The Reactions of Indoles in Aqueous Solution Initiated by One-electron Oxidation

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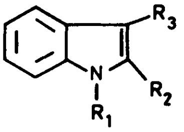
By pulse or gamma radiolysis in the presence of Br^-, 1-methylindole (**1b**) is oxidized to its radical cation (**2b**), which adds to (**1b**) to form a radical dimer (**8b**). Species (**2b**) also reacts with OH^- to yield the pseudo-base, proposed to be the C_2-OH adduct (**4b**). Identical end-product yields and spectral comparison reveal that the addition of $OH^{\bullet}$ radicals to (**1b**) also produces (**2b**) and (**4b**) but virtually no other $OH^{\bullet}$ adducts to (**1b**). The selectivity of OH towards the C_2 position appears to apply to the other indoles as well. The neutral indolyl radicals (**3**) disappear both by coupling and dismutation. Discrimination between these modes is governed by the substituent. A methyl group at the C_3 position favours C_3–C_3' coupling. Molecular oxygen adds to neutral indolyl radicals at the C_3 position. The measured and estimated rates of O_2 addition relate to the redox potentials of the indolyl radicals. The reaction between $O_2^{-\bullet}$ and the indolyl radical cation (**2**) produces (**1**) and O_2. On the other hand, $O_2^{-\bullet}$ couples to (**3**) to yield C_3-OOH hydroperoxides (**14**). The latter decompose into C_2–C_3 opened amides (**13**) with concomitant chemiluminescence.

Early photophysical[1,2] and pulse radiolysis studies[3–5] aimed at the spectral characterization of one-electron oxidized indole and tryptophan. It was shown that inorganic oxidants such as $Br_2^{-\bullet}$, $(SCN)_2^{-\bullet}$ *etc.* yield indolyl radical cations which are in acid–base equilibrium with the neutral form.[4,6] The $OH^{\bullet}$ radical was shown to generate a mixture of species including the indolyl radical. Armstrong *et al.*[3] characterized the transient spectra obtained upon $OH^{\bullet}$ addition to variously methylated indoles. In later studies the indolyl radicals of a number of methylated indoles were systematically investigated. One-electron reduction potentials[7] and acid–base equilibria[8] of the indolyl radical cations were determined. Furthermore, the yield of indolyl radicals per $OH^{\bullet}$ initially present was established.[8] In a few cases the product yield subsequent to the reaction of $OH^{\bullet}$ with tryptophan or indole was investigated both in the presence[9,10] and absence[3,11] of O_2. In studies conducted in Fenton's reagent[12–14] based on Fe^{2+}/H_2O_2 or Ti^{3+}/H_2O_2, indoles OH-substituted in the 4-, 5-, 6- and 7-positions were detected among the end products. The yield of benzene-hydroxylated products seemed to vary with experimental conditions, ranging from 10–35%. Several other products were also identified. However, nowhere was a reasonably complete product balance obtained and a comprehensive understanding of the reaction mechanisms is still lacking. The reaction of the $CCl_3OO^{\bullet}$ radical with tryptophan has been studied by pulse radiolysis.[15] Recently, a more detailed investigation of the reactions of $CCl_3OO^{\bullet}$ with several indoles has been carried out.[16] The formation of a 3-adduct was demonstrated and another adduct observed was proposed to be the 2-adduct. A reaction mechanism was suggested to rationalize the end-product pattern in terms of the properties of these adducts. The present work investigates in detail the reaction mechanism underlying one-electron oxidation of (**1b**) and, to a lesser extent, of other indoles. The main oxidant used is $Br_2^{-\bullet}$ but oxidation by $OH^{\bullet}$ will also be considered for comparison.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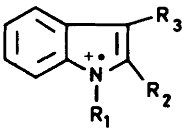
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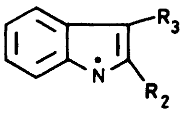
(3)

	R_1	R_2	R_3
a	H	H	H
b	CH_3	H	H
c	H	H	CH_3
d	H	CH_3	CH_3
e	H	H	Ala

Experimental

The pulse radiolysis equipment consists of a microtron accelerator[17] (7 MeV) and a computerised optical detection system.[18] For dosimetry, air-saturated 10^{-2} mol dm^{-3} KSCN solutions were employed. The $G\varepsilon$ value of the $(SCN)_2^{-\bullet}$ radical was taken to be 2.2×10^{-4} m^2 J^{-1} at 500 nm.[19] The AECL ^{60}Co gamma source had a dose rate of 0.50 Gy s^{-1}. HPLC analyses were performed on reversed-phase (RP-18) columns with water–methanol mixtures as eluant, using optical detection between 200 and 500 nm. TLC was run on silica with CH_2Cl_2–MeOH mixtures as eluants. Product identification was achieved after TLC separation by means of NMR and MS spectroscopy. Compound (**9b**) was identified by means of its NMR spectrum and by comparing its UV–VIS spectrum with that of authentic (**9a**). With authentic samples available, (**5c**), (**11c**), (**13c**), (**13d**), (**14d**), and (**16d**) were quantified by HPLC. As the HPLC peak of (**11c**) appears closely after that of (**1c**), quantification of the former entails a large error margin (see Table 5). The yields of (**5b**) and (**9b**) were estimated by absorption spectroscopy subsequent to TLC separation. The errors inherent in this process are considerable.

