Kinetic studies on the peroxyoxalate chemiluminescent reaction: imidazole as a nucleophilic catalyst

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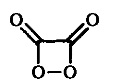
The peroxyoxalate system undergoes one of the most efficient chemiluminescence reactions and is the only one supposed to involve an intermolecular chemically induced electron exchange luminescence (CIEEL) mechanism, with proven high efficiency.

In this work we report kinetic results on the reaction of bis(2,4,6-trichlorophenyl) oxalate (TCPO) with hydrogen peroxide, catalysed by imidazole (IMI), in the presence of chemiluminescence activators. The kinetics were followed by measurement of the intensity of light emission and the 2,4,6-trichlorophenol (TCP) release, observed as an absorption change. From the dependence of the observed rate constants on the concentrations of TCPO, imidazole, hydrogen peroxide and activator, we attribute rate constants to three elementary steps in the proposed simplified mechanistic scheme. The initial step consists of attack of the nucleophilic imidazole on TCPO. A bimolecular ($k_{1(2)}$ = 1.4 ± 0.1 dm^3 mol^{-1} s^{-1}) and a trimolecular [$k_{1(3)}$ = (9.78 ± 0.08) 10^2 dm^6 mol^{-2} s^{-1}] rate constant can be attributed to this step. The imidazolide subsequently suffers imidazole-catalysed peroxide attack, leading to a peracid derivative; trimolecular rate constants $k_{2(3)}$ = (1.86 ± 0.06) 10^4 dm^6 mol^{-2} s^{-1} and $k_{2(3)}'$ = (8.7 ± 0.2) 10^3 dm^6 mol^{-2} s^{-1} can be obtained for this step from the peroxide and the imidazole dependence, respectively. The cyclization of the peracid affords the reactive intermediate, which we believe to be 1,2-dioxetanedione; a rough estimate of k_3 ~ 0.2 s^{-1} (at [IMI] = 1.0 mmol dm^{-3}) is obtained for this step. Finally, the interaction of the activator with the reactive intermediate, probably involving the CIEEL sequence and leading ultimately to light emission, is extremely fast and cannot be observed kinetically.

The peroxyoxalate chemiluminescence system undergoes one of the most efficient chemiluminescent reactions with quantum yields (Φ_{CL}) of up to 0.3 E mol^{-1}.[1,2] This system consists of a base-catalysed reaction of activated oxalic phenyl esters with hydrogen peroxide in the presence of highly fluorescent aromatic hydrocarbons with low oxidation potentials (E_{ox}) as chemiluminescent activators. Early works by Rauhut *et al.*[1] indicated the involvement of an electron transfer between the activator and a high energy intermediate in the excitation step.[3] This kind of excitation mechanism was generalized later by Schuster *et al.*[4] as 'chemically induced electron exchange luminescence—(CIEEL),' a widely accepted mechanism for excited state generation from the decomposition of certain types of peroxide, *i.e.*, diphenoyl peroxide[5] and α-peroxy lactones.[6] More recently, doubts about the efficiency of this mechanism arose from a redetermination[7] of the quantum yields from diphenoyl peroxide decomposition, the standard CIEEL system, leading to a value of 10^{-4} E mol^{-1}, in striking contrast with the 0.1 E mol^{-1} determined earlier.[5] Therefore, the peroxyoxalate reaction is the only chemiluminescence system, assumed to involve an intermolecular CIEEL mechanism, of proven high efficiency, as indicated by a recent careful redetermination of the quantum yield.[2] These facts reinforce the importance of mechanistic studies on this system.

The mechanism of this complex reaction sequence, with a great variety of possible subsequent and parallel steps, is not clearly understood, especially with respect to the excitation step, the elementary step in which the electronically excited species is formed. Rauhut *et al.*[1,3] postulated-1,2-dioxetanedione (**1**) as the high-energy intermediate responsible for activator excitation. This intermediate is accumulated in the absence of the activator, under the experimental conditions used, and reacts extremely fast upon activator addition, resulting in a flash of light. Subsequent kinetic studies,[8-13] using various oxalic esters and a variety of experimental conditions led to the proposal of other possible reactive intermediate structures, based merely on indirect evidence.



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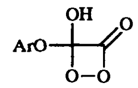

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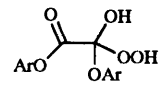

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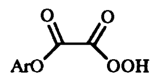

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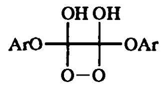

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Catherall *et al.*[8] identified the reaction between the oxalic ester and hydrogen peroxide anion as the rate-determining step, which evidently does not involve the activator. The 1,2-dioxetanone (**2**) was regarded as being the reactive intermediate, from indirect evidence. Contrary to the results of Rauhut *et al.*,[1] these authors were not able to detect a metastable intermediate, but could confirm the dependence of Φ_{CL} on the E_{ox} of the activator. Alvarez *et al.*[9] observed the apparently oscillating character of the light emission kinetics in anhydrous organic solvents under certain experimental conditions. The authors presented structures **1** or **2** and **3** or **4** as the two reactive intermediates capable of excited-state generation upon interaction with the activator, and structure **5** as an intermediate not capable of light generation.

