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Chemical and photochemical oxidation of tetrahydrobetacarboline

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Abstract

The photophysics and the mechanisms of the photochemical and chemical aromatization of 1,2,3,4-tetrahydro-7H-pyrido/3,4-b/indole (THBC) in 40% v/v methanol–water media have been investigated. The primary photophysical processes of THBC resemble those of indoles and related alkaloids. The photochemical oxidation has been carried out in the presence of atmospheric oxygen and light. The chemical oxidation has been studied using sodium peroxodisulphate (PDS) as the electrophilic agent. In both cases, strong acid media, i.e., sulphuric acid concentrations higher than 0.5 mol dm^{-3} are needed for the dehydroderivative (DH) to be formed. In the photochemical oxidation, the rate constants for the disappearance of THBC increase linearly with the concentration of acid and the intensity of the exciting radiation. However, the formation of DH only depends on the acidity of the media. In the chemical oxidation a similar behaviour is observed. In this case, the rate constants for the disappearance of THBC increase linearly with both PDS and acid concentrations, and the appearance of DH solely varies with the acid concentration. A two step mechanism is proposed for these oxidation reactions. In the first step, excited or ground state THBC reacts with ground state oxygen or PDS, respectively, to give an indolenine intermediate. This intermediate slowly rearranges, in a second acid catalysed step to yield DH.

Keywords: Tetrahydronorharmane; Photophysics; Oxidation; Kinetics

1. Introduction

The tetrahydrobetacarboline ring, 1,2,3,4-tetrahydro-7H-pyrido/3,4-b/indole (THBC) constitutes the basic element of numerous natural indolic alkaloids such as those belonging to the Harmala or Rauwolfia families [1–4]. These alkaloids occur in some plants like Peganum Harmala, Rauwolfia Serpentine, and in various marine organisms [5–7].

Several tetrahydrobetacarbolines are endogenous, albeit trace, constituents of the mammalian brain [8–10]. These so-called mammalian THBC alkaloids probably arise endogenously from the condensation of central nervous system indolamines (or their precursor amino acid L-tryptophan) with an aldehyde or α-keto acid via Pictet–Spengler reaction [11]. Other THBCs also appear to be formed after ingestion of ethanol [12], and the hypothesis that these alkaloids contribute to the behavioural changes, physical dependence and addictive properties of ethanol has been advanced. Although the mammalian metabolism of THBCs has not been studied, the fact that their oxidation reactions do occur in vivo might be implied from the observation that these alkaloids and their aromatized derivatives are present in human urine after alcohol consumption [12]. Also, THBCs are thought to be oxidized to a reactive intermediate which can cross-link proteins in aging human lenses suggesting that they might be involved in cellular aging phenomena [13].

In spite of the interest of the oxidation chemistry of THBCs, there have been no systematic studies on these reactions. However, it appears to be rather widely believed [14] that oxidation of these alkaloids should ultimately result in the formation of their partial or totally aromatized derivatives. For this reason, in this report we have studied the aromatization reactions of THBC, the most representative member of the tetrahydrobetacarboline's family. Because this alkaloid can be chemically and photochemically oxidized, we have analysed both oxidation processes and the factors that favour the aromatization reactions. The photochemical study has been carried out in the presence of atmospheric oxygen and light, while for the chemical study the oxidizing agent, sodium peroxodisulphate (PDS), has been used.

2. Experimental

2.1. Reagents

THBC was purchased from Sigma-Aldrich Química and was used as received. Stock solutions of the substrate,

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prepared in 40% v/v methanol–water, were stored in the dark and frequently renewed. Potassium PDS was Anala R grade (Merck). The acidity of the media was adjusted by the addition of appropriate amounts of standardized sulphuric or perchloric acid solutions. In the chemical oxidation, the ionic strength, *I*, was kept constant at a value of 4 mol dm^{-3} by the addition of $NaClO_4$.

As known, experimental conditions of high acid concentrations and ionic strength are far from being covered by simple thermodynamic approaches. Therefore, the kinetic results should be interpreted using proton activities rather than proton concentrations. Unfortunately, experimental data on hydrogen ion activities in 40% v/v methanol–water solutions at different acid concentrations and ionic strengths are not available in the literature. Thus, we checked the results obtained using proton activities in water [15,16] or directly proton concentrations. Because the resulting kinetic laws were independent of the approach used, we decided, for the sake of simplicity, to use proton concentrations instead of proton activities.

