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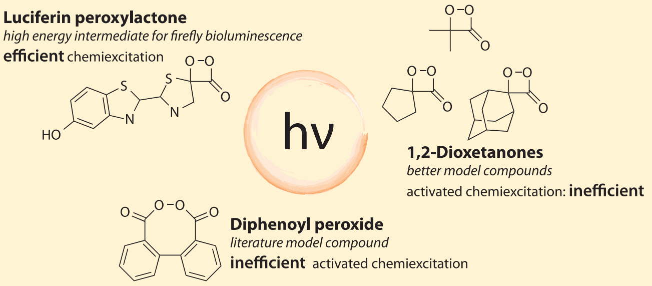
Revision of Singlet Quantum Yields in the Catalyzed Decomposition of Cyclic Peroxides[†]

Marcelo Almeida de Oliveira, Fernando Heering Bartoloni,[‡] Felipe Alberto Augusto,
Luiz Francisco Monteiro Leite Ciscato,[‡] Erick Leite Bastos, and Wilhelm Josef Baader*

Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

Supporting Information

ABSTRACT: The chemiluminescence of cyclic peroxides activated by oxidizable fluorescent dyes is an example of chemically initiated electron exchange luminescence (CIEEL), which has been used also to explain the efficient bioluminescence of fireflies. Diphenoyl peroxide and dimethyl-1,2-dioxetanone were used as model compounds for the development of this CIEEL mechanism. However, the chemiexcitation efficiency of diphenoyl peroxide was found to be much lower than originally described. In this work, we redetermine the chemiexcitation quantum efficiency of dimethyl-1,2-dioxetanone, a more adequate model for firefly bioluminescence, and found a singlet quantum yield (Φ_S) of 0.1%, a value at least 2 orders of magnitude lower than previously reported. Furthermore, we synthesized two other 1,2-dioxetanone derivatives and confirm the low chemiexcitation efficiency ($\Phi_S < 0.1\%$) of the intermolecular CIEEL-activated decomposition of this class of cyclic peroxides. These results are compared with other chemiluminescent reactions, supporting the general trend that intermolecular CIEEL systems are much less efficient in generating singlet excited states than analogous intramolecular processes ($\Phi_S \approx 50\%$), with the notable exception of the peroxyoxalate reaction ($\Phi_S \approx 60\%$).



1. INTRODUCTION

Chemiluminescence is the emission of light resulting from a chemical reaction.[1] The chemiluminescence quantum efficiency (Φ_{CL}, in einstein mol^{-1}) is defined as the ratio of the number of photons emitted by a reaction to the number of molecules of the limiting reactant consumed. The Φ_{CL} can also be described as the product of the quantum yields of two independent processes: (i) the formation of electronic excited-state products from ground-state reactants, i.e., chemiexcitation, and (ii) the radiative decay of the excited product.

Efficient chemiexcitation depends on strict energetic and geometric requirements, which have been extensively revised elsewhere.[1] The "energy sufficiency criterion" is fulfilled by the decomposition of chemical species with high energy content, referred to as high-energy intermediates (HEI). 1,2-Dioxetanes are isolable HEI whose thermal decomposition may lead to carbonyl products in both singlet and triplet excited states, depending on the substitution pattern.[1−4] Although the chemiluminescence efficiency of this unimolecular decomposition is usually low ($\Phi_{CL} < 0.01\%$) due to the high triplet/singlet state ratio, it might be enhanced by adequate fluorescent energy acceptors.[1] In the latter case, the chemiluminescence is *indirect* because it corresponds to the fluorescence of the energy acceptor, which was excited by singlet−singlet or triplet−singlet electronic energy transfer. In this process, the energy acceptor does not affect the decomposition rate constant of the cyclic peroxide.[1,3]