Later on, the same research group performed an elaborate kinetic study[10] on the same system, using imidazole as catalyst and aqueous acetonitrile as solvent. The authors claimed that, only in 'media containing substantial fractions of water,' may a simplified kinetic approach be applied and the dynamics of the emission can be determined by only three parameters, the rise constant, the decay constant and the maximum intensity of the emission curve typically for a consecutive reaction. The authors proposed a simplified kinetic scheme and suggested that the rise constant is composed of the imidazole-catalysed peroxide attack on the oxalic ester, leading to the corresponding peracid, and of the nucleophilic substitution of the ester by imidazole. The fall constant is attributed to the non-chemiluminescent decomposition of the peracid, as well as to the transformation of this compound into the reactive intermediate, which interacts with the activator leading to light emission. Following the suggestion of Catherall *et al.*,[8] **2** is tentatively attributed as being the reactive intermediate.

A study of the reaction of bis(2,6-difluorophenyl) oxalate with hydrogen peroxide in the presence of an activator, using chemiluminescence emission and ^{19}F NMR spectroscopy to follow the time course of the reaction, led to the proposal of a hydroperoxy ester analogous to **4** as the most likely reactive intermediate capable of excited-state generation upon interaction with the activator.[12] This work provides the most direct structural evidence for the intermediate as the authors claim to be able to observe it directly by NMR spectroscopy.

Some important mechanistic features of the peroxyoxalate reaction can be extracted from the studies published up to date. (*i*) The first step of the reaction sequence (attack of hydrogen peroxide on the oxalate ester) is the slowest and can be observed kinetically. A hydroperoxy ester analogous to **4** is proposed in all the studies as an intermediate, but not necessarily as the reactive intermediate which interacts with the activator, leading to light emission. (*ii*) Interaction of the reactive intermediate with the activator occurs in an extremely fast step, not observable kinetically. (*iii*) There exists a correlation between the half-wave oxidation potential of the activator and the chemiluminescence quantum yield, indicating the involvement of an electron transfer in the activation step, as postulated in the CIEEL mechanism. (*iv*) A controversy exists with respect to the metastable nature of the reactive intermediate (accumulation of this intermediate in the absence of the activator). Some authors claim to have detected a metastable intermediate,[1,3] whereas others report that they were not able to detect a metastable intermediate at all.[8,14] (*v*) Several structural hypotheses have been proposed for the reactive intermediate (**1** to **5**[1,8–12] and others[13]), capable of activator excitation. In all cases, only rather indirect evidence is presented with respect to the structure of this intermediate.

With the objective of understanding better the peroxyoxalate reaction mechanism, we performed a kinetic study of this system in an anhydrous organic solvent, to prevent undesired hydrolysis of the oxalate ester, using imidazole as catalyst, which was shown to act mainly as nucleophilic catalyst. Contrary to former reports,[9,10] we (*i*) observed simple kinetic behaviour of the system under anhydrous conditions, (*ii*) propose a simplified mechanistic scheme and (*iii*) determine rate constants for two elementary steps and obtain a rough estimate for a third.

Results

Here we present our results of a kinetic study on the peroxyoxalate chemiluminescence of the system bis(2,4,6-trichlorophenyl) oxalate (TCPO), hydrogen peroxide, imidazole (IMI; as catalyst) and 9,10-diphenylanthracene (DPA; as chemiluminescence activator) in anhydrous ethyl acetate. The kinetics were followed by measuring the activator chemiluminescence emission and the absorption of released 2,4,6- trichlorophenol (TCP).

Dependence on [TCPO]

The chemiluminescence emission curve of the complete system consists of a rapid increase followed by a slower decay of emission intensity (Fig. 1), and its spectral distribution corresponds to the fluorescence spectrum of the activator (DPA). Kinetic curves can be fitted by the sum of two exponential functions yielding two observed rate constants k_{obs1} and k_{obs2}. The higher value k_{obs2}, contained mainly in the fast increase, depends on the [TCPO] ($k_{obs2} = 0.03$–0.12 s^{-1} for [TCPO] = 0.01–0.10 mmol dm^{-3}), whereas the lower value k_{obs1}, which corresponds to the slower decay, does not depend on the TCPO concentration, providing a mean value of $k_{obs1} = (6.0 \pm 0.2)\ 10^{-3}$ s^{-1} for standard reaction conditions ([TCPO] = 0.01–0.10 mmol dm^{-3}).

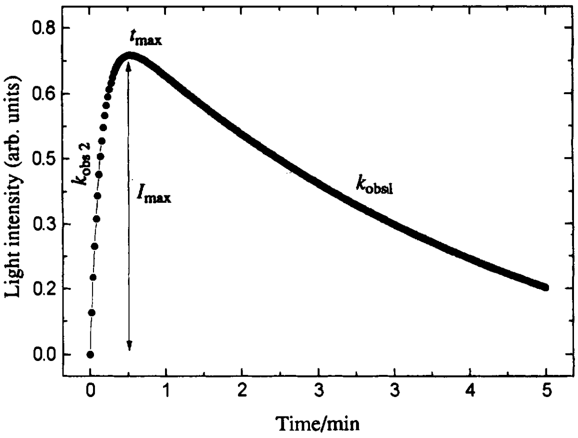


Fig. 1 Emission time course of the peroxyoxalate chemiluminescence: [TCPO] = 0.10 mmol dm^{-3}, [H_2O_2] = 10 mmol dm^{-3}, [IMI] = 1.0 mmol dm^{-3} and [DPA] = 1.0 mmol dm^{-3}

The rate of TCP formation from TCPO was determined from absorption changes at 288 nm and 295 nm. It was independent of the [TCPO] with a mean value of $k_{obs1} = (4.5 \pm 0.4)\ 10^{-3}$ s^{-1}, under standard conditions ([TCPO] = 0.01–0.10 mmol dm^{-3}). Only one rate constant could be obtained from the kinetic curves and the final absorption change corresponds to the release of both phenolic units from TCPO. The direct reaction of IMI with TCPO, observed by monitoring the absorption at 295 nm and 288 nm in the absence of hydrogen peroxide and the activator, provides only one rate constant. The final absorption change indicates the release of both phenolic units from TCPO. The rate constants are independent of the TCPO concentration ([TCPO] = 0.01–0.10 mmol dm^{-3}) with a mean value of $k_{obs} = (1.5 \pm 0.1)\ 10^{-3}$ s^{-1}.