H_2O_2 was determined by means of Cu^{2+}-catalysed luminol chemiluminescence after HPLC separation. O_2 consumption was measured with an oxygen electrode immersed in the reaction vessel during irradiation. All indoles (Aldrich, purest grade available), NaN_3, KSCN, KBr and the various buffers (Merck, pa quality) were employed without purification. Reference material for product identification was obtained from Dr J. Bergman, or was synthesized according to published procedures. Water was triply distilled in quartz.

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Results

When water is γ or electron-beam irradiated the primary species e^-_{aq}, $H^\bullet$ and $OH^\bullet$ are formed the yields of which are taken to be (in 10^7 mol J^{-1}) 2.8, 0.6, and 2.8, respectively. By the following set of reactions the primary species can be transformed.

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

$$e^-_{aq} + O_2 \longrightarrow O_2^{-\bullet}$$

$$H^\bullet + O_2 \longrightarrow HO_2^\bullet$$

$$OH^\bullet + 2Br^- \longrightarrow Br_2^{-\bullet} + OH^-$$

Thus in N_2O or N_2O/10% O_2-saturated solutions 90% of the radicals will be present as $OH^\bullet$, the remainder being $H^\bullet$ or $HO_2^\bullet/O_2^{-\bullet}$. In air- or O_2-purged solutions $H^\bullet$ and e^-_{aq} are transformed into superoxide (*ca.* 55% of all radicals). Addition of Br^- converts $OH^\bullet$ into the 'pure' one-electron oxidant $Br_2^{-\bullet}$.[20]

All species are labelled by current numerals with a letter attached to them to indicate substituents at the 1, 2 or 3 position. When a general reaction type is discussed the letter is omitted.

Pulse Radiolysis.—(i) *Oxidation of* (**1b**) *by* $Br_2^{-\bullet}$. The (**2b**) radical cation produced upon one-electron oxidation of the parent by $Br_2^{-\bullet}$ decayed in a second order reaction to yield a product absorbing slightly between 500 and 700 nm. Varying the pH between 3 and 11 or the dose between 20 and 200 Gy affected neither the rate constant ($2k$ 9 × 10^8 dm^3 mol^{-1} s^{-1})[8] nor the nature of the transient product. However, at doses of *ca.* 8 Gy the decay rate of (**2b**) increased when [(**1b**)] was varied from 5 × 10^{-4} mol dm^{-3} to 2 × 10^{-3} mol dm^{-1}. From the variation of $t_{\frac{1}{2}}$ *versus* [(**1b**)] we estimated the rate constant for reaction (1) presented in Table 1.

$$(\mathbf{2b}) + (\mathbf{1b}) \longrightarrow (\mathbf{8b}) \qquad (1)$$

Addition of $Fe(CN)_6^{3-}$ altered the course of the reaction. The disappearance of (**2b**) followed first order kinetics with rates proportional to the concentration of added $Fe(CN)_6^{3-}$. Also, the product of this reaction displayed no absorbance whatsoever above 500 nm. The (**2b**) radical cation was shown to react with nucleophiles, such as CN^- or OH^-, with rates as presented in Table 1. The spectrum of the pseudo-base radical, (**4b**), as obtained in the presence of 0.1 mol dm^{-3} OH^- is presented in Figure 1.

(ii) *Oxidation of other indoles by* $Br_2^{-\bullet}$. With (**1a**), (**1c**) and (**1d**) the $Br_2^{-\bullet}$ radical produced the corresponding indolyl radical cations (**2a**), (**2c**), and (**2d**) which deprotonated in agreement with previously determined pK_a values.[8] Irrespective of the pH, the radicals disappeared in second order reactions; the rate constants for the neutral radicals are given in ref. 8. The rates of recombination of the indolyl radical cations were slightly lower having values of 5–10 × 10^8 dm^3 mol^{-1} s^{-1}. The neutral indolyl radicals did not react with OH^- or other nucleophiles on the pulse radiolytic time scale.

The Reaction of the $OH^\bullet$ *Radical with* (**1b**).—Hydroxy radicals produce only 44% indolyl radical cations[21] on reaction with (**1b**) between pH 6 and 11. This yield increases with increasing acidity to attain a final value corresponding to *ca.* 93% of the total radical yield.

The spectrum observed in 0.1 mol dm^{-3} NaOH on the addition of $O^{-\bullet}(OH^\bullet)$ to (**1b**) is presented in Figure 1 along with the spectrum of the pseudo-base radical (**4b**). It is seen that >300 nm, the two spectra are virtually identical. The pK_a of $OH^\bullet$ is 11.9 thus in 0.1 mol dm^{-3} NaOH solutions, 93% is present as $O^{-\bullet}$. Combining the pK_a with the rate constants of $OH^\bullet$ (ref. 8) and $O^{-\bullet}$ (Table 1) with (**1b**) we find that 80% of the attack is by $O^{-\bullet}$. The proton-catalysed decomposition of the OH-adduct(s) into (**2b**) was investigated by varying the acidity of the solutions and by observing, respectively, the absorbance decay and build-up at 420 and 580 nm. The derived rate constant for reaction (2) is presented in Table 1.

Table 1. Pulse radiolytically measured rate constants.

Reaction	k/dm^3 mol^{-1} s^{-1} unit
(**1b**) + $O^{-\bullet}$	3.8 × 10^9
(**2b**) + OH^-	6 × 10^6
(**2b**) + CN^-	2 × 10^6
(**2b**) + (**1b**)	6 × 10^5
(**2b**) + O_2^-	4 × 10^9
(**2b**) + H_2O	<10^{2}[a]
(**3d**) + O_2	2 × 10^8
(**4b**) + O_2	8 × 10^8
(**4b**) + H^+	2.5 × 10^9

[a] s^{-1}.

