Reactions of Indolic Radicals Produced upon One-electron Oxidation of 5,6-Dihydroxyindole and its N(1)-Methylated Analogue

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The reactions of indole semiquinone radicals produced following one-electron oxidation of 5,6-dihydroxyindole (DHI) and its *N*-methyl-substituted analogue (MeDHI) have been studied using pulse radiolysis with spectrophotometric detection in the pH range 5–10 using different dose/pulse values (1–20 Gy/pulse). Using a dose/pulse of 18.5 Gy the semiquinone radicals of DHI and MeDHI decay predominantly by second order kinetics. The second order rate constants for disappearance of the semiquinone radicals are dependent upon the pH. Values of rate constants for decay of the semiquinone radical of DHI ($pK_a = 6.8$) at pH 5.5 and 9.1 are 3.8×10^9 and 1.0×10^8 dm^3 mol^{-1} s^{-1} respectively at room temperature. In contrast, at a lower dose/pulse of ≤ 2 Gy, the semiquinone radical decays predominantly by first order kinetics which are dependent upon the concentration of the indole when pH $\gg$ pK_a of the semiquinone radical. The second order rate constants for interaction of the semiquinone radical of DHI and MeDHI with DHI and MeDHI at pH 8.8 were determined to be 1.6×10^6 and 2.3×10^6 dm^3 mol^{-1} s^{-1} respectively. The decay of the semiquinone radical results in the formation of a semi-permanent product(s) with a lifetime of *ca.* 10 ms. These products are discussed in terms of the formation of a reactive quinone-methide and/or - imine.

The oxidation of indolic compounds has received much attention due to the involvement of the resulting products in significant biological processes.[1–16] In particular, the oxidation of hydroxylated indoles studied by pulse radiolysis[10,11,14–16] has recently become of interest due to their role as intermediates in biological processes which lead to the formation of melanin; processes as yet not well characterised. Recently we have shown[14] that the $N_3^{\bullet}$ radical formed by radiolysis of aqueous solutions of azide ion[3–5,17] readily oxidised 5,6-dihydroxyindole (DHI) and its *N*-methyl analogue (MeDHI) by a one-electron transfer process. This yields, following deprotonation at the hydroxy oxygen, the corresponding semiquinone radical ($pK_a = 6.8$), consistent with previous observations[10,16] at pH 7.4. One-electron oxidation of several methylated indoles yields either the indolyl radical cation or the corresponding indolyl radical as a result of deprotonation at N(1).[1–9,12–14,16] The radical cation of 1-methylindole interacts with 1-methylindole.[13]

The present study was undertaken to investigate further the reactions of the semiquinone radicals of DHI and MeDHI at various times after their generation using different doses/pulse and pH-values. These conditions systematically vary the initial concentrations of the semiquinone radical and its state of protonation. Under high dose/pulse conditions at pH 7.4, it has been reported that the semiquinone radicals of DHI undergo radical–radical reactions to form semi-permanent products.[10] Chemical analysis shows that following oxidation of DHI both dimeric and trimeric products are formed.[18–21] The material balance is poor, emphasising the difficulties in investigating the oxidation chemistry of hydroxylated indoles by analysis of a limited number of products. Further, the interaction of the semiquinone radical of DHI with DHI, similar to that observed with 1-methylindole,[13] could act as a chain initiation step leading to trimeric and higher polymeric products.