2.2. Spectral measurements

Absorbances were measured on a Perkin-Elmer Lambda-5 spectrophotometer equipped with thermostatted cell holders. Steadystate fluorescence or phosphorescence emission and excitation measurements were made in a Perkin-Elmer 650-40 spectrofluorometer equipped with a phosphorescence accessory Perkin-Elmer 650-0175 for recording the phosphorescence spectra and phosphorescence decays. The emission spectra were corrected by using a Perkin-Elmer Data Processor 650-0178 (Rhodamine-B as the quantum counter). Except for the phosphorescence spectra, which were recorded in absolute ethanol at 77 K, all the spectra were obtained in 1 cm quartz cells at 25.0±0.1°C. Sample absorbances for fluorescence measurements were adjusted to <0.1 at the corresponding excitation wavelengths to avoid inner filter effects and reabsorption phenomena.

Absolute fluorescence quantum yields, ϕ, were determined at 280 nm excitation wavelength by comparison of the corrected emission spectra with the spectrum of tryptophan. Sample quantum yields were calculated by using a value of 0.14 for tryptophan [17] in phosphate buffered solutions of pH 7.

Fluorescence decays were collected by time correlated single photon counting on a FL-900CD Edinburg Analytical Instrument. The excitation source was a nanosecond nF900 flash lamp filled with H_2 (0.4 bar) and operating at 40 kHz with ~6 kV applied across 1 mm electrode gap. Fluorescence decays from the samples were acquired to $(1–1.5)\times10^4$ counts in the peak. Fluorescence decay data were fitted by reference deconvolution to a sum of exponentials. Goodness of the individual fits was judged by the magnitude of the reduced χ_r^2 and the shape of the autocorrelation function of the weighted residuals [18].

2.3. Characterization of the reaction product

In the media of high sulphuric acid concentrations used in this work, TLC experiments showed the formation of a sole reaction product. This compound was undoubtedly characterized as the DH by comparison of its absorption, λ_{abs}=354 nm, and emission, λ_{em}=445 nm, spectra [19] with those of a pure sample of DH obtained by an independent procedure. In the photochemical reactions iodometric titrations also showed the stoichiometric formation of H_2O_2, through the global reaction: $THBC+O_2 \rightarrow DH+H_2O_2$.

2.4. Kinetic measurements

Photochemical reactions. The source of irradiation for the photochemical studies was the 250 W Hamamatsu xenon lamp of our spectrofluorometric system. As photoreactor we used a micro quartz cell Hellma (model 75.050). The small volume of the cell ensured the total irradiation of the solutions and minimized the problems resulting from the diffusion of the reagents. The solutions of THBC were placed in the cell holder of the spectrofluorometer and their fluorescence spectra were recorded periodically to analyse the evolution of the reaction mixture with time. The high absorbances of the solutions used in the photochemical experiments ensured the total absorption of the incident light.

The light intensity of the irradiation was varied by changing the slit of the excitation monochromator and it was evaluated with an actinometer of potassium ferrioxalate [20]. The actinometer solution was irradiated before and after the irradiation of the sample for an appropriate time (≈30 min). Generally during 2–4 h of irradiation the intensity of the lamp remained practically constant. However, when meaningful drifts in the lamp intensity were observed, the experimental measurements were rejected. All the kinetic experiments were carried out under temperature controlled conditions at 25±0.1°C. The excitation wavelength was always 278 nm, the maximum in the absorption spectra of the substrate. Intensity data were collected at 352 and 445 nm, the emission wavelengths of cationic THBC and DH, respectively. Pseudo-first-order rate constants were obtained by a nonlinear least-squares fitting of the intensity–time data to the following equation:

$$I_t = I_\infty + (I_0 - I_\infty)\exp(-k_{obs}t) \quad (1)$$

Always, the measured rate constants, at fixed oxygen and proton concentrations, were the same over a range of THBC concentrations. Finally, some kinetic measurements carried out in the presence of increasing amounts, up to 0.5 mol dm^{-3}, of sodium azide showed that this singlet oxygen quencher has no effect on the rate constant values.