Dependence on [IMI]

The decay constant, k_{obs1}, shows a complex concentration dependence. The experimental values can be fitted by the binomial function $k_{obs1} = k_{1(2)}[\mathrm{IMI}] + k_{1(3)}[\mathrm{IMI}]^2$, leading to the bimolecular constant $k_{1(2)} = (1.4 \pm 0.1)$ dm^3 mol^{-1} s^{-1} and the trimolecular constant $k_{1(3)} = (9.78 \pm 0.08)\ 10^2$ dm^6 mol^{-2} s^{-1} (Fig. 2). The rise constant k_{obs2} depends linearly on the [IMI], leading to a second-order rate constant $k_{2(2)} = (87 \pm 2)$ dm^3 mol^{-1} s^{-1} (Fig. 2). t_{max} as well as I_{max} depend on the [IMI], the I_{max} values show a linear dependence on the [IMI] (plot not shown), whereas the quantum yields (Φ_{CL}) decrease substantially with rising imidazole concentration (Table 1). The quantum yields obtained at low [IMI] are in excellent agreement with the values reported in the literature.[1,2]

It should be noted here that the lowest [IMI] used in these experiments is 0.2 mmol dm^{-3}. Under these conditions a 'normal' emission curve is observed, analogous to that shown in Fig. 1. In contrast, at [IMI] $\leqslant$ 0.1 mmol dm^{-3}, where imidazole is no longer in excess, complex kinetic curves are obtained, which, evidently, cannot be treated by simple first-order kinetics [Fig. 3(*a*)]. On the other hand, when only catalytic amounts of IMI are used, apparently simple emission curves are

Table 1 Effect of imidazole concentration on the fall rate constant (k_{obs1}), the rise rate constant (k_{obs2}), the relative maximum intensity (I_{max}), the time of maximum intensity (t_{max}) and the quantum yield (Φ_{CL}) in the peroxyoxalate chemiluminescence[a]

[IMI]/mmol dm^{-3}	$k_{obs1}/10^{-1}$ s^{-1} [b]	$k_{obs2}/10^{-1}$ s^{-1} [b]	I_{max}/a.u.	t_{max}/s	$\Phi_{CL}/10^{-2}$ E mol^{-1}
20	4.2 ± 0.4	*c*	*c*	*c*	*c*
15	2.4 ± 0.2	*c*	*c*	*c*	*c*
10	1.1 ± 0.1	*c*	*c*	*c*	*c*
9.0	0.91 ± 0.09	*c*	*c*	*c*	*c*
8.0	0.77 ± 0.03	7.2 ± 0.1	2.0	3	1.0
7.0	0.57 ± 0.01	6.6 ± 0.1	2.1	4	1.4
6.0	0.45 ± 0.01	5.4 ± 0.1	2.0	5	1.7
5.0	0.32 ± 0.01	5.1 ± 0.1	1.7	6	2.0
4.0	0.24 ± 0.02	3.7 ± 0.1	1.5	8	2.6
3.0	0.15 ± 0.01	3.1 ± 0.1	1.3	10	3.4
2.0	0.079 ± 0.004	2.3 ± 0.3	1.1	16	5.2
1.0	0.035 ± 0.004	1.3 ± 0.1	1.0	29	9.2
0.90	0.032 ± 0.004	1.2 ± 0.1	0.91	31	10
0.80	0.026 ± 0.003	1.1 ± 0.1	0.84	35	11
0.70	0.021 ± 0.003	1.2 ± 0.1	0.79	35	13
0.60	0.018 ± 0.003	1.1 ± 0.1	0.71	39	13
0.50	0.015 ± 0.003	0.94 ± 0.02	0.63	45	16
0.40	0.011 ± 0.003	0.78 ± 0.09	0.53	53	18
0.20	0.0062 ± 0.0001	0.40 ± 0.01	0.50	102	24

[a] [DPA] = 1.0 mmol dm^{-3}, [H_2O_2] = 10 mmol dm^{-3}, [TCPO] = 0.10 mmol dm^{-3}. [b] Error limits from at least three determinations. [c] No rise in the emission curve was observed.

Table 2 Effect of hydrogen peroxide concentration on the fall rate constant (k_{obs1}), the rise rate constant (k_{obs2}), the relative maximum intensity (I_{max}), the time of maximum intensity (t_{max}) and the quantum yield (Φ_{CL}) in the peroxyoxalate chemiluminescence[a]

[H_2O_2]/mmol dm^{-3}	$k_{obs1}/10^{-3}$ s^{-1} [b]	$k_{obs2}/10^{-1}$ s^{-1} [b]	I_{max}/a.u.	t_{max}/s	$\Phi_{CL}/10^{-2}$ E mol^{-1}
10	3.8 ± 0.3	1.5 ± 0.3	1.00	27	9.2
5.0	2.6 ± 0.1	0.94 ± 0.07	0.85	39	12
2.5	2.1 ± 0.1	0.48 ± 0.01	0.71	70	13
1.0	1.7 ± 0.1	0.26 ± 0.03	0.60	114	14
0.50	1.6 ± 0.1	0.14 ± 0.01	0.53	180	14
0.25	1.4 ± 0.2	0.087 ± 0.002	0.43	250	13
0.10	1.4 ± 0.1	0.045 ± 0.001	0.33	390	12

[a] [DPA] = 1.0 mmol dm^{-3}, [IMI] = 1.0 mmol dm^{-3}, [TCPO] = 0.10 mmol dm^{-3}. [b] Error limits from at least three determinations.