Experimental

The 4.3 MeV linear accelerator and the optical detection system with data handling have previously been described.[22] Solutions were prepared using deoxygenated water, which had been purified using a Multi-Q system, to minimise the auto-oxidation of the indoles, which is especially facile in alkaline solution. The pH-values of the solutions were adjusted using either $HClO_4$ or NaOH. Solutions were saturated with N_2O prior to irradiation in order to convert radiolytically generated e^-_{aq} into $^{\bullet}OH$ radicals. The solutions generally contained 0.1 mol dm^{-3} sodium azide which, by a reaction with the generated $^{\bullet}OH$ radicals, produces $N_3^{\bullet}$ within < 1 μs. This concentration of azide ensured that virtually all of the $^{\bullet}OH$ radicals react with N_3^- to form $N_3^{\bullet}$. Solutions contained in a quartz cell of 0.2 dm pathlength were irradiated at 296 ± 2 K with electron pulses of 1.6 μs duration and a dose/pulse of 1–20 Gy. Optical filters were used to minimise photochemical effects. Radiation doses (1–20 Gy/pulse) were determined using KSCN dosimetry at λ 480 nm assuming $G = 0.3$ μmol J^{-1} and $\varepsilon = 710$ m^2 mol^{-1}. The optical absorption spectra have not been corrected for depletion of the substrates following pulse irradiation.

The indoles, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxy-1-methyl indole (MeDHI) (> 99.5% pure based upon elemental analysis and HPLC) were prepared as previously described.[14] Sodium azide and all other reagents were of 'Analar' grade and used as supplied.

Results and Discussion

One-electron oxidation of DHI or MeDHI results[14] initially in the formation of the indolic semiquinone radical. In order to investigate the subsequent reactions of these semiquinone radicals, the optical absorption spectra were determined 10 μs,

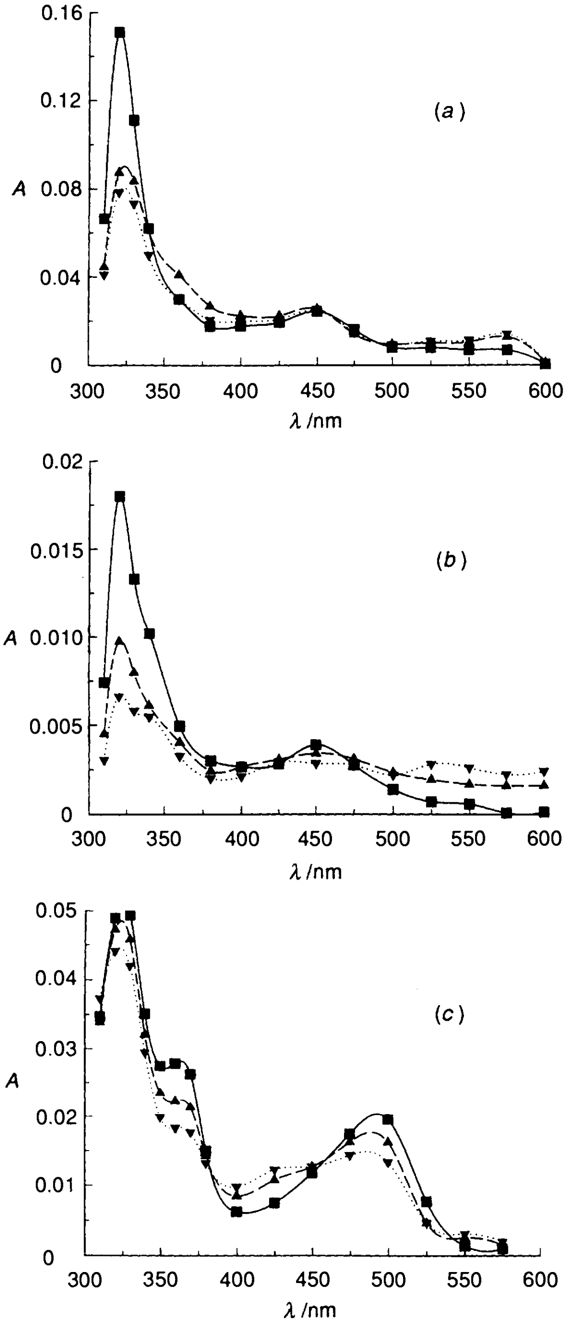


Fig. 1 The optical absorption spectra determined 10 μs (■), 7 ms (▲) and 20 ms (▼) after pulse irradiation of N_2O-saturated aqueous solution containing 10^{-4} mol dm^{-3} DHI and 10^{-1} mol dm^{-3} azide at pH 8.8 and radiation dose of (*a*) 18 Gy/pulse and (*b*) 2 Gy/pulse, and (*c*) at pH 5.2 where the spectra were recorded 4 μs (■), 10 μs (▲) and 150 μs (▼) after pulse irradiation (dose of 3 Gy per pulse)

