

Characterisation of the Intermediates Produced Upon One-electron Oxidation of 4-, 5-, 6- and 7-Hydroxyindoles by the Azide Radical

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One-electron oxidation of a series of monohydroxylated indoles (HI) by the azide radical in the pH range 5–9 has been studied using the technique of pulse radiolysis with spectrophotometric detection. One-electron oxidation of 4-, 5-, 6- and 7-hydroxyindoles results in the formation of the corresponding indoloxyl radicals, the optical absorption spectra of which are independent of pH (5–9). It is confirmed, using the *N*(1)-methyl substituted analogue of 6-HI, that deprotonation of the resulting radical cation of the hydroxyindoles occurs preferentially from the hydroxy group to yield the corresponding indoloxyl radical. Such deprotonation would be consistent with the resulting indoloxyl radical having a low pK_a .

With the exception of 4-HI, the indoloxyl radicals decay bimolecularly in the dose/pulse range of 1–30 Gy to yield semi-permanent products ($2k = 2-4 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). With 4-HI, the decay of the indoloxyl radical changes from second-order to first-order kinetics on lowering the dose/pulse. At 1 Gy/pulse, the first-order kinetics are dependent upon the concentration of 4-HI. The second-order rate constant for reaction of the indoloxyl radical with 4-HI was determined to be $4.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. The decay of these semi-permanent products from the indoloxyl radicals is first order and depends upon the concentration of azide. The second-order rate constants determined for this reaction depend markedly on the hydroxyindole used ($k = 159-821 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The semi-permanent product arising from the bimolecular decay of the indoloxyl radicals is discussed in terms of the formation of reactive quinone-methides and/or -imines.

The oxidation of hydroxylated indoles has received considerable attention in the last few years since these aromatic compounds are the basis of action of several biologically active substances (e.g. serotonin, tryptophan) which have been suggested to act as endogenous antioxidants.¹⁻¹³ This antioxidant property is based largely upon their ability to act as one-electron reductants. Recently the oxidation of dihydroxyindoles and hydroxylated methoxyindoles using a one-electron oxidant has received attention due to the involvement of these compounds in biological processes which lead to the formation of melanin.⁸⁻¹¹ For example, 5,6-dihydroxyindole (DHI) and 6-hydroxy-5-methoxyindole are readily oxidised to the corresponding semiquinone and 5-methoxyindol-6-oxyl radicals, respectively⁸ which subsequently decay by radical-radical interactions to yield a semi-permanent product(s).^{8,10,11} From the similarity of their optical absorption spectra it was inferred that a reactive quinone-methide is formed.¹¹ A quinone-imine is thought to be produced as a result of one-electron oxidation of the isomeric 5-hydroxy-6-methoxyindole.

From product analysis studies, oxidation of monohydroxyindoles results in the formation of stable oligomeric products^{2,3} but with low material balance. The involvement of free radical intermediates, together with the involvement of quinone-methides/imines, has been proposed to explain the spectrum of products formed.^{2,3} Previous EPR studies^{12,13} have shown that 5-hydroxyindole is readily oxidised in alkaline solution to yield free radicals which were characterised as semiquinone-imine radicals.

The present study was undertaken to characterise the radicals produced upon one-electron oxidation of a series of monohydroxyindoles and to investigate their subsequent reactions. It has previously been shown⁷ that one-electron oxidation of 5-hydroxyindole yields the indol-5-oxyl radical with a redox potential of 0.55 V at pH 9.1. A knowledge of the chemistry of

the one-electron oxidised species of monohydroxylated indoles should help elucidate the role of quinone-methides/imines in the formation of melanin.