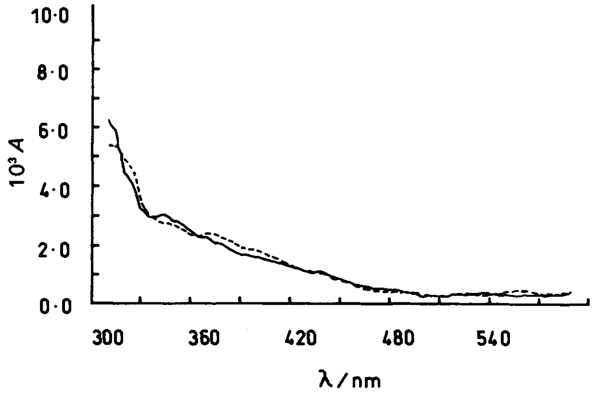


Figure 1. Full line (——): spectrum obtained upon pulse radiolysis of N_2O-saturated solutions containing 2 × 10^{-3} mol dm^{-3} (**1b**) and 0.1 mol dm^{-3} NaOH. Broken line (- - -): same as above but with 5 × 10^{-2} mol dm^{-3} Br^- added. The spectra were run at doses 6–9 Gy and normalized to 7 Gy.

$$(\mathbf{4b}) + H^+ \longrightarrow (\mathbf{2b}) + H_2O \qquad (2)$$

Solutions Purged with O_2/N_2O *Mixtures or Pure* O_2.—The decay of the indolyl radicals (**3a**), (**2b**) and (**3e**) formed by $Br_2^{-\bullet}$ oxidation was not affected by the amount of dissolved O_2 in the solutions. In contrast, the decay of the (**3d**) radical was clearly first order in [O_2]. The rate constant is given in Table 1. The (**3c**) radical showed a slight rate increase in 50% O_2/N_2O saturated solutions as compared with N_2O saturated ones. The reaction of O_2 with (**4b**), the pseudo-base of (**2b**), was measured between pH 11.8 and 13. Similarly, the oxygen reaction of the $OH^\bullet$ adduct of (**1b**) was studied between pH 7 and 13. Irrespective of the primary oxidant ($Br_2^{-\bullet}$ or $OH^\bullet/O^{-\bullet}$) the rate constants measured were identical and independent of the pH.

In O_2^- saturated solutions of (**1b**) the (**2b**) radical and O_2^- form in roughly equal amounts (45 and 55%). We observed a rapid second order decay of the 580 nm absorbance resulting in almost total restoration of the absorbance observed prior to the pulse. This shows that, in the main, reaction (3) is a one-electron transfer.

$$(\mathbf{2b}) + O_2^{-\bullet} \longrightarrow (\mathbf{1b}) + O_2 \qquad (3)$$

Table 2. Consumption, as a percentage of the initial radical yield, of various indoles when subjected to γ-radiolysis in the presence of 5×10^{-2} Br^- or N_3^-.

		% (– ind) Saturating gas			
Substance	pH	N_2O	N_2O/O_2[a]	Air	[indole]/mol dm^{-3}
(1a)	2	47	55	49	10^{-3}
	7	55	65	30	10^{-3}
	7	53[b]	63[b]	35[b]	10^{-3}
	11	40	72	45	10^{-3}
(1b)	2.7	90	120	32	10^{-3}
	7	113	104	28	10^{-3}
	11	105	122	51	10^{-3}
(1c)	2.6	72	86	60	10^{-3}
	7	75	112	104	10^{-3}
	11	95	145	160	10^{-3}
(1d)	2.7	65	—	—	5×10^{-4}
	7	72	351	366	5×10^{-4}
	11	96	383	417	5×10^{-4}

[a] $N_2O/10\%$ O_2. [b] Dose rate 0.043 Gy s^{-1}.

Table 3. Consumption of O_2 as a percentage of initial radical yield upon γ-radiolysis of various indoles in the presence of 5×10^{-2} mol dm^{-3} Br^-.

Substance	pH	N_2O/O_2[a]	Air	O_2	[indole]/mol dm^{-3}
(1a)	2	10	27	—	10^{-3}
	7	32	27	48	10^{-3}
	11	67	70	—	10^{-3}
(1b)	7	17	15	—	10^{-3}
	11	105	52	—	10^{-3}
(1c)	2	23	53	—	10^{-3}
	7	108	105	155	10^{-3}
	11	127	67	—	10^{-3}
(1d)	2	18	40	—	5×10^{-4}
	7	317	292	450	5×10^{-4}
	11	317	250	—	5×10^{-4}
(1e)	7	18	32	40	10^{-3}

[a] $N_2O/10\%$ O_2.

Table 4. Oxygen consumption in percent of initial radical yield upon γ-radiolysis of indole in the presence of Br^- [10^{-3} mol dm^{-3} indole, (**1a**), 5×10^{-2} Br^-].

% O_2 in N_2O	%(– O_2)	pH
10	32	7
20	40	7
50	52	7
10	67	11
20	72	11
50	98	11

The reaction between the (**3a**) or (**3c**) radical with $O_2^{-\cdot}$ was also observed. However, these reactions were not electron transfers like reaction (3) but yielded an intermediate hydroperoxide to be discussed later.