However, *oxidizable* fluorescent dyes, known as activators (ACT),[5] react with 1,2-dioxetanones (α-peroxylactones), resulting in higher Φ_{CL} when compared to their unimolecular thermal decomposition. The rate constant of this bimolecular *activated* chemiluminescence (k_{CAT}) changes with the concentration of ACT and depends on its oxidation potential.[1,6,7] On the basis of this experimental evidence, Schuster proposed that the reaction between the ACT and these peroxides involves an electron transfer from the ACT to the peroxide and called this mechanism chemically initiated electron exchange luminescence (CIEEL).[6] The CIEEL mechanism was originally proposed to explain the high chemiexcitation efficiency (Φ_S = 0.1 einstein mol^{-1})[8,9] of the chemiluminescent decomposition of both diphenoyl peroxide (**1**) and 3,3-dimethyl-1,2-dioxetanone (**2**), activated by perylene.[6,9−11] It also provided a rationale for the bioluminescent oxidation of the firefly luciferin (Φ_{BL} = 0.41 einstein mol^{-1})[12] by proposing a 1,2-dioxetanone as the HEI.[13] Furthermore, the study of CIEEL-active peroxides led to the development of protected electron-rich 1,2-dioxetanes, whose chemiluminescent decomposition is highly efficient (Φ_S up to 1.0 einstein mol^{-1})[14−26] and can be induced by adequate deprotection reagents, e.g., enzymes.[27]

However, the chemiexcitation efficiency of activated diphenoyl peroxide decomposition, one of the model systems

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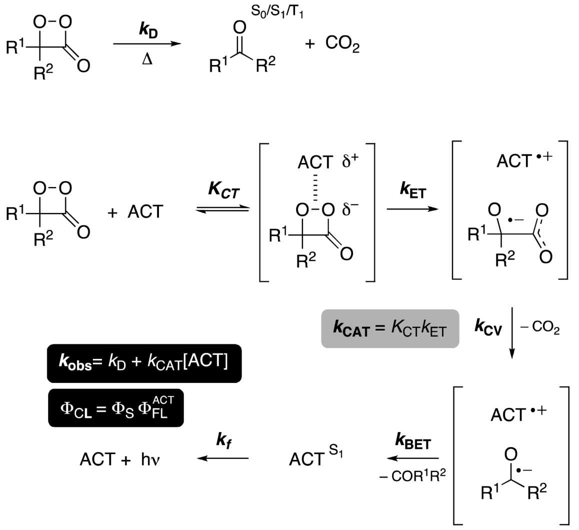
used by Schuster to propose the CIEEL mechanism, was further investigated by Catalani and Wilson, and it was found to be much lower than initially determined, i.e., $\Phi_S = 2 \times 10^{-5}$ einstein mol^{-1} [28] instead of 0.1 einstein mol^{-1}.[8,9] This result questioned the validity of the CIEEL mechanism in producing electronically excited states with high efficiency.[7,20] However, although 1,2-dioxetanones are much better models for the HEI occurring in firefly bioluminescence than diphenoyl peroxide, the chemiexcitation quantum yield for the activated decomposition of α-peroxylactones ($\Phi_S = 0.1$ einstein mol^{-1})[8,29] has never been redetermined, probably due to difficulties in the synthesis of this class of highly unstable cyclic peroxides.

In this work, we describe studies on the activated decomposition of three 1,2-dioxetanones, including the 3,3-dimethyl-1,2-dioxetanone used by Schuster[8] and a novel spirocyclopentyl derivative, determine their chemiexcitation quantum efficiencies, and compare them with that of diphenoyl peroxide and available literature data on the chemiexcitation quantum yields of representative chemiluminescence and bioluminescence systems.

Background on the CIEEL Mechanism. The unimolecular decomposition of cyclic peroxides (e.g., 1,2-dioxetanones) can produce excited carbonyls with a rate constant k_D (Scheme 1). However, a charge transfer complex between the peroxide and the ACT can be formed, with an equilibrium constant (K_{CT}) depending on the redox properties of these reagents.[1,3,6] The endothermic electron transfer (k_{ET}) from the ACT to the antibonding σ orbital of the peroxide bond (facilitated by O−O-bond elongation) is essentially irreversible due to concerted O−O bond cleavage.[1] Subsequent cleavage of the C−C bond, with a rate constant k_{CV}, leaves the new radical ion pair (carbonyl radical anion and the ACT radical cation) still within the solvent cage. A back-electron-transfer (i.e., annihilation, k_{BET}) from the $CO^{\bullet-}$ to the antibonding orbital of the $ACT^{\bullet+}$ leads to the formation of the ACT in the electronically excited singlet state, which decays to the ground state, giving rise to fluorescence emission (Scheme 1, k_f).