Chemical reactions. Kinetic measurements were done under pseudo-first-order conditions with a large excess of PDS. As mentioned before, because the reactants involved in these reactions can be charged, the ionic strength of the media was

kept constant by the addition of $NaClO_4$. The spectra were recorded in the Perkin-Elmer 650-40 spectrofluorometer. The excitation wavelength and the wavelengths for recording the intensity data were those used in the photochemical reactions, i.e., 278, 352 and 445 nm, respectively. However, in these experiments the samples were irradiated only during the time necessary for the obtention of the emission spectra. Pseudo-first-order rate constants were also obtained by a nonlinear least-squares fitting of the intensity–time data to Eq. (1). After elimination of a small induction period, the reproduction of the rate constant values was excellent.

The possibility that the hydrolysis of PDS to peroxomonosulphate could compete with the oxidation of the substrates has been tested by independent measurements. These experiments showed that, under the acidity conditions used in the work, the hydrolitic reactions are much slower than the oxidation reactions. Also, the presence of radical promoters or radical traps did not affect the oxidation rates. This excludes the involvement of radical species on the reactions.

3. Results and discussion

3.1. Photochemical oxidation

Before broaching the photochemical reactivity of THBC, we will briefly discuss the photophysics of this compound. Owing to the basic properties of its piperidinic nitrogen, THBC can exist in aqueous solutions as neutral or cationic species. In the acid solutions used in this work, the spectrum of the cationic species is characterized by a weakly structured band with a maximum absorption at 278 nm. On the other hand, the steady-state fluorescence emission spectra of the cations are broad and featureless. The emission maximum occurs at 352 nm with a quantum yield of 0.27. These cationic species decay monoexponentially with lifetimes of 6.2 ns. Moreover, deoxygenation of the solutions of THBC has a very little effect on the fluorescence quantum yield as well as on the fluorescence lifetime.

As shown in Fig. 1, the fluorescence intensity of the cationic species is appreciably quenched in the presence of a strong acid as $HClO_4$, although its absorption and excitation spectra remain unchanged. However, the fluorescence spectrum is not affected when the inorganic counter-ion concentration is changed by the addition of $NaClO_4$ or Na_2SO_4 salts up to 2 mol dm^{-3}. This behaviour, already observed for other indole derivatives [21–24], has been attributed to an acid catalysed protonation of the indole ring or to a collisional quenching by hydrogen ions in the excited state. Although, we have previously reported the formation of very weak fluorescent indoleninic dications of THBC [25], λ_{em}=424 nm, in $H_2SO_4 \approx 18$ mol dm^{-3}, there is no evidences on their formation under the acidity conditions used in this work.

In the presence of H_2SO_4 or $HClO_4$, the fluorescence decay curves give always good single exponential decays with lifetimes decreasing as the acid concentration increases. The presence of SO_4^{2-} or ClO_4^- anions have no effect on the measured lifetimes. Furthermore, as shown in the inset of Fig. 1 for $HClO_4$ acid, the plots of ϕ_0/ϕ and τ_0/τ against $[H^+]$ are linear. The slopes of these Stern–Volmer plots are practically identical, 38±2 and 39±2 $mol^{-1}\,dm^3$, respectively. Therefore, steadystate as well as time resolved fluorescence measurements show that the quenching of the fluorescence of THBC cations by protons is a dynamic process. The average value for the quenching rate constant in $HClO_4$ acid calculated from the Stern–Volmer plot is 6.2×10^9 $mol^{-1}\,dm^3\,s^{-1}$. This value is similar to those previously reported for the quenching of tryptamine 6.6×10^9 $mol^{-1}\,dm^3\,s^{-1}$ [22], tetrahydroharmane 5.9×10^9 $mol^{-1}\,dm^3\,s^{-1}$ [24], and the Rauwolfia alkaloid yohimbine 5.6×10^9 $mol^{-1}\,dm^3\,s^{-1}$ [26].

