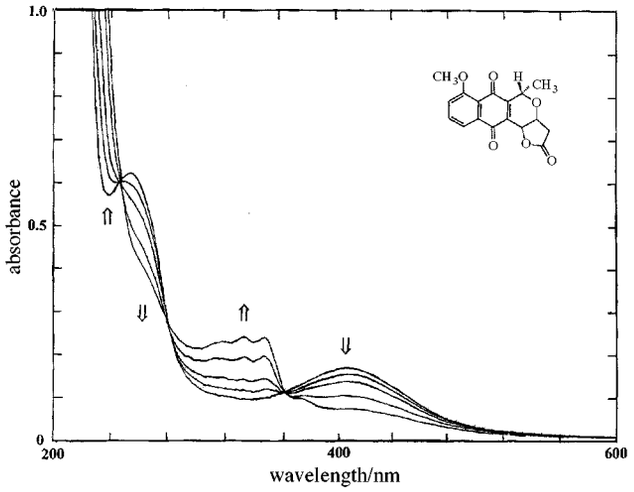


Fig. 3 Absorption spectra recorded following step-wise reduction by ^{60}Co γ-irradiation of compound **4** (40 μmol L^{-1}) in deaerated aqueous solution containing 0.1 mol L^{-1} sodium formate. Spectra measured for 0, 15, 30, 60 and 90 Gy absorbed doses. Spectral changes upon cumulative doses are in the directions indicated by the arrows.

radiolysis experiments. In the case of kalafungin itself, **1**, the spectra indicated that a second compound was also formed on the admission of air.

Chemistry of 2-(methoxymethyl)benzo-1,4-semiquinone and 2-(phenoxymethyl)benzo-1,4-semiquinone

Whereas dissociation of a number of radicals formed by the one electron reduction of nitroaromatic compounds to give benzyl (methide) type radicals has been reported,[22] O'Shea and Fox's finding that the above two semiquinones can undergo equivalent reactions is the first reported example for substituted quinones. In the light of our not finding any evidence for first-order decay of the semiquinones of kalafungin and analogues at pH 7 or lower pH it is appropriate to look more closely at their results.[10] They report that at low pH the protonated forms of these semiquinones decayed with mixed first- and second-order kinetics and gave the values of the rate constants. At pH 7 the decays were by pure second-order. However decay kinetics of the similar 2-(chloromethyl) and 2-(bromomethyl) substituted benzo-1,4-semiquinones were pure second order at both pH 7 and at low pH. As chloride and bromide are normally much better leaving groups than methoxide or phenoxide these results are surprising. The authors rationalised their conclusion by invoking hydrogen bonding between the protonated semiquinone and the methoxy or phenoxy oxygens, this presumably lowering the activation energy for dissociation just as protonation of OH or OR groups make them better leaving groups in normal nucleophilic substitution or elimination reactions. However as discussed above there is little evidence for significant hydrogen bonding from the pK_a values. Furthermore O'Shea and Fox reduced their quinones with e^-_{aq} using *tert*-butyl alcohol to scavenge $^{\bullet}OH$ radicals. Thus their measured decay kinetics would not be simple bimolecular disproportionation reactions of the protonated or unprotonated semiquinone radicals as they assumed. Reactions between semiquinones and the $^{\bullet}CH_2C(CH_3)_2OH$ radical, formed by reaction of $HO^{\bullet}$ with *tert*-butanol, could also be occurring invalidating their conclusions based entirely on the kinetics of semiquinone decay. To test this we compared the second-order decay of the semiquinone of compound **2** in neutral solution formed in the presence of propan-2-ol (where $^{\bullet}OH$ radicals are converted into reducing $(CH_3)_2C^{\bullet}OH$ radicals which reduce the quinone) with that in the presence of *tert*-butanol (the radical of which is not reducing). At the same initial electron adduct concentration the decay in the *tert*-butanol system was more than an order of magnitude greater. To confirm that a reaction can occur

between the semiquinone and the $^{\bullet}CH_2C(CH_3)_2OH$ radical we ran spectra after steady-state γ-radiolysis at different doses. The propan-2-ol system showed simple disproportionation to the hydroquinone and quinone, whereas in the *tert*-butanol system no hydroquinone was formed and the spectrum of a different product was evident. We found a similar result with menadione, disproportionation occurring under formate/N_2O conditions whereas under *tert*-butanol/N_2 conditions a new product, probably the aryl-alkyl ether from radical–radical reaction between the semiquinone and β-alcohol radicals, was found. We have also found that in pulse radiolysis studies of the second-order kinetics of many nitroaromatic radical anions, the apparent rate of decay of these radical anions is typically 100 times greater in the *tert*-butanol/N_2 system than in the formate/N_2O system.

Thus we believe that rate constants for the bimolecular decay of radicals formed by reaction of aquated electrons cannot be measured with any certainty when radicals from the scavenging of hydroxyl radicals with *tert*-butanol are present at similar concentrations. Hence we believe the kinetic evidence on which the proposed elimination of the methoxy and phenoxy groups is based is flawed and that the conclusions made are probably invalid.

Conclusions

The possible utilisation of pyranonaphthoquinones to deliver active methide moieties *via* ring opening to overcome chemoresistant hypoxic cancer cells is an attractive idea. In such a scenario hypoxia-selectivity arises from back oxidation of the semiquinones in normal oxic cells by oxygen. Although we have not found evidence of first-order decays of the electron-adducts (semiquinones) of the kalafungin series of compounds, this does not necessarily mean that ring opening *in vivo* does not occur. The concentrations of the semiquinones generated *in vivo* would be orders of magnitude less than in our pulse radiolysis experiments and hence their life-times before bimolecular decay would be very much greater. However no evidence for the elimination of leaving groups upon the one-electron reduction of a related series of substituted 2- and 6-methylnaphtho-1,4-quinone compounds has been found[23] even though such compounds possess antitumor activity.[24]

Factors other than the possible, but unobserved, slow ring opening with methide formation are important. The $E(1)$ of the compounds are similar or higher in potential to that of oxygen which result in slow rates of electron transfer from the semiquinones to oxygen. Such high $E(1)$ values could on first appearance undermine hypoxia-selectivity if dismutation to form the hydroquinone occurs in oxic solutions and is the pathway for the release of the toxic species. It has been pointed out[25] that in the *in vivo* situation oxygen can inhibit the reduction of quinones of higher $E(1)$ than of oxygen because the efficient removal of superoxide, $O_2^{\bullet -}$, by superoxide dismutase drives eqn. (14) to the right even when K_{14} is <1. However, to improve hypoxia-selectivity, future lead compounds of much lower $E(1)$ need to be developed. Also, while the unmasking of a potent cytotoxin upon one-electron reduction is desirable in some anticancer treatment regimes, such as in the release of cytotoxins in a radiation field,[26,27] the selective production of a toxic species following two-electron reduction is a desirable objective in the development of bioreductive alkylating agents.

Acknowledgements

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