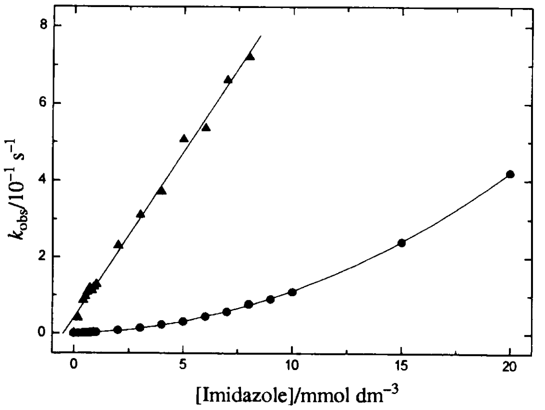


Fig. 2 Dependence of the rate constants k_{obs1} (●) and k_{obs2} (▲) on imidazole concentration, under standard conditions. The k_{obs1} values were fitted using the equation: $k_{obs1} = k_{1(2)}[IMI] + k_{1(3)}[IMI]^2$, where: $k_{1(2)} = (1.4 \pm 0.1)$ dm^3 mol^{-1} s^{-1} and $k_{1(3)} = (9.78 \pm 0.08)\ 10^2$ dm^6 mol^{-2} s^{-1}. The k_{obs2} values show a linear correlation ($r = 0.997$): $k_{2(2)} = (87 \pm 2)$ dm^3 mol^{-1} s^{-1}.

obtained. At concentrations between 10^{-7} and 10^{-5} mol dm^{-3}, the kinetics appear to be extremely slow, but reproducible, and exhibit considerable lag phases [Fig. 3(*b*)]. These curves cannot be treated using first-order kinetics, indicating the contribution of higher-order terms. Although the rate of the reaction is about two orders of magnitude lower, the quantum yields are comparable to those determined under standard conditions (data not shown). It should be emphasized that it was not possible to obtain reproducible emission curves in the complete absence of IMI, a fact also reported in the literature.[8] On the other hand, with catalytic amounts of the IMI, the results showed high reproducibility.

Dependence on [H_2O_2]

Both observed rate constants depend on the concentration of H_2O_2, although to a different extent (Table 2). k_{obs1} shows only a slight dependence, significant mainly at high concentrations, as indicated by the double logarithmic plot in Fig. 4. However, k_{obs2} depends linearly on the peroxide concentration, leading to a bimolecular rate constant $k_2 = 18.6 \pm 0.6$ dm^3 mol^{-1} s^{-1}, obtained from the slope of the straight line (Fig. 4). Both t_{max} and I_{max} depend on [H_2O_2], and Φ_{CL} values show a slight decrease at higher concentrations.

Reaction of H_2O_2 with the imidazolide

As indicated earlier, imidazole may react directly with TCPO, leading to the formation of an intermediate imidazolide. Preliminary experiments, where peroxide was added after the other reagents, indicated the formation of the imidazolide intermediate, which reacts rapidly with H_2O_2, leading to light emission in the presence of the activator (Fig. 5). Analysis of the fast decay provided observed rate constants of $(2.6 \pm 0.6) \times 10^{-1}$ s^{-1} and $(2.0 \pm 0.1) \times 10^{-1}$ s^{-1} from two independent series of experiments under standard conditions. These values are comparable to $k_{obs2} = 1.2 \times 10^{-1}$ s^{-1}, determined for the complete system, also under standard conditions. The maximum intensity of the fast-decay curve, following the addition of H_2O_2, is a measure of the imidazolide concentration, accumulated in the absence of peroxide. The time course of the intermediate concentration (I_{max} values *versus* delayed H_2O_2 addition time), obtained from two independent series of experiments, is shown in Fig. 5 inset.

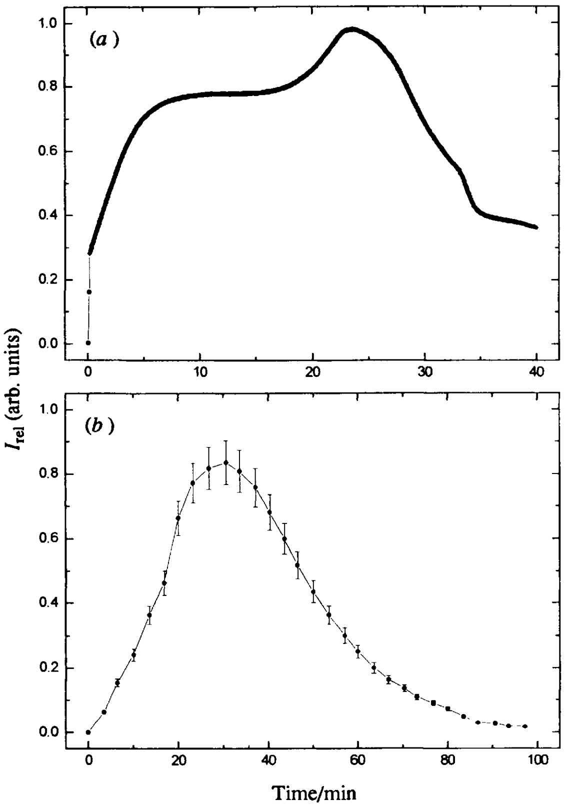


Fig. 3 Emission curves obtained using low imidazole concentration: (*a*) [IMI] = 0.1 mmol dm^{-3}, standard conditions; (*b*) [IMI] = 10^{-7}–10^{-5} mol dm^{-3}, standard conditions (identical kinetic curves were obtained to within experimental error)