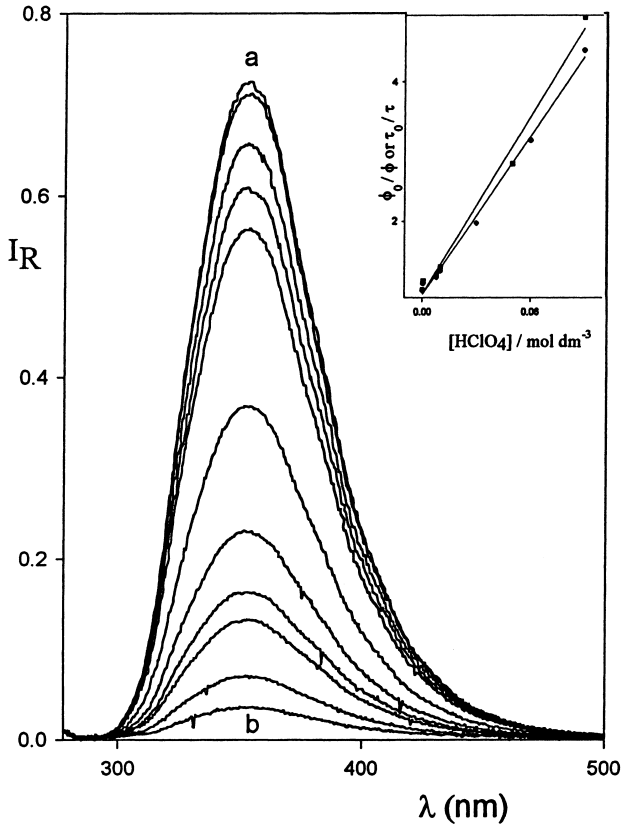


Fig. 1. Quenching of THBC fluorescence by $HClO_4$: (a) $[HClO_4]$= 0.001 mol dm^{-3}; (b) $[HClO_4]$=0.3 mol dm^{-3}. In the inset, Stern–Volmer plots of ϕ_0/ϕ (■) and τ_0/τ (●) versus $[HClO_4]$.

The luminescence spectrum of THBC in ethanol glasses at 77 K is shown in Fig. 2. It consists of fluorescence and phosphorescence emissions. As in the case of other simplest indoles, the fluorescence emission of THBC at 77 K, λ_{max}= 325 nm, is blue shifted and intensified with respect to that observed at 298 K. The phosphorescence emission displays well-resolved bands at 410, 437 and 464 nm characteristics of the indole ring [19]. The addition of acid produces a slight blue shift of the luminescence spectrum but neither its

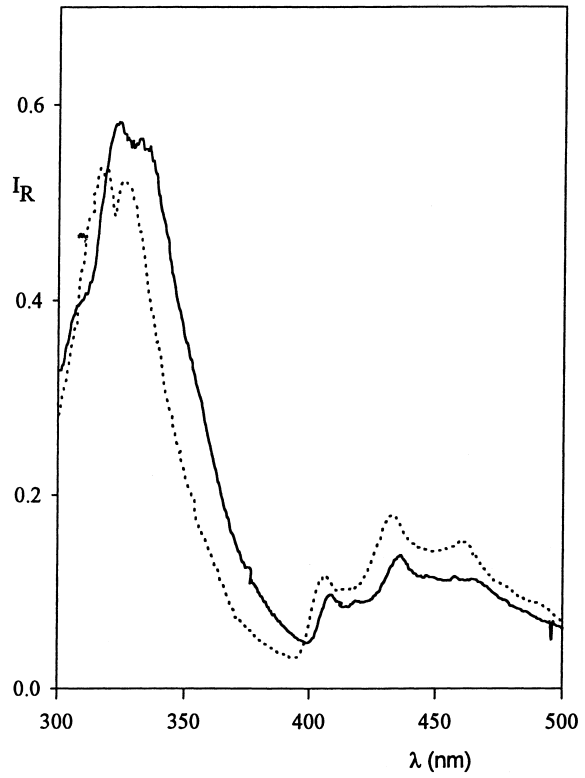


Fig. 2. Luminescence spectra of THBC in ethanol glasses at 77 K. Full line $[H_2SO_4]=0\,mol\,dm^{-3}$, dotted line $[H_2SO_4]=1.8\,mol\,dm^{-3}$.