Scheme 1. CIEEL Mechanism for the Activated and Unimolecular Decomposition of 1,2-Dioxetanones



The observed rate constant for the chemiluminescence decay (k_{obs}) can be obtained by nonlinear fitting of the emission intensity decay curves over time and is defined by eq 1. Consequently, the rate constants k_{CAT} and k_D correspond to the angular and linear coefficients of the k_{obs} vs [ACT] plot, respectively (eq 1)

$$k_{obs} = k_D + k_{CAT}[ACT] \qquad (1)$$

where

$$k_{CAT} = K_{CT}k_{ET} \qquad (2)$$

The chemiluminescence quantum yield (Φ_{CL}) is obtained from the division of the area under the emission intensity curve (AUC, in einstein) by the number of peroxide molecules consumed.[1] The efficiency of the excitation of the ACT to its first singlet electronic excited state (singlet chemiexcitation quantum yield, Φ_S) can be calculated by dividing the Φ_{CL} by the fluorescence quantum yield of the ACT (Φ_{FL}^{ACT}). To obtain the chemiexcitation quantum yield for the activated decomposition (Φ_S^{CAT}), the calculated Φ_S has to be divided by the fractioning factor (χ_{CAT}), which indicates the fraction of peroxide that is decomposed by the activated pathway, in the specific experimental conditions utilized (eqs 3 and 4).

$$\Phi_S^{CAT} = \frac{\Phi_S}{\chi_{CAT}} \qquad (3)$$

$$\chi_{CAT} = \frac{k_{CAT}[ACT]}{k_D + k_{CAT}[ACT]} \qquad (4)$$

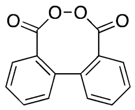
However, for an experimental situation, where the unimolecular decomposition is predominant ($k_D \gg k_{CAT}[ACT]$) and the bimolecular rate constant cannot be determined graphically from the ACT concentration dependence of k_{obs}, the chemiexcitation quantum yield at an *infinite* concentration of the ACT (Φ_S^{∞}) can be calculated from the double-reciprocal plots of Φ_S and the ACT concentration (eq 5).[30,31] The parameters Φ_S^{CAT} and Φ_S^{∞} are analogous and comparable, as neither depend on the ACT concentration, and in both cases, the yields are calculated in a hypothetical condition where all peroxide is decomposed by the activated pathway.

$$\frac{1}{\Phi_S} = \frac{1}{\Phi_S^{\infty}} + \left(\frac{k_D}{k_{CAT}\Phi_S^{\infty}}\right)\frac{1}{[ACT]} \qquad (5)$$

2. RESULTS

The following cyclic peroxides were prepared according to adapted literature procedures: diphenoyl peroxide (**1**), 3,3-dimethyl-1,2-dioxetanone (**2**), spiro-adamantyl-1,2-dioxetanone (**3**) and spiro-cyclopentyl-1,2-dioxetanone (**4**).[32−39] All cyclic peroxides, including the hitherto unknown α-peroxylactone derivative **4**, were characterized by low-temperature ^1H and ^{13}C NMR spectroscopy. In the case of the known compounds **1**−**3**, these were fully characterized for the first time only recently.[39−43] Due to the difficulty of preparing such unstable compounds, some critical details on their storage, purification, and characterization are given elsewhere.[39]

The emission kinetics for the decomposition of **1**−**4** catalyzed by rubrene (RUB, E^{ox} = 0.61 V vs SCE)[30] or perylene (PER, E^{ox} = 0.88 V vs SCE)[30] in aprotic solvents were acquired. Monoexponential fitting of the emission intensity versus time profiles allowed the calculation of the initial emission intensity (I_0) and observed rate constants (k_{obs}) (Supporting Information, Figures S1−S3). The emission