γ-Irradiation and Product Analysis.—All yields are given relative to the total yield of radicals which is taken to be 6.2×10^{-7} mol J^{-1}. It then follows that the maximum yield of a product formed in a dismutation or recombination reaction is 50%.

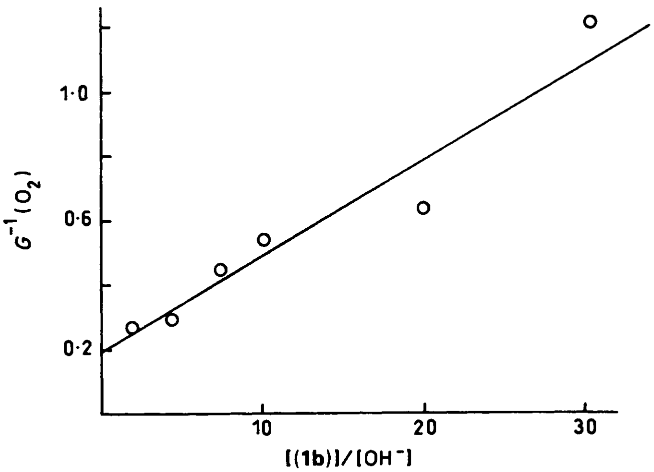


Figure 2. Oxygen consumption as a function of the ratio [(**1b**)]/[OH^-] observed during γ-radiolysis of $N_2O/10\%$ O_2-saturated aqueous solutions containing 5×10^{-2} mol dm^{-3} Br^-.

γ-Irradiation was performed on a number of indoles in the presence of $Br^{-\cdot}$. The consumption of the parents was determined by HPLC and is presented in Table 2. Without going into detail we observed that the consumption yields of the indoles with a hydrogen atom at the 3-position were very sensitive to the total dose delivered and diminished markedly with irradiation time. Although the values presented refer to a conversion degree of only 5–10%, the above finding prompts caution in interpreting the results, since evidently irradiation products interfere with the reaction mechanism at an early stage. Thus the consumption values of (**1a**) and (**1b**) should be viewed as lower limits. The consumption of O_2 as presented in Tables 3 and 4 refers to the initial slope of the response. With (**1b**) as the parent the oxygen consumption as a function of the ratio between [(**1b**)] and [OH^-] was measured. The competition kinetics are depicted in Figure 2. In neutral and basic solutions purged with N_2O or $N_2O/10\%$ O_2 the yield of H_2O_2 was found to be $< 0.6 \times 10^{-7}$ mol J^{-1} in all cases, which does not exceed the primary radiation chemical yield (0.6×10^{-7} mol J^{-1}) in dilute aqueous solutions.

Irradiation of indoles in N_2O- or O_2/N_2O-saturated solutions mostly results in a large number of end products as evidenced by TLC and HPLC analysis. Several of these products are unstable and consequently only a few of them have been positively identified. In Table 5 are compiled the measured yields of detected and identified end products. The yields of (**13d**) and (**14d**) were measured within 10 min from the onset of irradiation. This was done to minimize the conversion of (**14d**) into (**13d**) (see below). After 10 min the amount of (**13d**) formed from (**14d**) made up less than half of the total amount of (**13d**) found. When (**1d**) is irradiated (saturated N_2O, Br^-) one major and several minor products are formed. The mass spectrum of the major product shows peaks with *m/z* 288 (M^+), 273 ($M^+ - CH_3$) and 144 ($M^+/2$). Similar experiments on (**1c**) show, apart from the major product (**11c**), a minor product with *m/z* 260 (M^+), 245 ($M^+ - CH_3$) and 130 ($M^+/2$). These products are only consistent with 1-1′, 1-3′ or 3-3′ coupling, but as the major product of (**1c**) is the 3-3′ coupled (**11c**) we suggest them to be (**10d**) and (**10c**).

γ-Irradiation of N_2O-saturated solutions of (**1a**) do not generate products hydroxylated on the benzene ring. This was established by comparison with authentic samples of 4-, 5-, 6- and 7-OH indoles.

When N_2O-saturated solutions of (**1b**) at pH 11 were γ-irradiated in the presence or absence of Br^- the end products and their amounts were found identical in both systems, as evidenced by HPLC and TLC analysis. This identity in end-product composition persisted when similar comparison was made on irradiated solutions purged with $N_2O/10\%$ O_2

Table 5. End products formed in γ-radiolysis of various indole solutions as a percentage of initial radical yield.[a]

Indole	Purging gas	pH	Product (%)	Product (%)	Product (%)	Oxidant
(**1b**)	N_2O	3	(**9b**) (27 ± 3)			$Br_2^{-\bullet}$ or $OH^\bullet$
(**1b**)	N_2O	11	(**5b**) (24 ± 7)			$Br_2^{-\bullet}$ or $OH^\bullet$
(**1b**)	$N_2O/10\%\ O_2$	11	(**7b**) (27 ± 3)	(**5b**) (14 ± 5)		$Br_2^{-\bullet}$ or $OH^\bullet$
(**1c**)	N_2O	7	(**11c**) (36 ± 10)	(**5c**) (6 ± 0.2)		$Br_2^{-\bullet}$
(**1c**)	$N_2O/10\%\ O_2$	7	(**13c**) (26 ± 1)	(**5c**) (13 ± 0.5)		$Br_2^{-\bullet}$
(**1c**)	$N_2O/10\%\ O_2$	11	(**13c**) (27 ± 1)	(**5c**) (5 ± 0.2)		$Br_2^{-\bullet}$
(**1d**)	$N_2O/10\%\ O_2$	7	(**14d**) (135 ± 10)	(**16d**) (108 ± 10)	(**13d**) (28 ± 1)	$Br_2^{-\bullet}$

[a] The maximum yield of an end product formed through radical recombination or dismutation is 50%.

mixtures. The product (**7b**) was inferred from the UV–VIS spectrum[16] showing the presence of the 3-oxy group. The nature of the functional group at the 2-position has not been verified but we suggest that it is the OH group.