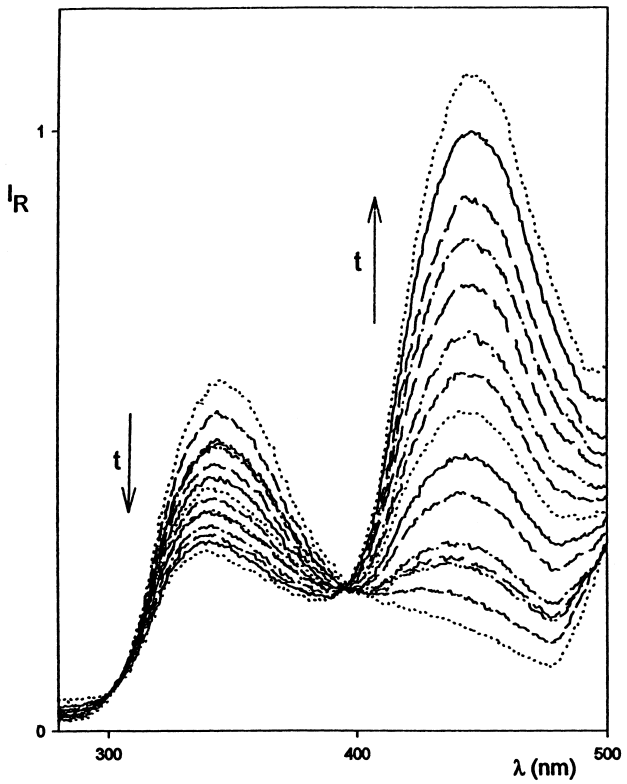


Fig. 3. Evolution with time of the fluorescence spectra of THBC solutions. $\lambda_{exc}=278$ nm, $[H_2SO_4]=1.8\,mol\,dm^{-3}$.

intensity and shape nor the phosphorescence lifetimes are modified.

In the light of the results mentioned, we can now discuss the photochemical reactivity of THBC in acid media. As shown in Fig. 3, when the acidified THBC solutions in 40% v/v methanol–water are continuously irradiated with near-UV light, the fluorescence band of the cationic species at 352 nm disappears and simultaneously a new band at 445 nm, characteristic of the DH, grows up. Interestingly, irradiation of the nonacidified solutions of THBC leads to the photodecomposition of the substrate, but DH is not formed. Thus, acidity is essential for this product to be formed. When the experiments are conducted in the dark or with deoxygenated solutions the emission spectrum of THBC remains unchanged. Therefore, apart from the acid media, oxygen as well as light are necessary for this oxidation reaction.

Under the acidity conditions used in this work, the rate constants for the disappearance of THBC, k_{obs1}, and the appearance of DH, k_{obs2}, vary linearly with the acid concentration, see Table 1. However, k_{obs1} values are always greater than those of k_{obs2} which indicates that these processes are not coupled, i.e., DH is formed through a two step mechanism involving a long lived intermediate. A similar behaviour has already been observed in the case of yohimbine, a Rauwolfia alkaloid possessing a tetrahydrobetacarboline ring in its skeleton [26].

Apart from the influence of acidity, the rate constants k_{obs1} also depend on the excitation wavelength. At wavelengths higher than 250 nm, the rate for the disappearance of THBC roughly correlates with the molar absorption coefficients of the substrate at the irradiation wavelengths. Thus, when the substrate does not absorb, no reaction is observed. Moreover, as shown in Fig. 4, the disappearance of THBC varies linearly with the intensity of the exciting radiation. According to these results it can be concluded that the photochemical oxidation of THBC is not a thermal reaction, but it proceeds through an excited state of THBC formed upon absorption of light.

Table 1
Observed rate constants for the photochemical disappearance of THBC, k_{obs1}, and appearance of DH, k_{obs2}, at different H_2SO_4 concentrations[a]

$[H_2SO_4]$ (mol dm^{-3})	$10^4\,k_{obs1}$ (s^{-1})[b]	$10^5\,k_{obs2}$ (s^{-1})[c]
1.0	0.63	0.98
1.2	1.46	3.72
1.3	1.97	5.66
1.4	2.3	7.42
10^4 m (mol^{-1} dm^3 s^{-1})[d]	4.4±0.4	1.6±0.4

[a] $[THBC]=2\times10^{-4}\,mol\,dm^{-3}$, $T=298$ K.
[b] Values calculated at 352 nm.
[c] Values calculated at 445 nm.
[d] Slopes of the plots of k_{obs} versus acid concentration.