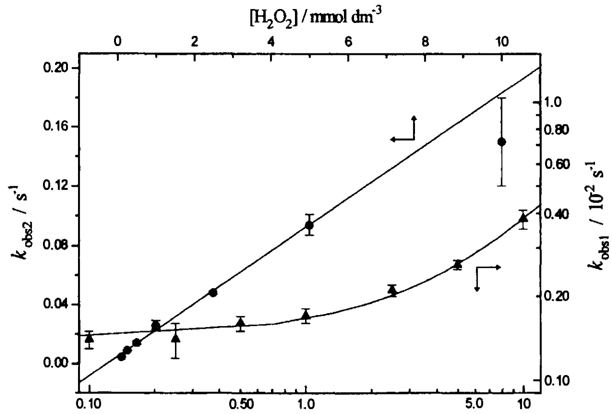


Fig. 4 Double logarithmic plot of k_{obs1} (▲) and linear plot of k_{obs2} (●) *versus* peroxide concentration, under standard conditions. The value at $[H_2O_2]$ = 10 mmol dm^{-3} was not used in the linear regression of the k_{obs2} values.

Dependence on [DPA]

The quantum yields increase with increasing activator concentrations; a double reciprocal plot of the quantum yields *versus* [DPA] provides a linear correlation (data not shown). On the other hand, both observed rate constants (k_{obs1} and k_{obs2}) proved to be independent of the activator concentration.

The metastable intermediate

Finally, we performed some experiments with the aim of confirming the formation of a metastable reactive intermediate in the peroxyoxalate reaction. An extremely fast decaying flash of light was observed in initial studies upon delayed addition of an activator to the reaction system, under standard conditions. The emission intensity depended on the delay time and showed an increase with the pre-incubation time up to 5 min and a decrease at higher pre-incubation times. In several further assays we were unable to reproduce these results, under identical conditions. Delayed addition of the activator only led to the 'normal' emission curve, with no initial flash of light. These results, together with the conflicting results reported in the literature,[1,8,14] clearly show the highly unstable nature of the reactive intermediate.

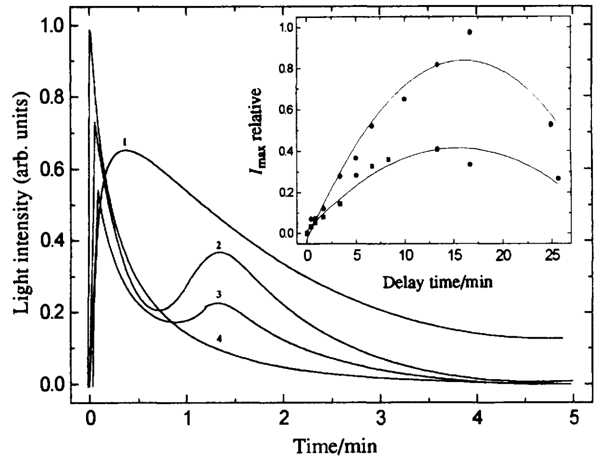


Fig. 5 Qualitative emission time course upon delayed hydrogen peroxide addition. Standard conditions; delay time: 1, 0 s; 2, 10 s; 3, 60 s; 4, 600 s. Inset: relative maximum flash intensities obtained on hydrogen peroxide addition after different delay times, for two independent experiments.

Discussion

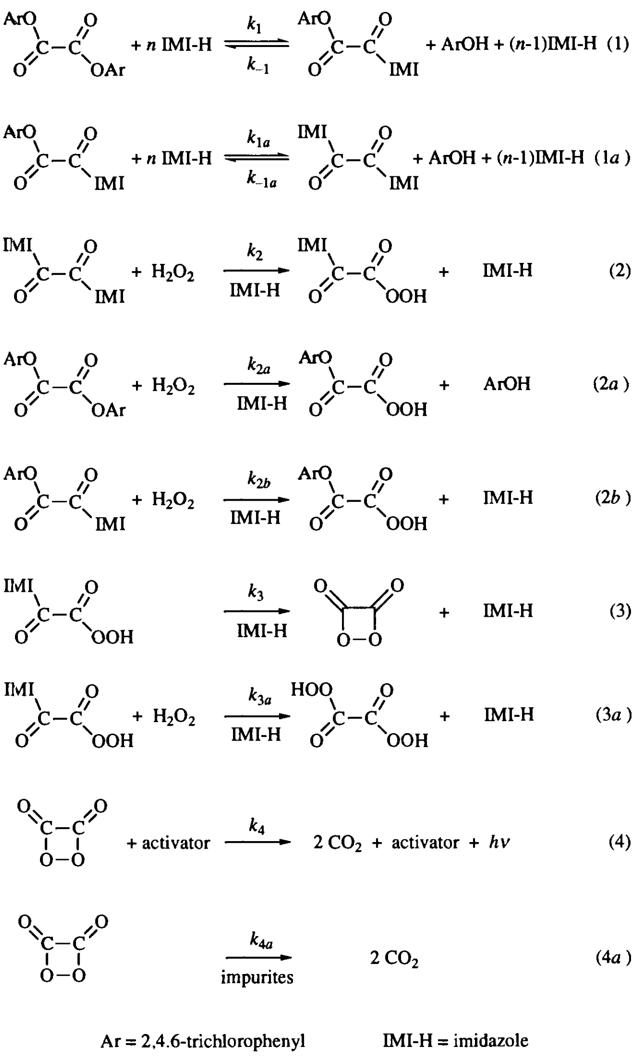
A plausible reaction sequence, divided into four principal steps (1)–(4) and which contains some of various possible side steps—although many more are possible—is shown in Scheme 1.