Next, air-saturated neutral solutions containing Br^- and any of (**1a**), (**1c**) or (**1d**) were γ-irradiated. After irradiation these solutions produced observable chemiluminescence at *ca.* 430 nm [$t_{\frac{1}{2}}$ (**1a**), 1.5 min; (**1c**), 0.7 min; (**1d**), 1 h]. HPLC-analysis of such spent solutions revealed the presence of C(2)–C(3) ring-opened amides (**13**) from (**1c**) and (**1d**). Compound (**14d**), synthesized according to ref. 22 was found to decompose into (**13d**) during chemiluminescence ($t_{\frac{1}{2}}$ 1 h). In the absence of O_2, (**13d**) formed quantitatively.

In pulse-irradiated O_2-saturated solutions containing Br and (**1c**) the major route for radical annihilation is the reaction of $O_2^{-\bullet}$ with (**3c**). In such solutions at pH 11 the major product (>35%) is (**13c**).

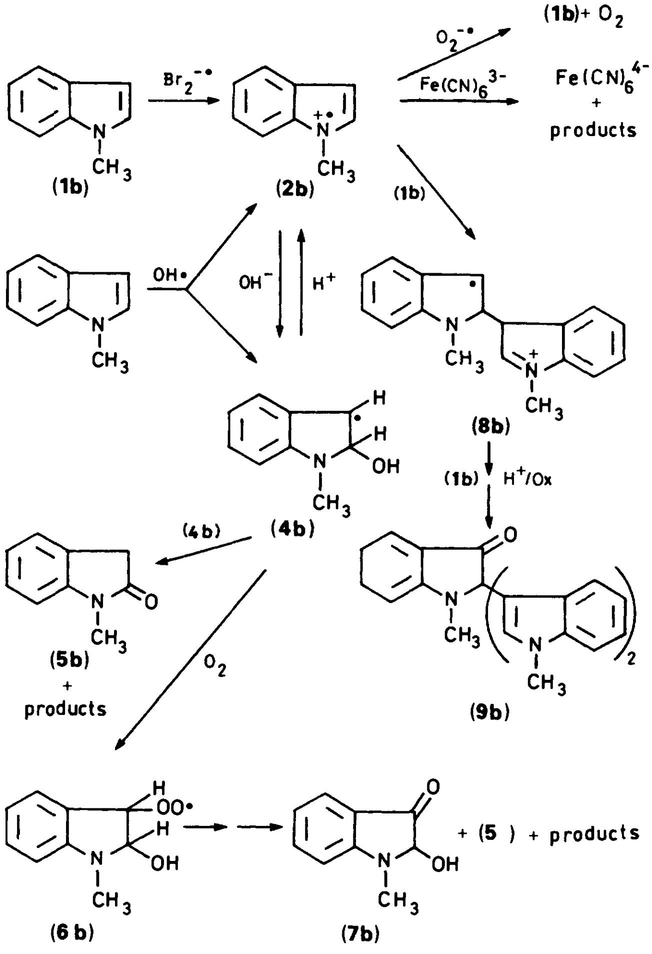
In O_2-saturated 0.1 mol dm^{-3} $NaHCO_2$ solutions, in which initially only $O_2^{-\bullet}$ forms, no consumption could be observed for any indole.

Discussion

Despite much effort to quantify the reaction mechanism only parts of it could be unravelled in the present investigation. This is mainly due to the high reactivity of the initial products from whence the product pattern becomes complex and difficult to interpret. The discussion will reflect our line of reasoning and thus indicate possible completing experiments to confirm or discard certain conclusions.

The Reaction of Indolyl Radical Cations.—Cations (**2a**), (**2c**), (**2d**) and (**2e**) exist only in acid solution.[8] However, we believe that (**2b**) is a good probe with which to pin-point the site of nucleophilic addition to all the cations. Scheme 1 comprises our findings with (**2b**). The observation that self-recombination and oxidation by $Fe(CN)_6^{3-}$ yield different transient products reveals that the former reaction is a radical–radical coupling process while the latter should be an overall one-electron oxidation, with water or OH^- probably participating to yield pseudo-bases with the incipient indole dication.

In this context we recall that the transient spectrum observed subsequent to radical recombination of (**2b**) absorbs at much higher wavelengths than the corresponding coupling product of the (**3c**) radical, (**10c**). This suggests that (**2b**) presumably undergoes considerable 2-3′ coupling as 3-3′ coupling is hard to reconcile with the formation of a coloured product. Several of the reactions in the scheme involve nucleophilic addition to (**2b**). Utilizing electrochemical oxidation or photosensitized electron-transfer Yoshida[23,24] has shown that in the attack of CN^- on (**2b**) there is a fivefold preference of CN^- for the 2-position as compared with the 3-position. The formation of the end-products (**5b**) and (**9b**) are in agreement with the 2-position being the electrophilic centre on (**2b**). As the consumption of O_2 in Figure 2 is dependent on the ratio [(**1b**)]/[OH^-] it follows that there is competition between OH^- and the parent where the product of reaction (1) does not react with O_2. The Figure enables us to determine the ratio between the rate constants of reactions (1) and (4).