Experimental

The 4.3 MeV linear accelerator and the optical detection system with data handling have previously been described.¹⁴ All solutions were prepared using water which had been purified using a Multi-Q system. The pH values of the solution were adjusted using either HClO_4 or NaOH. Solutions were saturated with N_2O (BOC, zero grade) prior to irradiation in order to convert e^-_{aq} into OH^\bullet radicals. The solution generally contained 0.1 mol dm^{-3} azide (NaN_3) which reacts effectively with all of the OH^\bullet radicals to produce N_3^\bullet within time $< 1 \mu\text{s}$.⁸ This concentration of azide ensures that the interaction of OH^\bullet with azide competes with the reaction of OH^\bullet with the hydroxyindoles ($10^{-4} \text{ mol dm}^{-3}$). The low optical absorption of N_3^\bullet at $\lambda > 300 \text{ nm}$ is an important advantage since it allows straightforward measurement in spectral regions where the one-electron oxidised transient species absorb. Radiation doses (1–30 Gy/pulse) were determined using KSCN dosimetry at 480 nm assuming $G = 0.3 \text{ mmol J}^{-1}$ and $\epsilon = 710 \text{ m}^2 \text{ mol}^{-1}$. The optical absorption spectra measured have not been corrected for depletion of the substrates following pulse irradiation.

The indoles, 4-hydroxyindole (4-HI) and 5-hydroxyindole (5-HI) were supplied by Aldrich Chem. Co. and Janssen Chimica, respectively. 6-Hydroxyindole (6-HI), 7-hydroxyindole (7-HI) and 6-hydroxy-1-methylindole (6-HMeI) were prepared as described in refs. 15 and 16. All the indoles were used without further purification. Sodium azide and all other reagents were of 'AnalaR' grade and used as supplied. The structures of the indole derivatives used in the present study are given in Fig. 1 and their pK_a values are presented in Table 1.

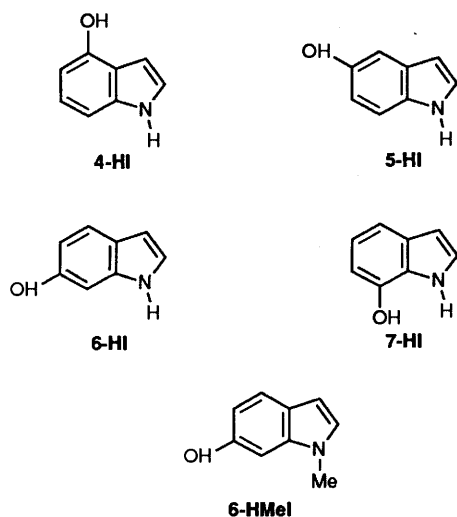


Fig. 1 Structures of the monohydroxyindoles

Table 1 Rate constants (k_1) determined for interaction of $N_3^{\cdot-}$ with the monohydroxyindoles at pH 9.0 and the rate constants ($2k_2$) for decay^a of the resulting indolyl radicals

Indole	pK_a	$k_1/10^{10}$ $\text{dm}^3 \text{mol}^{-1} \text{s}^{-1} \epsilon^b$	$2k_2/10^9$ $\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$
4-HI	9.9	1.2	2 548
5-HI	10.6	1.1	n.d.
6-HI	10.3	1.3	10 641
7-HI	9.5	1.7	2 114

^a Determined at initial radical concentration of *ca.* $18 \mu\text{mol dm}^{-3}$.

^b Extinction coefficient at wavelength used for determination of second-order kinetics (500 nm).

Results and Discussion

The one-electron oxidation of the monohydroxyindoles, 4-HI, 5-HI, 6-HI and 7-HI, was achieved using the azide radical $N_3^{\cdot-}$ at pH 5.1 and 9.1. It is assumed that $N_3^{\cdot-}$ oxidises the indole analogues primarily *via* electron transfer as previously suggested for phenols and 5-hydroxyindole.^{7,17} The rate constants for interaction of $N_3^{\cdot-}$ with the hydroxyindoles which were determined at pH 9.0, are essentially diffusion controlled and are presented in Table 1.

The optical absorption spectra obtained *ca.* 10 μs after pulse irradiation of a N_2O -saturated solution containing $10^{-1} \text{mol dm}^{-3}$ azide and $10^{-4} \text{mol dm}^{-3}$ monohydroxyindole at pH 9 are shown in Fig. 2. The optical absorption spectra of the one-electron oxidised species of 4-, 5-, 6- and 7-hydroxyindoles differ significantly. In the pH range studied of 5–10, the optical absorption spectra of these one-electron oxidised species are essentially independent of pH. The occurrence of a deprotonation step at pH 9.0, as reported previously for the radicals formed upon one-electron oxidation of 5,6-dimethoxyindole (DMOI) and DHI, was not observed.⁸ Upon one-electron oxidation of the hydroxyindoles it is therefore suggested that deprotonation of the resulting radical cation occurs preferentially from the hydroxy group to yield the corresponding indoloxyl radical as shown in Scheme 1, using 6-HI as an example. Such a deprotonation process for the radical cation is consistent with the resulting indoloxyl radical having a low pK_a value, as previously shown for substituted phenoxyl radicals.¹⁸ For 5-HI, this assignment is also consistent with that from EPR studies.¹³