7 ms and 20 ms after pulse irradiation of an N_2O saturated aqueous solution containing 10^{-1} mol dm^{-3} azide and 10^{-4} mol dm^{-3} DHI at pH 8.8. These spectra are shown in Figs. 1(*a*) and 1(*b*) for radiation doses of 2 Gy/pulse and 18 Gy/pulse. The spectrum at longer times, following loss of the semiquinone radicals, is dependent upon the dose/pulse, and therefore the initial concentration of semiquinone radicals. At the higher concentration of semiquinone radicals, formation of a semi-permanent product was observed within 7 ms. The intensity of the absorption spectrum/unit dose at $\lambda > 500$ nm at $t \geq 20$ ms is greater for lower concentrations of semiquinone radicals (*e.g.* 2 Gy/pulse). The optical absorption spectra at pH 5.2, where the semiquinone radical of DHI is predominantly protonated,[14] were also determined at various times after the pulse and are shown in Fig. 1(*c*). At this pH-value, the profile of the product spectra at longer times after loss of the semiquinone radicals is essentially independent of the initial concentration of semiquinone radicals (2–20 Gy/pulse) and are similar to those recently reported[10] following one-electron oxidation of DHI at pH 7.4. Similar spectral changes with time were also observed with MeDHI, and therefore methyl substitution of N-1 does not significantly influence the subsequent reactions of the semiquinone radical.

The decay kinetics of the semiquinone radical anion depend upon their initial concentrations as seen from the changes of optical absorbance with time at λ 325 and 500 nm. Fig. 2 shows data following pulse irradiation of a DHI solution containing 10^{-1} mol dm^{-3} azide at pH 8.8 with a dose/pulse of either 18.5 or 2 Gy yielding concentrations of the semiquinone radical anion of DHI of *ca.* 10 and *ca.* 1.2 μmol dm^{-3} respectively. The first half life for disappearance of the semiquinone radical at λ 325 nm increases upon decreasing its initial concentration but eventually becomes independent of the semiquinone concentration (see later). At the higher dose/pulse, the decay of the semiquinone radical at λ 325 nm is predominantly second order as shown in Fig. 3. The second order rate constant was determined to be *ca.* 1.8×10^8 dm^3 mol^{-1} s^{-1} at pH 8.8 assuming an ε_{325} for the semiquinone radical of 945 m^2 mol^{-1}. The corresponding rate constant for loss of the semiquinone radical of MeDHI at pH 8.8 was determined to be *ca.* 1.0×10^8 dm^3 mol^{-1} s^{-1}.

Differences in the rate of decay of the semiquinone radical due to changes in pH were also observed, especially when the pH is greater than the pK_a of the semiquinone radical. The dependence of the second order rate constant for bimolecular reaction of the semiquinone radicals on pH is shown in Fig. 4 under conditions with high initial concentrations of semiquinone radicals. When the pH is less than the pK_a of the semiquinone radical of DHI, it decays predominantly by second order kinetics even at the lowest dose/pulse. The second order rate constant for loss of the semiquinone radical at pH 5.5 was determined to be 3.8×10^9 dm^3 mol^{-1} s^{-1}. This value is similar to that previously determined[10] from the bimolecular disappearance of the semiquinone radical of DHI at a radiation dose of *ca.* 9 Gy but at pH 7.4. From our data it would be expected that at pH 7.4 the bimolecular rate constant should be at least a factor of 2 less than that at pH 5.5, as shown in Fig. 4. Similar dependences on pH have been established for the rate constants for the reaction of several radical species which are in equilibrium with their protonated form, *e.g.* nitro-radical anions[23] and $O_2^{\cdot-}/HO_2^{\cdot}$.[24]