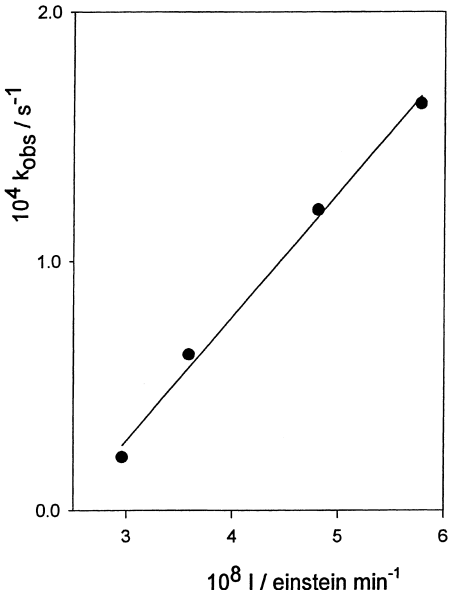
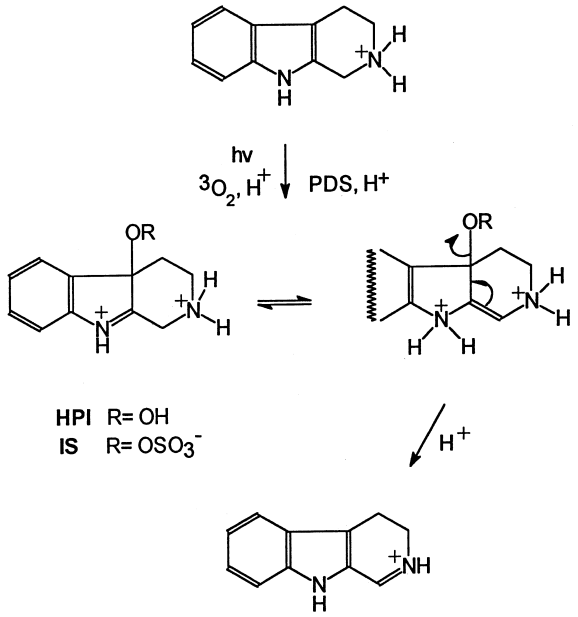


Fig. 4. Influence of the intensity of the exciting radiation at 298 nm on the rate constant for the disappearance of THBC.



Scheme 1.

Due to its short lifetime, singlet excited states of THBC can hardly be involved in the reaction. Furthermore, quenching of THBC singlets by O_2 should be ruled out since the fluorescence of THBC is not appreciably quenched in aerated methanol–water solutions. It seems, therefore, more plausible that the reacting species are the triplets of THBC or other species formed from them in the acid media where the photochemical oxidations are being studied. In relation to this, it should be mentioned that flash photolysis experiments have shown that, upon excitation of indole and tryptophan in sulphuric acid solutions from 1 to 3 mol dm^{-3}, a new transient is formed [27]. Because photoionization did not apparently occur, these transients were thought to be the triplets of their pyrrolic protonated cations. In a similar way, the acid dependence observed for the disappearance of THBC could be attributed to the formation of the corresponding triplets of THBC.

In relation to the oxygen species involved in the oxidation reactions, the fact that, as mentioned, the addition of sodium azide has no effect on the rate constant values, k_{obs1}, indicates that singlet oxygen species are not involved in the reaction. This means that the reaction studied is a type I process, i.e., molecular oxygen, $^3O_2$, reacts with the triplets of protonated THBC.

Taking into account the nature of the reactants, we assume that, as proposed for other indole derivatives [28–31], the reaction intermediate is the nonfluorescent 7-hydroperoxo-7H-THBC, HPI. Once this indolenine intermediate is formed, and internal reorganization step, from the enamonium tautomer with acid catalysed hydrolysis of the leaving group gives DH, see Scheme 1. In the disappearance of this intermediate, light is not involved. Thus, when the samples are irradiated and subsequently the light is cut off, DH is still being formed for a long period of time.

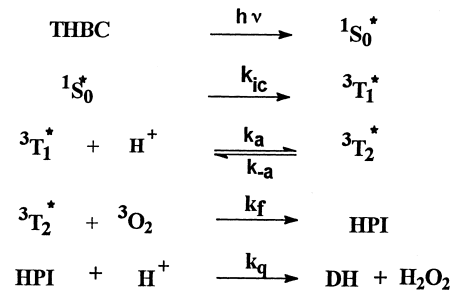
In the light of these results, we propose the most probable mechanistic route for this photochemical oxidation, in Scheme 2. The reacting species, $^3T_2{}^*$, are formed in an acid catalysed step from the triplets $^3T_1{}^*$. Applying the equilibrium condition to the formation of $^3T_2{}^*$ at fixed intensity of the exciting radiation, k_{obs1} can be expressed by

$$k_{obs1} = \frac{k_a k_f}{k_{-a}}[^3O_2][H^+] \quad (2)$$

and the rate law for the formation of DH is given by

$$k_{obs2} = k_q[H^+] \quad (3)$$

According to Eq. (2), k_{obs1} values should, as observed, vary linearly with both oxygen and proton concentrations with zero intercept at the origin. Also, k_{obs2} values in Eq. (3), should only depend on proton concentrations. From the k_{obs2} against $[H^+]$ plot, a value of (1.6±0.2)×



Scheme 2.