Verification that the deprotonation site of the radical cation occurs at the oxygen of the hydroxyl group and not at N(1) was obtained through one-electron oxidation of (1)-*N*-methyl-6-

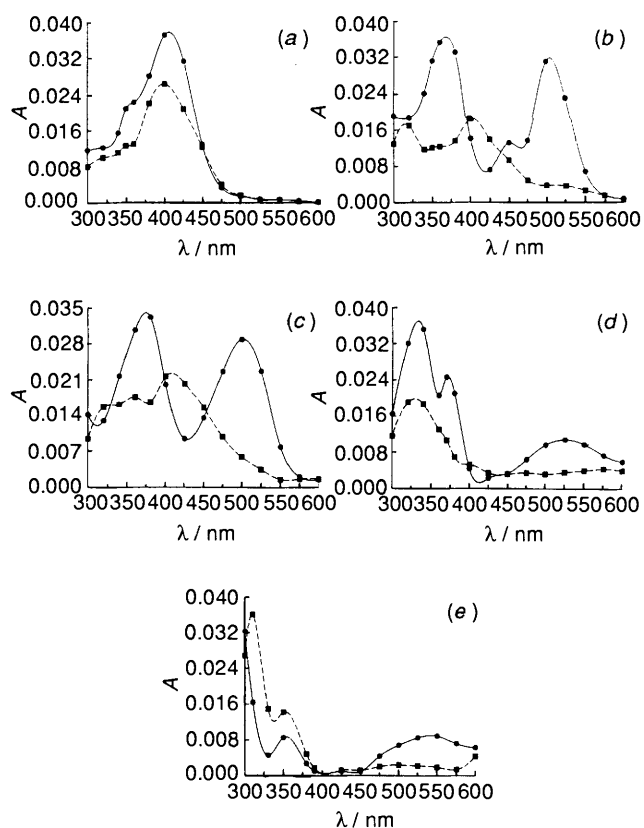
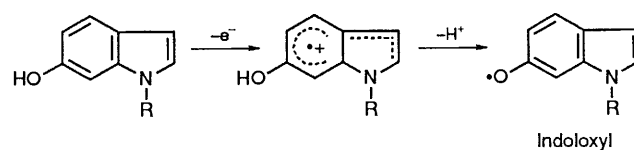


Fig. 2 The optical spectra determined 10 μs (●) and 5 ms (■) after pulse irradiation of an N_2O -saturated, aqueous solution containing $10^{-1} \text{mol dm}^{-3}$ azide and $10^{-4} \text{mol dm}^{-3}$ (a) 5-HI, (b) 6-HI, (c) 6-NMeI, (d) 4-HI and (e) 7-HI at pH 9.0

hydroxyindole (6-HMeI), as shown in Fig. 2. From the similarity of the optical absorption spectra of one-electron oxidised 6-HI and 6-HMeI and their independence of pH in the range 5–9, it is confirmed that deprotonation of the radical cation occurs at the hydroxy group (see Scheme 1).



Scheme 1

The fate of the indoloxyl radicals of these monohydroxyindoles was followed from the time-dependent changes in the optical absorption spectra at different dose/pulse (1–30 Gy). At a dose of 30 Gy/pulse (equivalent to a radical concentration of *ca.* $18 \mu\text{mol dm}^{-3}$) the rates of decay of the indoloxyl radicals for 4-, 6- and 7-HI follow second-order kinetics and are independent of pH (5–10). The second-order rate constants for loss of indoloxyl radicals through radical–radical interactions are presented in Table 1. With 5-HI, it was not possible to determine accurately a rate constant for loss of its indoloxyl radical since the corresponding product has a similar optical absorption spectrum (see Fig. 2). However, the first half-life for loss of the indoloxyl radical of 5-HI was dependent upon its initial concentration, consistent with second-order kinetics. The radical–radical interaction of the indoloxyl radicals of 5-HI, 6-HI and 6-NMeI results in the formation of semi-permanent products whose optical absorption spectra (Fig. 2) are essentially independent of pH in the range 5–9. Furthermore the spectra of the products resulting from oxidation of 6-HI and 6-HMeI are similar, consistent with the reaction involving a