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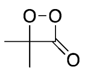

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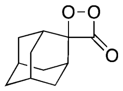

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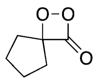

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intensity curves were extrapolated to near-zero intensity, integrated, and transformed in the chemiluminescence quantum yields by emission intensity calibration using the luminol standard (Supporting Information, Table S1−S6).[30]

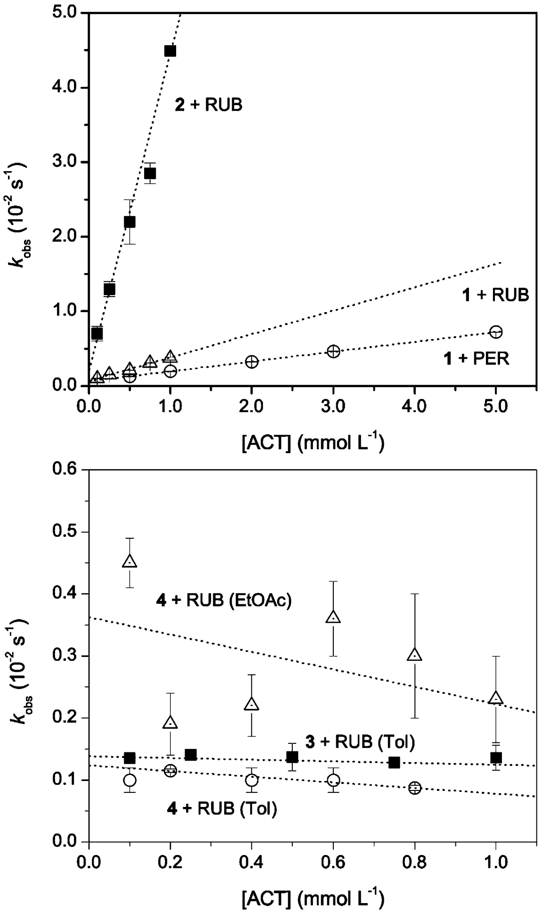


Figure 1. Dependence of the observed rate constant (k_{obs}) with the activator concentration ([ACT]) for the catalyzed decomposition of **1** (1.0 mmol L^{-1}) by PER (CH_2Cl_2 at 32.5 °C) or RUB (toluene at 25 °C), **2** (0.11 mmol L^{-1}) by RUB (CH_2Cl_2 at 25 °C), **3** (0.25 mmol L^{-1}) in the presence of RUB (toluene at 35 °C), and spirocyclopentyl-1,2-dioxetanone **4** (3.0 μmol L^{-1}) in the presence of RUB (in toluene or EtOAc at 25 °C).

The k_{obs} values change linearly with the [ACT] for compounds **1** and **2**, but they do not depend on the [RUB] for peroxides **3** and **4** (Figure 1). The dependence of k_{obs} on the [ACT] is one piece of evidence for the occurrence of the CIEEL mechanism and allows the graphical determination of the unimolecular (k_D) and catalyzed (k_{CAT}) peroxide decomposition rate constant (Table 1).[6,8,44−49] Therefore, the occurrence of CIEEL in the decomposition of peroxides **3** and **4** must be carefully investigated as the mean values of k_{obs} are similar in the presence or absence of ACT [**3**, $k_D = (1.4 \pm 0.6) \times 10^{-3}$ s^{-1} in toluene at 35 °C; **4**, $k_D = (1.3 \pm 0.3) \times 10^{-3}$ s^{-1} in toluene at 25 °C and $k_D = (6 \pm 1) \times 10^{-3}$ s^{-1} in ethyl acetate at 25 °C; **3** + **RUB**, $k_{obs} = (1.35 \pm 0.05) \times 10^{-3}$ s^{-1} in toluene at 35 °C; **4** + **RUB**, $k_{obs} = (9 \pm 1) \times 10^{-4}$ s^{-1} in toluene at 25 °C and $k_{obs} = (3 \pm 1) \times 10^{-3}$ s^{-1} in ethyl acetate at 25 °C].