10^{-4} mol^{-1} dm^3 s^{-1} can be obtained for k_q. This value is in excellent accordance with the value of 1.3×10^{-4} mol^{-1} dm^3 s^{-1} previously reported for the same rate constant of the related substrate yohimbine [26].

3.2. Chemical oxidation

As shown typically in Fig. 5, the changes with time of the THBC spectrum upon addition of PDS are completely similar to those observed in the photochemical oxidation of this substrate. Thus, as in the last reaction, under the acidity conditions used, DH is the unique reaction product. As mentioned in Section 2, we have followed both, the disappearance of the substrate, k_{obs1}, and the appearance of the reaction product, k_{obs2}. The kinetics was always first order, and as observed in the photochemical oxidation, the values of k_{obs1} greater than those of k_{obs2}.

At fixed proton concentration, 1 mol dm^{-3}, the rate constants k_{obs1} vary linearly with [PDS] with slope of $(4.5\pm0.2)\times10^{-2}$ mol^{-1} dm^3 s^{-1} and zero intercept at the origin, see Fig. 6. Contrarily, the rate constants for the appearance of DH do not depend on PDS concentration. As shown in Table 2, at fixed [PDS], 8×10^{-3} mol dm^{-3}, the values of k_{obs1} and k_{obs2} vary linearly with proton concentration with zero intercept at the origin.

To account for these experimental results, we postulate, as in the case of the photochemical reaction, a mechanism with two consecutive steps. The first step is the electrophilic attack by PDS at the C7 atom of the substrate, k_{obs1}, to give a sulphate indolenine intermediate, Is in Scheme 1. Thus, these rate constants should, as observed, depend on [PDS]. The dependence of k_{obs1} values on proton concentration can be ascribed to the protonation of $HS_2O_8^-$ anions to give $H_2S_2O_8$ species which are much better electrophilic agents. Because, unfortunately, we have no reliable value for the pK_a of this peroxoanion, we have confirmed this assumption by independent kinetic measurements. We have analysed the dependence on proton concentration of the reaction between 2-phenyl-3-methyl indole and PDS. This substrate was selected for the following two reasons. Firstly, the observed rate constants correspond to the attack of PDS at the indole ring to form the indolenine intermediate as the final product [32]. Secondly, because this substrate cannot be protonated in these media [33], any influence of the acidity on the rate constants can be attributed to the oxidant. As expected, the

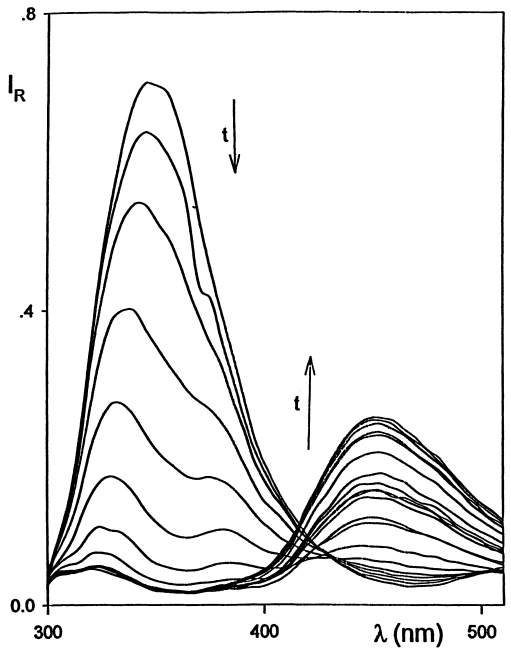


Fig. 5. Changes in the fluorescence spectra of THBC for a typical reaction mixture in 40% v/v methanol–water: λ_{exc}=278 nm, [THBC]=2×10^{-4} mol dm^{-3}, [PDS]=8×10^{-3} mol dm^{-3}, [H_2SO_4]=1 mol dm^{-3}, I=4 mol dm^{-3}, Δt=10 min.