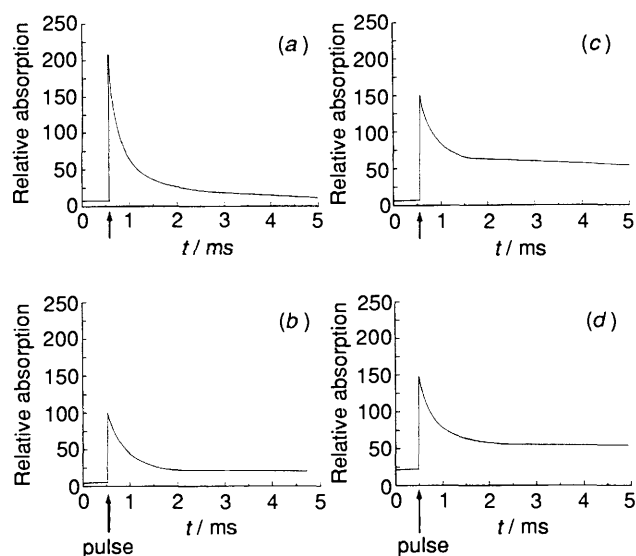


Fig. 3 The optical absorption changes with time at pH 5 (a,b) and pH 9 (c,d) for the decay of the indoloxyl radical from 4-HI (a,c) and 7-HI (b,d)

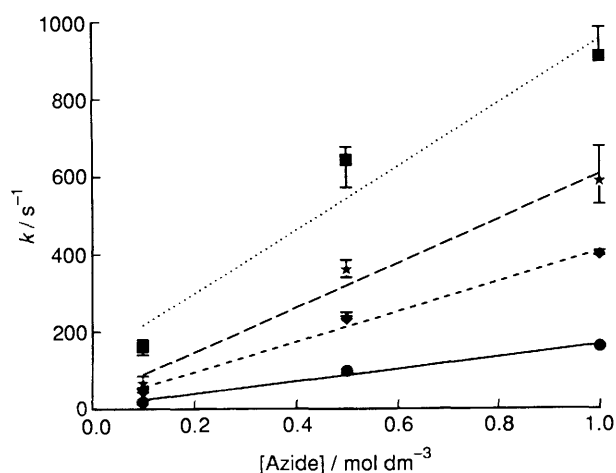


Fig. 4 Dependence of the first-order rate constant pH 9.0 for the loss of the semi-permanent product derived from 4-HI (◆), 5-HI (●), 6-HI (■) and 7-HI (✱) on the concentration of azide at a dose/pulse of 30 Gy

Table 2 Rate constants (k_3) for interaction of the product, formed from radical-radical decay of the indoloxyl radicals, with azide ion at pH 9.0. k_0 represents the azide-independent rate constant for loss of the products. Value in parentheses is the value determined in the absence of azide (see text).

Indole	$k_3/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	k_0/s^{-1}
4-HI	392	17 (10–15)
5-HI	159	8 (7.0)
6-HI	821	134 (127)
7-HI	576	31.5 (24)

similar indoloxyl radical, and not critically dependent upon the presence of a proton at N(1). With 4-HI and 7-HI (Fig. 2), the products present after 5 ms absorb predominantly at wavelengths below 400 nm, in contrast to the absorption centred around 400–425 nm for the products from 5-HI and 6-HI. The product spectra with 4-HI and 7-HI are dependent on pH as observed from the changes of optical absorbance with time at 525 nm as shown in Fig. 3. These pH effects probably reflect the presence of ionisable hydroxy groups within the products.