From the following scheme for loss of the semiquinone radicals ($SQ^{\cdot-}/SQH^{\cdot}$) we derive the following equation assuming that equilibrium (4) is established very rapidly compared to the disappearance of the semiquinone radicals. Using the ex-

$$2SQ^{\cdot-} \longrightarrow 2k_1 \qquad (1)$$

$$SQ^{\cdot-} + SQH^{\cdot} \longrightarrow k_2 \qquad (2)$$

$$2SQH^{\cdot} \longrightarrow 2k_3 \qquad (3)$$

$$SQH^{\cdot} \rightleftharpoons SQ^{\cdot-} + H^{+} K \qquad (4)$$

$$k_{obs} = \frac{\left\{2k_3 + \frac{k_2K}{[H^+]} + \frac{2k_1K^2}{[H^+]^2}\right\}}{(1 + K/[H^+])}$$

perimentally-determined rate constant ($2k_3$) for reaction (3) of 3.8×10^9 dm^3 mol^{-1} s^{-1} at pH 5.5, the best fit for the

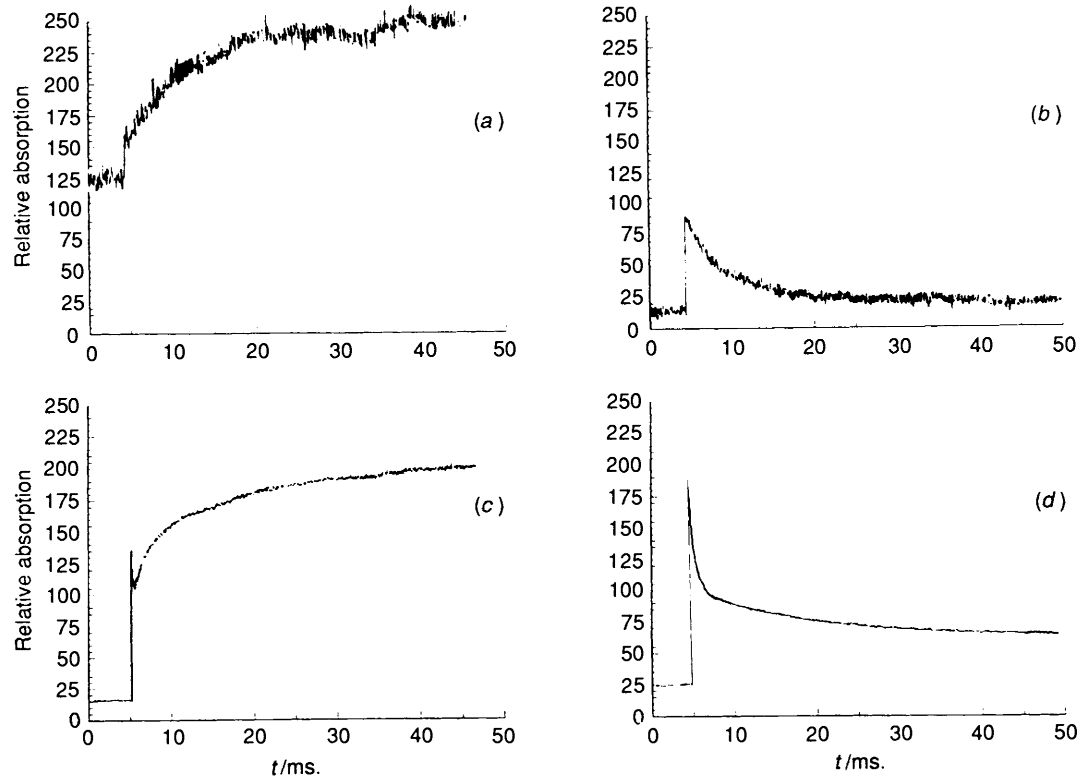


Fig. 2 The optical absorption changes with time at pH 8.8 for the formation of optical absorption at λ 500 nm (*a*, *c*) and the decay of $SQ^{\bullet -}$ from DHI (λ 325 nm) (*b*, *d*) at low (*a*, *b*) and high (*c*, *d*) concentrations of semiquinone radical (see text)