The initial step (1)

This step consists of the replacement of the TCP moiety by IMI [(1) or (1) and (1*a*)], as evidenced by the similarity exhibited by the rate constants obtained from the direct reaction of TCPO with IMI and from the release of TCP in the complete system, as well as the decay rate constant (k_{obs1}) calculated from the emission kinetics at low $[H_2O_2]$. The higher value obtained for the latter constant at higher $[H_2O_2]$ may be due to a minor contribution of the reverse reaction in steps (1) and (1*a*). The assignment of k_{obs1} to the first step is in agreement with it being fairly independent of the $[H_2O_2]$ (Fig. 4). The third-order rate constant $k_{1(3)}$ obtained from the [IMI] dependence of k_{obs1} (Fig. 2) represents the general-base catalysis by IMI of the nucleophilic IMI attack on TCPO.[15,16] The release of the two phenolic units and the observation of only one rate constant in the reaction of IMI with TCPO, in the absence of H_2O_2, indicates that step (1*a*) is much faster than step (1). Literature results on the hydrolysis of oxalate esters, where the substitution of the first ester unit was found to be faster than the second,[11,17] appear to be in contrast with our observation, but the high reactivity of imidazolides[15] may also indicate activation of the adjacent ester function, leading to an accelerated second substitution. Therefore, both phenolic units are initially replaced, leading to 1,1′-oxalyldiimidazole, and both k_{obs1} and the TCP release rate constant correspond to step (1).

The second step (2)

The most important consecutive step should be reaction (2) leading to an IMI-substituted peracid. The rise constant (k_{obs2}) corresponds to this step as indicated by its linear dependence on $[H_2O_2]$, and it can be observed directly by delayed H_2O_2 addition (Fig. 5), yielding a rate constant similar to k_{obs2} of the



Scheme 1 Reaction sequence for the peroxyoxalate chemiluminescence

complete system (Table 2). The dependence of k_{obs2} on [IMI] indicates IMI catalysis of step (2). Using the expression $k_{obs2} = k_{2(3)}$ [H_2O_2][IMI], trimolecular rate constants $k_{2(3)} = 1.86$ 10^4 dm^6 mol^{-2} s^{-1} from the [H_2O_2] dependence and $k_{2(3)'} = 8.7 \times 10^3$ dm^6 mol^{-2} s^{-1} from the [IMI] dependence can be obtained, which are in reasonable agreement with each other. Steps (*2a*) and (*2b*) should be of minor importance, at least at moderate to high [IMI], as might be inferred from the extremely slow reaction with catalytic amounts of IMI [Fig. 3(*b*)]. Extremely complex kinetics† are observed for [IMI] similar to [TCPO] [Fig. 3(*a*)]. Therefore, in our experimental conditions (with an excess of IMI and H_2O_2), the main chemiluminescence pathway proceeds through step (2).

The third step (3)

The IMI-catalysed cyclization of the peracid intermediate leads to a cyclic peroxide which we formulate as 1,2-dioxetanedione,[1] although other intermediates have been postulated.[8-13] This step cannot be observed kinetically under normal conditions. However, the deviation in the [H_2O_2] dependence of k_{obs2} from linearity at the highest [H_2O_2] studies (on using still higher [H_2O_2], we were not able to obtain reliable k_{obs2} values) indicates a change in the rate-limiting step and reaction (3) may be observed kinetically. Therefore, a rough estimate for the unimolecular rate constant of this step is $k_3 \sim 0.20$ s^{-1} at [IMI] = 1.0 mmol dm^{-3}. However, at high [H_2O_2] reaction (*3a*) should also contribute, as indicated by the decrease in the quantum yields in these conditions (Table 2), leading to an overestimation of the k_3 value.

The excitation step (4)

The interaction of the reactive intermediate, presumably involving the CIEEL sequence and leading ultimately to light emission, is certainly too fast to be observed kinetically. This fact is supported by the non-sensitivity of k_{obs} values to the activator concentration, in agreement with former reports.[8,10,11] The importance of the non-chemiluminescent decomposition of the intermediate [step (*4a*)], can be inferred from the dependence of the quantum yields on the activator concentration.

The structure of the reactive intermediate is still an open question. In our work, like in former kinetic studies,[8-11] it was not possible to obtain any direct evidence on the reactive intermediate's structure. Although the results reported by Chokshi *et al.*[12] indicate the interaction of an intermediate such as **4** with an activator, the peracid-type intermediates **3** and **4** should not lead to efficient excited-state generation, as it is known that peresters—which may be regarded as models for **3** and **4**—show extremely low quantum yields upon decomposition by the CIEEL mechanism.[18] The dioxetane structure **5** may be excluded, as it is well known[19] that dioxetanes do not undergo intermolecular CIEEL reactions and do not lead to efficient production of excited singlet states. On the other hand, 1,2-dioxetanones—which may serve as models for **2**—are well known to undergo the CIEEL mechanism,[6,19] although there might be doubts with respect to the quantum yields‡ obtained from this process. Additionally, tetrahedral intermediates are known to be extremely short-lived in interconversion reactions of carboxylic acid derivatives, especially in cases where excellent leaving groups are present, and, moreover, when the bifunctional catalyst imidazole is present.[20] Therefore, the 'tetrahedral' intermediates **2** and **5** should be too short-lived to interact with the activator. For these reasons and on the basis of our previous experience in the chemistry of highly unstable four-membered ring peroxides,[21] it appears to us that 1,2-dioxetanedione is still the most likely candidate for the reactive intermediate.