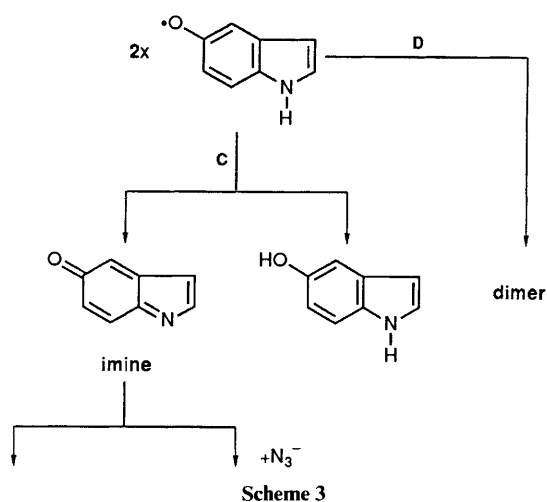
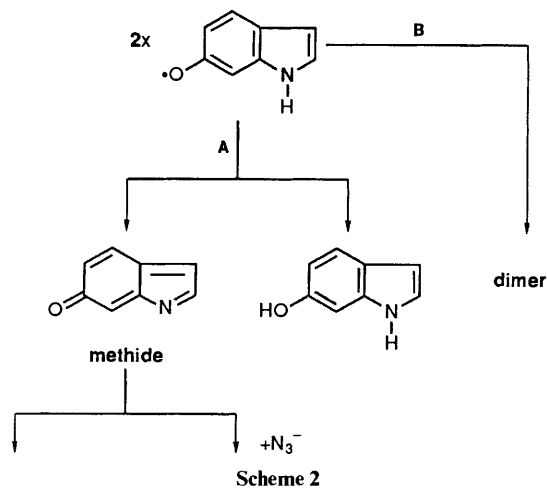
With the exception of 4-HI, the first half-lives for disappearance of the indoloxyl radicals of the monohydroxyindoles are dependent upon dose/pulse (1–30 Gy). The decay of the indoloxyl radical of 4-HI, however, becomes first order at a low dose/pulse of 1 Gy and dependent upon the concentration of 4-HI. The bimolecular rate constant determined from this dependence is $4.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. An interaction of the indoloxyl radical with the substrate was previously reported with DHI;¹¹ however, the rate constant determined with DHI is about one order of magnitude less than that determined with 4-HI. If the corresponding reaction occurs with the other monohydroxyindoles, then, based upon the first half-life for disappearance of these indoloxyl radicals at the lowest dose/pulse used, the rate constant is estimated to be $\ll 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. It is inferred that these differences in reactivity of the indoloxyl radicals with their parent compound reflect both the influence of the hydroxy groups upon the site of nucleophilic attack and steric effects. Similar reactions between the indole radical and its indole have been proposed¹⁹ to occur following one-electron oxidation of 1-methylindole.

The products formed within ca. 5 ms following radical-radical interaction of the indoloxyl radical of monohydroxyindoles (Fig. 2) are unstable. The decay of these semi-permanent products is first order and dependent upon the concentration of azide (0.1–1 mol dm⁻³ at pH 9.0). From the dependence of the first-order rate constants on azide concentration, as shown in Fig. 4, second-order rate constants for reaction of the nucleophilic azide ion with these products were determined and are presented in Table 2. As can be seen in Fig. 4, an intercept was observed which is suggested to represent interaction of these semi-permanent products with water (k_0) which is acting as a nucleophile. In the absence of high concentrations of added nucleophiles and using the hydroxyl radical to initiate formation of the indoloxyl radicals (unpublished data), the decay of the ensuing products at pH ca. 9 is first order (Table 2). The value of the rate constant is consistent with the intercept values determined from the dependences shown in Fig. 4. At the concentrations of azide used, even at pH 9, a significant percentage is present as N_3H , so that reaction of the semi-permanent product may involve N_3H . However, on lowering the pH from 9 to 7, the rate constants for loss of the semi-permanent products on reaction with azide are not modified. This independence of pH is consistent with the azide ion, and not N_3H , acting as the nucleophile in these studies. From investigations at pH ca. 10–11 in the absence of azide, it is evident that the lifetime of these products becomes dependent upon pH, indicative of an interaction with OH^- . In this pH range the situation is complicated by the hydroxyindoles undergoing a prototropic equilibrium (see Table 1 for pK_a values). The decay of these products is, however, independent of pH in the range 5–9, dose (1–30 Gy/pulse) and of the concentration of the indoles (25–100 $\mu\text{mol dm}^{-3}$).