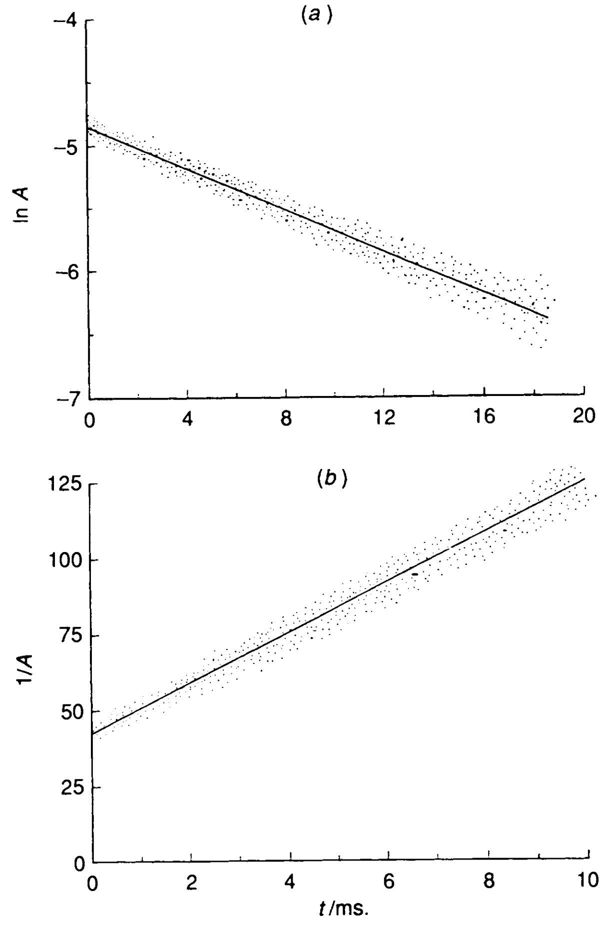


Fig. 3 The kinetics for loss of semiquinone radical of DHI by (*a*) first order (2 Gy/pulse) and (*b*) second order (18.5 Gy/pulse) kinetics at pH 8.8

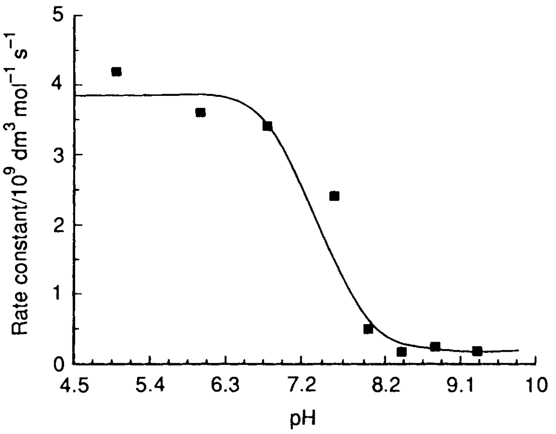


Fig. 4 The dependence of second order rate constant for loss of the semiquinone radical of DHI on pH

experimental data obtained using this equation is shown as the solid curve in Fig. 4. The best-fit values of $2k_1$, k_2 and K were determined to be 1.35×10^8, 8.9×10^9 dm^3 mol^{-1} s^{-1} and 1.26×10^{-7} mol dm^{-3} respectively. The calculated value of $2k_1$ is consistent with the experimentally determined value at pH 9.0. The calculated pK_a value of 6.9 is also in excellent agreement with the measured value[14] of 6.8.