Conclusions

Scheme 2 presents a simplified kinetic sequence for the chemiluminescent pathway of the peroxyoxalate reaction with imidazole as nucleophile and base catalyst. This agrees with our experimental results§ obtained in an anhydrous solvent,

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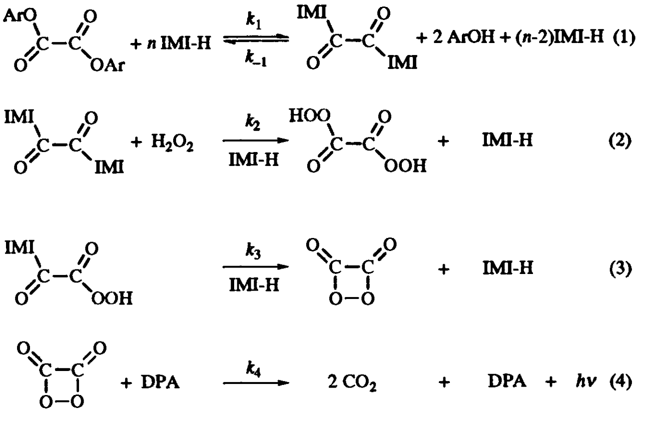
† These curves appear to indicate some kind of 'oscillatory behaviour' of the system under these conditions, analogous to that observed by Alvarez *et al.*,[9] but we point out that the shape of the curves, and the conditions under which this behaviour is observed, are completely different from those previously reported. The behaviour in our case might be explained by a complex competition between the steps (*1a*), (*2a*) and (*2b*) at non-excess [IMI], without it being necessary to postulate the involvement of various reactive intermediates.[9]

‡ The quantum yield of the rubrene-catalysed dimethyl-1,2-dioxetanone decomposition was determined by Schmidt and Schuster[6] to be $\Phi = 0.10$ E mol^{-1}, a yield comparable to that of the peroxyoxalate reaction. However, the same research group determined the quantum yield of diphenoyl peroxide decomposition, with perylene as the activator, to be $\Phi = 0.10$ E mol^{-1}. This quantum yield was shown recently to be three orders of magnitude lower (see the Introduction).[7] Due to this fact, doubts may also arise with respect to the quantum yield of the 1,2-dioxetanone. There is no report in the literature of an independent redetermination of this quantum yield.

§ The decrease in the quantum yields with increasing [IMI] (Table 1), indicates the occurrence of non-emissive side reactions at high [IMI]. Preliminary experiments show that, at high [DPA], the effect of IMI on the quantum yields is much less pronounced than at low [DPA], indicating a competition between DPA and IMI in the interaction with the reactive intermediate.



Ar = 2,4.6-trichlorophenyl IMI-H = imidazole

$k_{1(2)} = 1.4 \pm 0.1$ dm^3 mol^{-1} s^{-1} $k_{2(3)} = (1.86 \pm 0.06)\ 10^4$ dm^6 mol^{-2} s^{-1} $k_3 - 0.20$ s^{-1}
$k_{1(3)} = 978 \pm 8$ dm^6 mol^{-2} s^{-1} $k_{2(3)}' = (8.7 \pm 0.2)\ 10^3$ dm^6 mol^{-2} s^{-1}

Scheme 2 Simplified kinetic scheme for the main pathway of the peroxyoxalate chemiluminescence

without the need to postulate[9] various reactive intermediates. Side reactions (see Scheme 1) are omitted for simplicity.

The decay constant k_{obs1} corresponds to the replacement of TCPO by IMI [step (1)]; a second ($k_{1(2)}$) and a third-order rate constant ($k_{1(3)}$) are obtained from the [IMI] dependence. As the second substitution should be much faster than the first, the formation of 1,1′-oxalyldiimidazole is shown in one step. The rise constant k_{obs2} corresponds to the IMI-catalysed interaction of H_2O_2 with 1,1′-oxalyldiimidazole; third-order rate constants ($k_{2(3)}$ and $k_{2(3)'}$) are obtained for this step from the [IMI] and [H_2O_2] dependence. Our assignment of the observed rate constants to the reaction steps is different from that made in the literature,[10] however, we have obtained much more direct experimental evidence in favour of our assignment. The third step consists of the cyclization of the peracid to the reactive intermediate 1,2-dioxetanedione (see above); a rough estimate can be obtained for the rate constant (k_3) of this step; a unimolecular rate constant is given, although the reaction should be IMI-catalysed. The cyclization may occur in a concerted process, where imidazole acts as a general base and a general acid catalyst.[20] The general-base catalysed intramolecular peracid attack is accompanied by the general-acid catalysed elimination of the leaving group (imidazole in our system). In this way the formation of any partial negative charge at the peroxidic ring, which should lead to immediate cleavage,[¶] is avoided. Finally, the excitation step is certainly too fast to be observed kinetically and is thought to involve the CIEEL mechanism. For the reasons outlined above, we still favour 1,2-dioxetanedione as the most likely reactive intermediate in the chemiluminescence of peroxyoxalate.