Based upon this spectral and kinetic data, Schemes 2 and 3 are proposed to account for the loss of the indoloxyl radicals of the monohydroxyindoles using the 6- and 5-HI as examples.

With the exception of the indoloxyl radical of 4-HI, the indoloxyl radicals decay through radical-radical interactions involving disproportionation (step A,C) and/or dimerisation (step B,D). With 4-HI at low dose/pulse, the reaction of its indoloxyl radicals with 4-HI is apparent. This reaction is in competition with the competing radical-radical interactions.

With 4- and 6-HI, disproportionation (step A) results in the formation of a quinone-methide, which is expected to be susceptible to nucleophilic attack. With 5- and 7-HI, disproportionation (step C) results in the formation of the corresponding quinone-imines. From product studies,^{2,3} the involvement of quinone-imines has been inferred on oxidation of 5- and 7-HI.

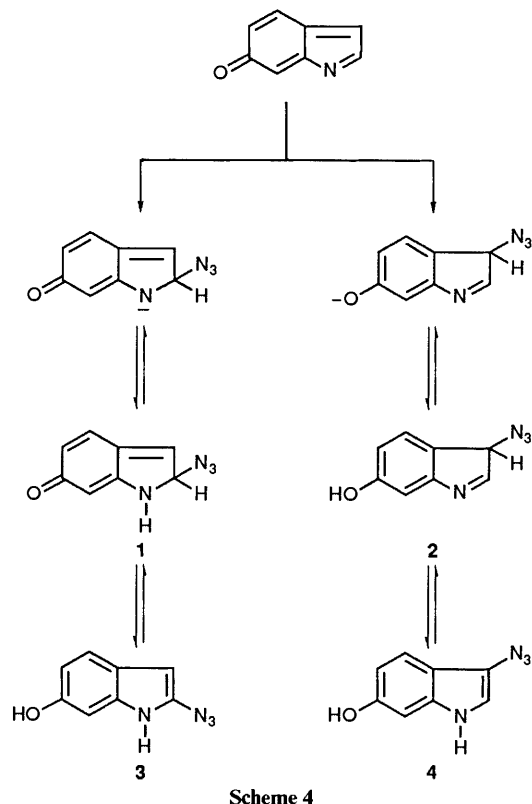


The formation of these reactive species is consistent with the experimental observation that the product(s) from the hydroxyindoles undergo nucleophilic reactions with azide and water (Table 2). The stability of the proposed quinone-methides from 4- and 6-HI in aqueous solution is similar to that reported for the corresponding quinone-methide from DHI.¹¹ It is apparent that the susceptibility of the quinone-methide and -imine to nucleophilic reactions is similar, with that of 6-HI being the most reactive.

Dimerisation of the indoloxyl radicals is also proposed, since stable dimers have been isolated,^{2,3} but since the material balance based upon the yield of stable products is low, it is concluded that dimerisation initiated through one-electron oxidation of these monohydroxyindoles is not a major pathway. Since delocalisation of the unpaired spin only occurs over the six-membered ring in 5- and 7-HI, the dimers identified^{2,3} involve coupling only through the benzenoid ring of the indoles. In contrast, with 4- and 6-HI, where the unpaired spin is distributed over the entire π system, additional products have been identified through coupling involving the pyrrole ring. From comparison of the optical spectra of the semi-permanent products, for example, from 5- and 6-HI, the similarities observed do not reflect the differences in the expected distribution of dimer products solely when considering the preferred sites for radical-radical coupling. The observed similarities in the optical spectra of the products are consistent with their assignment to quinone-imines/methides. This spectral similarity of the semi-permanent products of 5- and 6-HI is due to the conjugation through the *para*-position as compared to the *ortho* conjugation with the product from 4- and 7-HI. Since the dimer products identified^{2,3} are not expected to react with

azide ions, the species removed in the reaction with azide ions must therefore be of the quinone-methide/imine type.