At a low dose/pulse of 1 Gy the semiquinone radical at λ 325 nm was determined to decay predominantly by first order kinetics at pH 8.8 as shown in Fig. 3. At intermediate doses/pulse the decay of the semiquinone radical of DHI is of mixed order. At a dose of 1 Gy/pulse, the first order rate constant for loss of the semiquinone radical of DHI depends upon the concentration of DHI as shown in Fig. 5. From this dependence, the bimolecular rate constant for interaction of the semiquinone radical with DHI was determined to be 1.6×10^6 dm^3 mol^{-1} s^{-1}. Similar observations to these were made with MeDHI yielding a rate constant of 2.3×10^6 dm^3 mol^{-1} s^{-1}. Corresponding reactions, with a rate constant of 6×10^5 dm^3

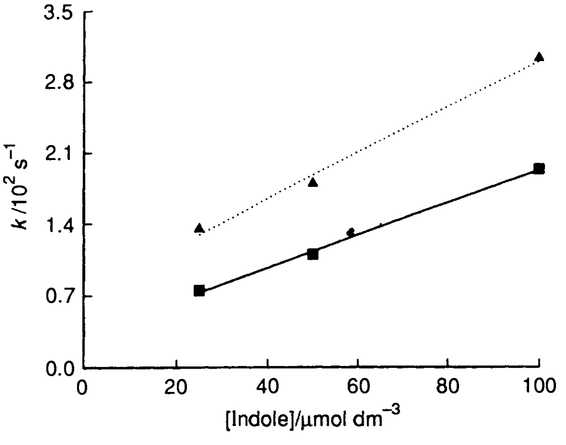


Fig. 5 Dependence of the first order rate constant at pH 8.8 for the loss of semiquinone radical of DHI (and MeDHI) on the concentration of DHI (■) and MeDHI (▲) at low dose/pulse of ≤ 1 Gy

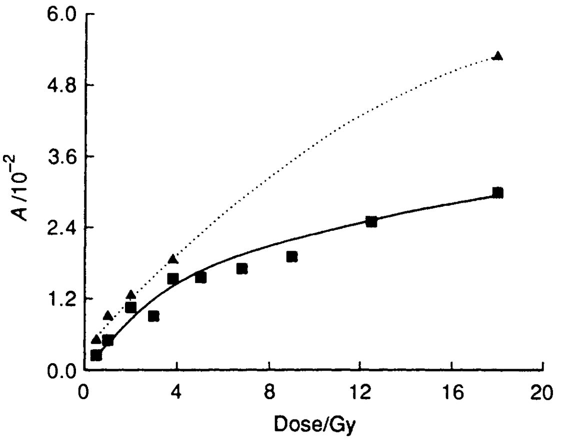
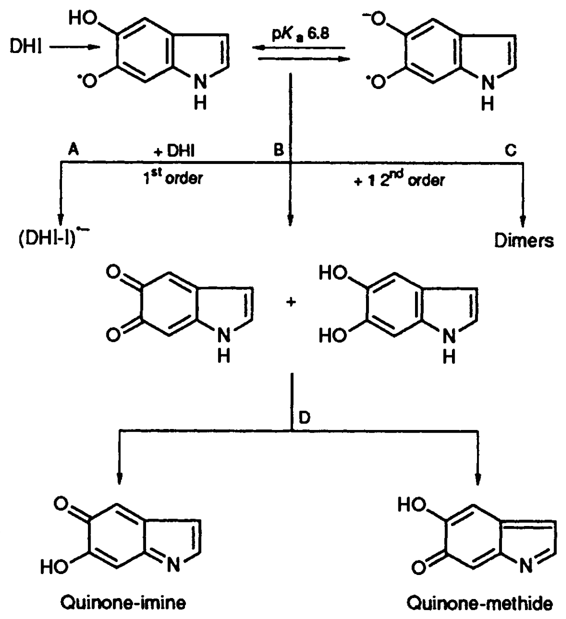


Fig. 6 Dependence of the optical absorption at λ 500 nm on dose/pulse determined 1 s after one electron oxidation of DHI (■) or MeDHI (▲) at pH 9.0

mol^{-1} s^{-1}, have been proposed to occur following one-electron oxidation of 1-methylindoles.[13] If the rate constant for interaction of the semiquinone radical with DHI (or MeDHI) is independent of pH, even at the lowest radiation doses used, radical–radical reactions of the semiquinone radical will predominate when pH ≤ pK_a of the semiquinone radical. This is consistent with the experimental observations. Occurrence of these reactions was not observed by Lambert *et al.*[10] since the radiation dose (*i.e.* high semiquinone radical concentration) and pH of 7.4 used in their work are not appropriate for observation of these empirical first order processes.