Experimental

Reagents

Bis(2,4,6-trichlorophenyl)oxalate (TCPO) was synthesized following the literature procedure[24] and recrystallized from chloroform–hexane (14:5), producing white needles (53%), mp 191 °C (lit.,[24] 190–192 °C). Ethyl acetate was dried for 3 days over $CaCl_2$, stirred for 30 min over NaOH (pellets; 40 g dm^{-3}) at 0 °C, distilled after filtration, kept for 1 day over 4 Å molecular sieves, carefully distilled over a 30 cm Vigreux column under a nitrogen atmosphere (bp 77 °C) and stored over 4 Å molecular sieves under nitrogen. Imidazole (IMI) and 9,10-diphenylanthracene (DPA) were obtained from Aldrich and were used without further purification. A stock solution of anhydrous hydrogen peroxide in ethyl acetate was prepared by adding 200 μl of an aqueous solution of H_2O_2 (60%) to 5 cm^3 of the solvent. The solution was dried with successive portions of $MgSO_4$ and finally kept for 1 day at 0 °C over $MgSO_4$. The peroxide concentration was determined by an iodimetric assay, using horseradish peroxidase as catalyst, and photometric determination of the [I_3^-].[25]

Apparatus

The chemiluminescence kinetics were followed on a Perkin-Elmer LS-5 luminescence fluorimeter (emission monochromator: 450 nm, slit 2.5 mm; sensitivity: C60) and on a Spex Fluorolog 1681 spectrometer (emission monochromator: 450 nm; slit: 1.0 mm; photomultiplier voltage: 750 kV). The emission quantum yields were determined using the luminol standard;[26] in this case the monochromator was set on mirror position. The kinetics of the release of 2,4,6-trichlorophenol (TCP) were followed on a Hitachi U-2000 spectrophotometer.

Data analysis

The kinetic curves were analysed using the program DISCRETE developed by Provencher,[27] which adjusts the sums of exponential functions to the values according to eqn. (1); a weighting function is contained to indicate the best fit.

$$f(t) = \sum_{i=0}^{n} A_i \exp(-k_i t) \quad (1 \leq n \leq 9) \qquad (1)$$

The emission curves render in all cases best fits for $n = 2$, whereas the absorption curves always afford mono-exponential best fits ($n = 1$). The two k values obtained from the emission curves contained pre-exponential factors (A_i) of opposite sign but comparable magnitude. The curves were also analysed by non-linear least-squares analysis using the eqns. (2) and (3)

$$f(t) = A_1 \exp(-k_1 t) - A_2 \exp(-k_2 t), \text{ for emission} \qquad (2)$$

$$f(t) = A_1[1 - \exp(-k_1 t)], \text{ for absorption} \qquad (3)$$

Kinetics

The experiments were carried out in quartz fluorescence or absorption cuvettes (10 × 10 mm; total volume 3.0 cm^3) at 25.0 ± 0.2 °C. The reactions in standard conditions were initiated by the addition of 1.5 cm^3 of solution A (2.0 mmol dm^{-3} imidazole, 20 mmol dm^{-3} H_2O_2 in ethyl acetate, pre-thermostatted to 25 °C) to 1.5 cm^3 of solution B (0.20 mmol dm^{-3} TCPO, 2.0 mmol dm^{-3} DPA in ethyl acetate) pre-thermostatted in the cuvette, followed by rapid mixing and the measurement of the emission intensity. The absorption experiments were performed in the same way in the absence of the activator and, in some experiments, in the absence of H_2O_2 [TCP: λ_{max}(ethyl acetate)/nm 295 (ε/dm^3 mol^{-1} cm^{-1} 2870) and 288 (2920); TCPO: λ_{max}(ethyl acetate)/nm 283 (ε/dm^3 mol^{-1} cm^{-1} 675) and 273 (900)]. In the experiments with delayed H_2O_2 addition, the other reagents were pre-incubated and 35 μl of a 0.85 mol dm^{-3} solution of H_2O_2 in ethyl acetate were added after the delay times indicated. In the same way, the activator DPA (0.3 cm^3 of a 10 mmol dm^{-3} solution) was delay-added after pre-incubation of the other reagents. The solutions were added by means of calibrated volumetric glass pipettes and Hamilton micro syringes for small volumes. The values reported are means of at least three determinations.

[¶] The CIEEL sequence is initiated by an electron transfer to the peroxide in the rate-limiting step, followed by an extremely fast cleavage of the peroxide radical anion.[4] Moreover, 1,2-dioxetanes show intramolecular CIEEL decomposition, if a negative charge is generated near to the peroxide ring.[22] Furthermore, the cyclization of a protected α-peroxy aldehyde under basic conditions leads to the immediate formation of cleavage products without any detectable intermediate.[23]

Standard conditions

In emission experiments [TCPO] = 0.10 mmol dm^{-3}, [DPA] = 1.0 mmol dm^{-3}, [IMI] = 1.0 mmol dm^{-3}, [H_2O_2] = 10 mmol dm^{-3}; in absorption experiments: [TCPO] = 0.10 mmol dm^{-3}, [IMI] = 1.0 mmol dm^{-3}, [H_2O_2] = 10 mmol dm^{-3}, and the reaction of IMI with TCPO: [TCPO] = 0.10 mmol dm^{-3}, [IMI] = 1.0 mmol dm^{-3}.

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