The reactive quinone-methides/imines have been demonstrated to interact with the nucleophilic azide ion. Ring positions C(2) and C(3) of the quinone-methide are considered as sites susceptible to nucleophilic addition. If azide adds to C(3) or C(2) using the quinone methide as an example (as shown in Scheme 4), it is envisaged that the azide conjugates **1** and **2** are formed at pH *ca.* 9.



It is assumed that the conjugates are protonated at this pH value. As the methylene protons are expected to be labile, the rearrangement results in restoration of the aromatic ring system, yielding products **3** and **4**. Based upon Scheme 4, the reaction of the quinone-methide with a nucleophile may be viewed as a ring substitution to yield the azide product. This rearomatization would be consistent with the loss of optical absorption of the quinone-methide. Similar considerations are proposed to account for the loss of the quinone-imine upon its interactions with nucleophiles. In the absence of azide, a similar scheme is proposed for the interaction of the quinone-methide with water resulting in the formation of the hydroxylated products at C(2) and C(3), equivalent to products **3** and **4**.

In summary, one-electron oxidation of monohydroxyindoles by N_3^- results in formation of their corresponding indoloxyl radicals. These radicals subsequently undergo radical-radical interactions to yield dimers and reactive intermediates assigned to quinone-imines/methides which are susceptible to nucleophilic reactions.

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References

- 1 M. Z. Wrona and G. Dryhurst, *J. Org. Chem.*, 1987, **52**, 2817.
- 2 A. Napolitano, M. d'Ischia and G. Protta, *Tetrahedron*, 1988, **44**, 7265.

- 3 A. Napolitano, M. d'Ischia, G. Prota, T. M. Schultz and L. J. Wolfram, *Tetrahedron*, 1989, **45**, 6749.
- 4 A. C. Chan and P. R. Hillard, Jr., *Tetrahedron Lett.*, 1989, **47**, 6483.
- 5 S. V. Jovanovic and M. G. Simic, *Life Chem. Rep.*, 1985, **3**, 124.
- 6 S. V. Jovanovic and M. G. Simic, *Biochim. Biophys. Acta*, 1989, **1008**, 39.
- 7 S. V. Jovanovic, S. Steenken and M. G. Simic, *J. Phys. Chem.*, 1990, **94**, 3583.
- 8 A. T. Al-Kazwini, P. O'Neill, G. E. Adams, R. B. Cundall, B. Jacquet, G. Lang and A. Junino, *J. Phys. Chem.*, 1990, **94**, 6666.
- 9 C. Lambert, J. N. Chacon, M. R. Chedekel, E. J. Land, P. A. Riley, A. Thompson and T. G. Truscott, *Biochim. Biophys. Acta*, 1989, **993**, 12.
- 10 C. Lambert, E. J. Land, P. A. Riley and T. G. Truscott, *Biochim. Biophys. Acta*, 1990, **1035**, 319.
- 11 A. T. Al-Kazwini, P. O'Neill, G. E. Adams, R. B. Cundall, G. Lang and A. Junino, *J. Chem. Soc., Perkin Trans. 2*, 1991, 1941.
- 12 D. C. Borg, *Proc. Natl. Acad. Sci.*, 1965, **53**, 829.
- 13 E. Perez-Reyes and R. P. Mason, *J. Biol. Chem.*, 1981, **256**, 2427.
- 14 P. O'Neill and P. W. Chapman, *Int. J. Radiat. Biol.*, 1985, **47**, 71.
- 15 Eur. Pat. Appl. EP 237781 A223, September 1987 (*Chem. Abstr.*, 108, **15**, 131859h).
- 16 M. Makosza, W. Danikiewicz and W. Wojciechowski, *Liebigs Ann. Chem.*, 1988, **3**, 203.
- 17 Z. B. Alfassi and R. H. Schuler, *J. Phys. Chem.*, 1985, **89**, 3359.
- 18 W. T. Dixon and D. Murphy, *J. Chem. Soc., Faraday Trans. 2*, 1976, **72**, 1221.
- 19 X. Shen, J. Lind, T. E. Eriksen and G. Merinyi, *J. Chem. Soc., Perkin Trans. 2*, 1990, 597.

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