Scheme 1.

$$(\mathbf{2b}) + OH^- \longrightarrow (\mathbf{4b}) \qquad (4)$$

By use of the rate constant k_4 we calculate k_1 to be 9×10^5 dm^{-3} mol^{-1} s^{-1} which is in good agreement with the estimated k_1 value presented in Table 1. Compound (**8**) is a reasonable precursor to the end product (**9b**) which thus supports reaction (1) to involve a C(2)–C′(3) coupling.

When (**1a**) is oxidized by Ti^{3+}–H_2O_2 in acidic solution, some 2-3′ coupled end-products are detected.[13] This system produces $OH^\bullet$ radicals which in the acidic environment will oxidize (**1a**) to (**2a**). Thus, similarly to reaction (1), nucleophilic addition of (**1a**) to (**2a**) could be the path to 2-3′ coupling.

The slight consumption of O_2 at pH 7 in the presence of 1×10^{-3} mol dm^{-3} (**1b**) (Table 3) could imply that *ca.* 10% of the cation radical undergoes hydrolysis [reaction (5)] in competition with dimerization reaction (1).

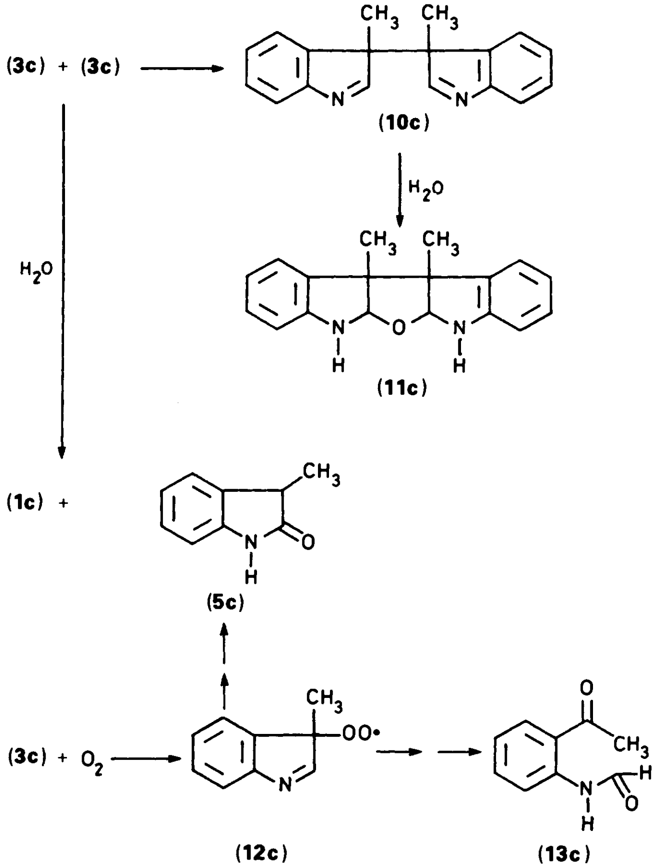
$$(\mathbf{2b}) + H_2O \longrightarrow (\mathbf{4b}) + H^+ \qquad (5)$$

Thus the upper limit of reaction (5) is calculated to be *ca.* 10^2 s^{-1}. Once the radical is hydrolysed it reacts rapidly with O_2 (see Table 1), probably forming (**6b**). The presence of a 3-oxy compound such as the suggested (**7b**) among the end-products is consistent with the dismutation of two (**6b**) peroxy radicals.

$OH^{\bullet}$ *Addition to Indoles.*—The finding that, irrespective of primary oxidant ($Br_2^{-\bullet}$ or $OH^{\bullet}$), the end products are the same in irradiated (**1b**) solutions implies that OH^- addition to (**2b**) and $OH^{\bullet}$ addition to (**1b**) yield the same intermediate which is proposed to be (**4b**). The essential identities of the spectra in Figure 1 show that $O^{-\bullet}$ forms (**4b**) as well. The above observations are somewhat surprising. Hydroxy radicals would be expected to form OH adducts in all available positions. Why then should only the 2-adduct survive? We suggest that the other adducts are very unstable as regards dissociation into the indolyl cation and OH^-. The fact that in N_2O/10% O_2 purged solutions the presence of Br^- does not modify the end-product composition can give us a clue to the maximum life time of any ephemeral $OH^{\bullet}$ adduct. Assuming the reaction between O_2 and any such adduct to proceed with a rate of *ca.* 10^9 dm^3 mol^{-1} s^{-1} and to modify the product pattern, we infer that none of these adducts should survive a few µs. The spectra in Figure 1 were recorded after completion of reaction (4). From the rate of reaction (4) it follows that the dissociation of the presumptive $O^{-\bullet}$ ($OH^{\bullet}$) adducts should have half-lives $< 10^{-6}$ s which supports the above estimate. In ref. 8 the yield of indolyl radicals at pH 7 produced in the $OH^{\bullet}$ attack was found to be 44 and 49% for (**1a**) and (**1c**) while the corresponding yields with (**1d**) and 2-methylindole were 67 and 68%, respectively. In the present work the re-measured yield of (**2b**) was found to be 44%. Given the steric hindrance of a methyl group to $OH^{\bullet}$ attachment the finding that only methylation of the 2-position increases the yield of indolyl radicals suggests equally that the latter form is at the expense of the $OH^{\bullet}$-adducts in the 2-position.