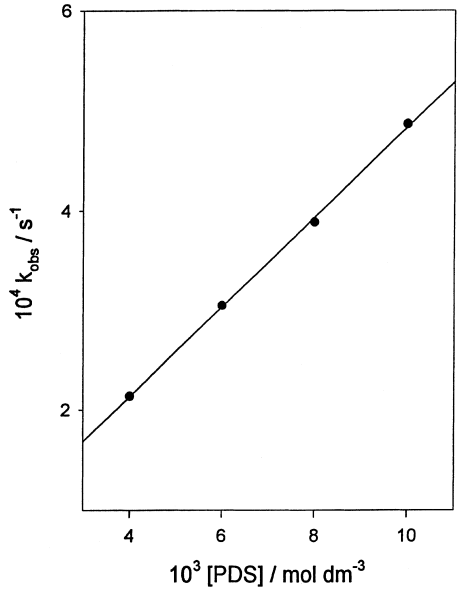
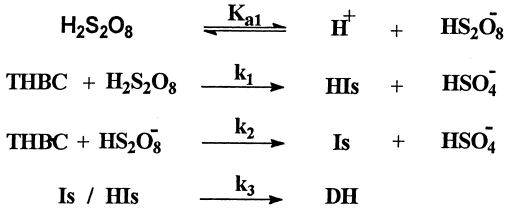


Fig. 6. Plot of the observed rate constants, k_{obs1}, for the chemical oxidation of THBC against PDS concentration. [THBC]=2×10^{-4}, [H_2SO_4]=1 mol dm^{-3}, I=4 mol dm^{-3}, T=298 K.

Table 2
Observed rate constants, k_{obs1} and k_{obs2} for the reaction between THBC and PDS at different H_2SO_4 concentrations[a]

[H_2SO_4] (mol dm^{-3})	10^4 k_{obs1} (s^{-1})[b]	10^5 k_{obs2} (s^{-1})[c]
0.7	3.28	1.05
0.8	3.49	1.13
1.0	3.89	1.25
1.2	4.45	1.4
10^4 m (mol^{-1} dm^3 s^{-1})[d]	2.3±0.4	0.69±0.08

[a] [THBC]=2×10^{-4} mol dm^{-3}, [PDS]=8.0×10^{-3} mol dm^{-3}, T=298K.
[b] Values calculated at 352 nm.
[c] Values calculated at 445 nm.
[d] Slopes of the plots of k_{obs} versus acid concentration.



Scheme 3.

observed rate constants for this system vary linearly with PDS and proton concentrations with zero intercept at the origin. Therefore, for the reaction between THBC and PDS, we postulate the mechanism shown in Scheme 3. According to this mechanism and considering that $[PDS]_0 = [HS_2O_8^-] + [H_2S_2O_8]$, the rate law in Eq. (4) should hold good

$$k_{obs1} = \frac{k_1[H^+] + k_2 K_{a1}}{K_{a1} + [H^+]}[PDS]_0 \quad (4)$$

Because we have checked that the rate constants for the reaction between THBC and PDS do not depend on the ionic strength, we assume that neutral PDS, better electrophilic than its conjugated base, is the species attacking the C7 atom of the substrate, i.e., $k_2 K_{a1} \ll k_1[H^+]$. On this basis, and considering that $K_{a1} \gg [H^+]$, Eq. (4) yields the following equation:

$$k_{obs1} = \frac{k_1[H^+][PDS]_0}{K_{a1}} \quad (5)$$

Hence, k_{obs1} values should, as observed, vary linearly with PDS and proton concentrations with zero intercept at the origin. The parameters obtained from these plots allow us to calculate a mean value of $(3.7 \pm 0.3) \times 10^{-2}\,mol^{-2}\,dm^6\,s^{-1}$ for the k_1/K_{a1} relation.

Once the long lived indolenine intermediate, Is, is formed, its reactivity depends only on proton concentration. That is, a second PDS attack is not observed. Therefore, DH is formed in an acid catalysed reorganization step. According to the mechanism in Scheme 3, the rate constants for this second step, k_{obs2}, should be given by the following equation:

$$k_{obs2} = k_3[H^+] \quad (6)$$

From the plot of k_{obs2} values against $[H^+]$, a value of $(6.9 \pm 0.6) \times 10^{-5}\,mol^{-1}\,dm^3\,s^{-1}$ can be obtained for the rate constant of this step, k_3.

We can, therefore, conclude that in acid media THBC can be oxidized, chemically or photochemically, to its DH. In both cases, a two step mechanism involving the initial formation of an indolenine intermediate operates. The nature of the indolenine intermediate depends on the electrophilic agent. It is postulated that when oxygen or PDS is the oxidizing agent, a hydroperoxo or a sulphate indolenine is obtained, respectively. Further, these indolenines are reorganized in a second acid catalysed step to give DH. The relative rate of this second step depends on the nature of the leaving group and the structure of the substrate [26].

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