Radical–radical reactions of the semiquinone radicals of DHI and MeDHI result in products which are not stable and which decay with first order rate constants of 78 ± 8 and 50 s^{-1} respectively to yield other, permanent products. The absorption spectra of these products are shown in Figs. 1 and 2 determined 20 ms after the pulse. The stability of the semi-permanent product(s) is essentially independent of the indole concentration (25–100 μmol dm^{-3}) and pH (5–10). The first order rate constant for disappearance of the semi-permanent products of DHI is similar in magnitude to that for loss of the semi-permanent product from 6-hydroxy-5-methoxyindole (70 s^{-1}) and corresponds to that determined for formation of the permanent product(s) (80 s^{-1}). It has previously been reported[10] that the semi-permanent products produced upon oxidation of DHI decay by two first-order processes at pH 7.4 with rate constants of 150 and 50 s^{-1}. The dependence of the absorbance at λ 500 nm [which represents permanent product(s) formed from DHI or MeDHI] on dose/pulse at pH 8.8 was determined *ca.* 50 ms after the pulse and is shown in Fig. 6. From these dependences it is proposed that the efficiency of formation of the permanent product(s) decreases as the radical–radical loss of the semiquinone radicals assumes importance at higher dose/pulse.

Based on the spectral and kinetic data, Scheme 1 is proposed to account for the decay of the semiquinone radical of DHI *via* three pathways which depend upon its initial concentration.



Scheme 1 Reactions of the semiquinone radicals of DHI

The reaction of the semiquinone radical with DHI (reaction A) is in competition with its loss through radical–radical interactions involving disproportionation (reaction B) and/or dimerisation (reaction C).

Disproportionation (reaction B) results in the formation of the indolequinone which may undergo a rapid rearrangement (reaction D) into the quinone-methide and/or quinone-imine. The formation of these two species, which are expected to be reactive, is consistent with the experimental observation of a semi-permanent product(s) from DHI with a lifetime of *ca.* 10 ms. Consistent with this reaction sequence, B followed by D, is the fact that disproportionation reactions involving the radicals from 6-hydroxy-5-methoxyindole (5,6-MHI) and 5-hydroxy-6-methoxyindole (5,6-HMI) yield directly the quinone-methide and quinone-imine respectively. The spectral characteristics and stability of the semi-permanent product from 5,6-MHI[14] are similar to those of the semi-permanent product from DHI which we therefore assign to the quinone-methide. A contribution of the quinone-imine to the optical absorption of the semi-permanent product of DHI formed in step D cannot, however, be ruled out. The quinone-methide/imine of DHI subsequently decays by a first order process, resulting in the formation of a permanent product monitored by its optical absorption centred around λ 500 nm.

Dimerisation (reaction C) of the semiquinone radicals is also proposed since stable dimers have been reported[18–21] to be formed following oxidation of DHI. Since the material balance is low, it is inferred that dimerisation is not the major pathway involving radical–radical loss of the semiquinone. Furthermore, the assignment of the semi-permanent product(s) to these dimers is ruled out on the grounds of their reported stability.[18–21] Similar considerations also apply to the reactions of the semiquinone radical of the N(1)-methyl analogue of DHI.

Summary.—The semiquinone radicals produced upon one-electron oxidation of DHI or MeDHI undergo reaction with DHI or MeDHI especially when pH is greater than the pK_a of the semiquinone radical. This reaction competes with the radical–radical reactions of the semiquinones which lead to dimers and a reactive intermediate(s) assigned to a quinone methide/imine.

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