From results obtained in ref. 16 it was inferred that the $CCl_3OO^{\bullet}$ radical forms 2-adducts with (**1a**), (**1c**), (**1d**), (**1e**) and 2-methylindole but not with (**1b**). The strong case for the existence of (**4b**) in the present system would seem to militate against the interpretation in ref. 16. A possible expedient would be to assume that initial radical addition occurs on the nitrogen to form 1-adducts. These adducts could then rapidly rearrange into 2-adducts, and undergo an inner-sphere electron transfer or cleave into nitroxide radicals and CCl_3OH. Should the last alternative occur, this would imply that the 460 nm absorbances observed[16] belong to the nitroxide radicals. The complete absence of products hydroxylated on the benzene ring is seemingly at variance with the results found in refs. 12 and 14 where such products were formed when Fe^{2+} H_2O_2 was used as Fenton's reagent. However, during the Fenton reaction the oxidized metal ion Fe^{3+} formed may oxidize the $OH^{\bullet}$ adduct in competition with water expulsion.

Reactions of Neutral Indolyl Radicals.—Unlike (**1b**), indoles with hydrogen in the 1-position form the neutral indolyl radicals [(**3a**), (**3c**), (**3d**), and (**3e**)] upon one-electron oxidation in neutral solution.[8] The main reaction pattern, (Scheme 2), of (**3c**) appears to be dimer formation through 3-3′ coupling followed by water incorporation to give (**11c**). The recombination should give both the *meso* and racemic forms of (**10c**) both of which might be in equilibrium with H_2O to form 2-OH compounds. The energetically most favourable conformation of the racemate requires only a slight distortion in order to cyclize into (**11c**). On the other hand, the *meso* form would have to attain the fully eclipsed form to do likewise. Thus the minor product suggested to be (**10c**) could be its *meso* form. The consumption yields for (**1c**) and (**1d**) at pH 7 show that most of the radicals undergo dimerization as opposed to dismutation. The latter pathway yields basically (**5c**). Dismutation seems to be the major route for (**1a**). Even so, 3-3′ coupling must occur to some extent as the detected end-product 3,3′-bi-indole in ref. 13 testifies. By use of the rate constants in Table 1 the equilibrium constant for reaction(s) [with (**2b**) as the reactant] can be calculated to be $K_1 < 10^{-7}$ mol dm^{-3}.



Scheme 2.

$$(2) + H_2O \rightleftharpoons (4) + H^+ \qquad (6)$$

We expect the electrophilicity of (**2**) to parallel its redox potential. Therefore, since the reduction potential of (**2b**) is equal to or higher[7] than those of (**2a**), (**2c**) and (**2d**), K_1 should be the upper limit for all radical cations. Introducing reaction (7)

$$(2) \rightleftharpoons (3) + H^+ \qquad (7)$$

we obtain reaction (8).

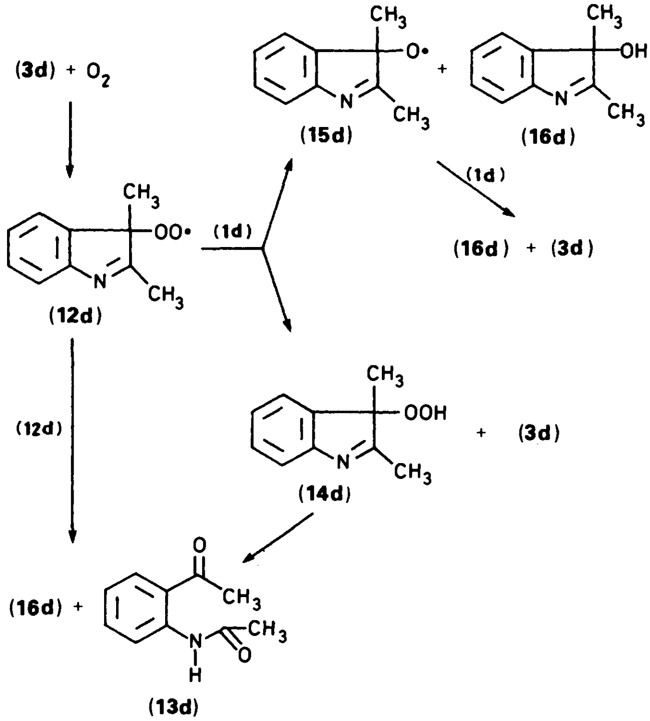
$$(3) + H_2O \rightleftharpoons (4) \qquad (8)$$

From the known pK_a values[8] it follows that $K_3 < 0.1$, *i.e.* all neutral indolyl radicals are resistant to water addition. It is safe to assume that water reacts much slower with neutral indolyl radicals (by a factor of 10^2-10^3) than with the indolyl cation. Therefore, even if K_3 is as large as 0.1, the indolyl radicals should remain completely unhydrolysed at pH 7 over *ca.* 0.1 s, the lifetime of the indolyl radicals in the γ-source. It is noted that O_2 consumption in the presence of (**3a**) increases from pH 7 to 11. This finding could imply that reaction (9) comes into

$$(\mathbf{3a}) + OH^- \xrightarrow{H_2O} (\mathbf{4a}) + OH^- \qquad (9)$$

competition with radical recombination. Although equilibrium (8) is unfavourable, a rapid reaction of O_2 with (**4a**) would quench the back reaction. From the results the rate constant of reaction (9) is predicted to be *ca.* 10^4 dm^3 mol^{-1} s^{-1}. In analogy with reaction (4) the 2-position is probably the site of addition in reaction (9) as well. However, it is also possible that the OH^- attack occurs on the (**12a**) radical to form (**6a**). Table 4 reveals that oxygen consumption is *ca.* twice as high at pH 11 as at pH 7. In particular, at sufficiently high concentration of oxygen, one O_2 molecule is incorporated into the end products at pH 11 while only *ca.* 0.5 O_2 is incorporated at pH 7. Therefore, we conclude that two radicals (**12**) regenerate one O_2 upon recombination while O_2 regeneration is barred once (**6a**) radicals have formed.

Oxygen Reaction and Chemiluminescence.—The reduction potential of (**2d**) is 0.93 V, the lowest of the indoles investigated.[7] In keeping with this, (**3d**) is observed to react with O_2 on the pulse scale. As the consumption values in Tables 2 and 3 reveal, one-electron oxidation of (**1d**) in the presence of oxygen results in a chain reaction. The large amount of (**14d**) formed points to (**12d**) as the chain carrier (Scheme 3). However, the substantial yield of (**16d**) reveals that the latter also forms in the chain reaction. We propose that the reaction between (**12d**) and (**1d**) is branched. In analogy to $CCl_3OO^{\bullet}$,[16] (**12d**) could add to the 3-position of (**1d**) and the adduct would probably suffer O–O bond cleavage. The small amount of (**13d**) is in keeping with it being formed in the termination step *i.e.* dismutation of (**12d**).



Scheme 3.

Similarly, the formation of (**13c**) as the major product from the reaction of (**3c**) with O_2 (where no chain reaction occurs) should derive from dismutation of (**12c**). For the (**3a**) radical the increase of O_2 consumption at pH 7 with increasing [O_2] (see Table 4) is an indication of its reaction with O_2 in competition with radical recombination on the γ-radiolytic time scale. If so, the rate for reaction (10) is calculated to be 10^5 dm^3 mol^{-1} s^{-1}

$$(\mathbf{3a}) + O_2 \longrightarrow (\mathbf{12a}) \qquad (10)$$

from the data in Table 4. For tryptophan, the redox potential of which is the same as that of indole, the slight O_2 consumption at pH 7 indicates a rate constant of *ca.* 5×10^4 dm^3 mol^{-1} s^{-1} for O_2 addition to (**3e**). Finally, from the consumption of O_2 in O_2/N_2O-saturated (**3c**) solutions at pH 2, the corresponding rate [allowing for the pK_a of (**2c**)] for reaction (11)

$$(\mathbf{3c}) + O_2 \longrightarrow (\mathbf{12c}) \qquad (11)$$

is calculated to be 2×10^7 dm^3 mol^{-1} s^{-1}. This rate is rapid enough to explain the rate enhancement of radical disappearance with O_2 concentration in the pulse radiolytic experiments. It also rationalizes the quantitative consumption of O_2 in γ-irradiation at pH 7. Although indirect, these rates relate very reasonably to the experimental redox potentials.[7] Finally, some words should be said about the autoxidation. The finding that reaction (3) occurs quantitatively is no surprise given the enormous driving force of 1.36 V for the reaction. (The reaction could easily produce some $^1O_2$ which, however, has not been explored). The matter is more complex for the reaction of the neutral indolyl radicals with $O_2^{-\bullet}$ which occurs according to reaction (12) rather than reaction (13). Now, the pK_a values[25] of

$$(3) + O_2^{-\bullet} \longrightarrow (14) \longrightarrow (13) + h\nu \qquad (12)$$

the indoles are *ca.* 17. Therefore, the driving force for reaction (13) is still large (0.45–0.7 V). However, given the low self-exchange rate of the $O_2/O_2^{-\bullet}$ couple[26] the predicted rate of reaction (13) is below the diffusion-controlled limit. Here, $(\mathbf{1})^-$

$$(3) + O_2^{-\bullet} \longrightarrow (1)^- + O_2 \qquad (13)$$

denotes the deprotonated indoles. This rationalizes the fact that the radicals couple to a hydroperoxide instead of undergoing an electron transfer. We take the identical chemiluminescent half lives of irradiated solutions of (**1d**) and the decomposition of authentic (**14d**) as proof that there are no other routes leading to light generation. Consequently we ascribe the observed chemiluminescence in irradiated solutions of (**1a**) and (**1c**) to some formation of the short-lived species (**14a**) and (**14c**). The chemiluminescence of the hydroperoxides probably involves the formation and breakdown of dioxetane intermediates by analogy with what has been inferred from autoxidation studies of deprotonated indoles in aprotic solvents.[27]

Conclusions

(*a*) Nucleophilic addition to indolyl radicals occurs at the C_2 position.

(*b*) Oxygen adds to indolyl radicals at the C_3 position. The reaction rates are well below the diffusion-controlled limit and appear to be governed by the redox potentials of the radicals.

(*c*) The ratio between dismutation and recombination of the indolyl radicals varies with the substituent. A methyl group at the C_3 position promotes recombination through C_3–C_3' coupling.

(*d*) Apart from producing indolyl radicals the $OH^{\bullet}$ radical yields C_2 adducts to the indoles. The amount of OH adducts to other indole positions appears insignificant.

(*e*) In its reaction with indole the $OH^{\bullet}$ radical affords no detectable end product to be hydroxylated at the six-membered ring.

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