Although the [ACT] has no effect on k_{obs} in the decomposition of **3** and **4**, the values of I_0 and Φ_{CL} increase significantly in the presence of the fluorescent dye; i.e., the [ACT] affects the emission intensity and the total amount of light emitted (Supporting Information, Tables S5 and S6). To rule out the occurrence of *indirect* chemiluminescence (resulting from electronic energy transfer from the excited carbonyl compound to the fluorescent dye) instead of CIEEL,[1,3] we compared the amount of light emitted by **3** and **4** in the presence of different fluorescent dyes having similar fluorescence quantum yields (Φ_{FL}) but different oxidation potentials.[3]

The decomposition of **3** and **4** in the presence of 1.0 mmol L^{-1} RUB (E^{ox} = 0.61 V vs SCE, Φ_{FL} = 0.98)[30] or 9,10-diphenylanthracene (DPA, E^{ox} = 1.06 V vs SCE, Φ_{FL} = 0.95)[30] were compared directly. Emission intensities in the decomposition of **3** in toluene are $I_0 = 4.4 \times 10^{-13}$ E s^{-1} for RUB and $I_0 = 3.8 \times 10^{-14}$ E s^{-1} for DPA, whereas the emission intensities in the decomposition of **4** under similar conditions ([ACT] = 0.6 mmol L^{-1}) proved to be $I_0 = 8.0 \times 10^{-16}$ E s^{-1} for RUB and $I_0 = 9.7 \times 10^{-17}$ E s^{-1} for DPA. In both cases, the emission intensity with RUB is 1 order of magnitude higher than with DPA, indicating the involvement of an electron transfer in chemiexcitation. If ACT excitation would have occurred by electronic energy transfer, the emission intensities should be similar for both ACTs, due to their comparable Φ_{FL}.

Therefore, these results clearly indicate that the decomposition of peroxides **1**−**4** in the presence of ACTs involves an electron transfer step according to the CIEEL mechanism, although for compounds **3** and **4**, $k_D \gg k_{CAT}$[ACT]. As a consequence, the values of the bimolecular rate constant k_{CAT} can be graphically determined only for **1** and **2**, but not for **3** and **4**. This fact limits the determination of the chemiexcitation quantum yield of the activated path (Φ_S^{CAT}) of **3** and **4** using eq 3.

To circumvent this restriction, the quantum yields of singlet excited state formation at an infinite activator concentration (Φ_S^{∞}) were determined using eq 5. This parameter represents the maximum emission efficiency of a hypothetical experimental system in which every molecule of the peroxide will react instantaneously with the ACT. Consequently, Φ_S^{∞} is analogous to Φ_S^{CAT} because it is independent of the [ACT]. Double-reciprocal plots of the singlet quantum yields (Φ_S) and the ACT concentration for the chemiluminescent decomposition of peroxides **3** and **4** catalyzed by RUB are depicted in Figure 2 (Supporting Information, Tables S4−S6). The values for Φ_S^{∞} can be obtained from the intercept of these linear correlations,[50] and these values are shown together with the quantum yield values obtained for **1** and **2** (Table 1).

The quantum yield values for **1** and **2** were determined in the same experimental conditions used before by other research groups; however, for both compounds the yields obtained are considerably lower than originally measured. Furthermore, for dimethyl-1,2-dioxetanone (**2**), the catalytic pathway is favored in the presence of the ACT (χ_{CAT} = 96.6% at 1 mmol L^{-1}, Supporting Information, Table S1), a result that conflicts with that reported previously.[8]

Table 1. Singlet Quantum Yields for the Catalytic Pathway (Φ_S^{CAT}) in the Activated Decomposition of **1** and **2** and Infinite Singlet Quantum Yields (Φ_S^{∞}) for the Decomposition of **3** and **4**

peroxide	ACT	k_D (10^{-3} s^{-1})	k_{CAT} (L mol^{-1} s^{-1})	Φ_S^{CAT} (einstein mol^{-1})	Φ_S^{∞} (einstein mol^{-1})
1[a]	PER	0.65 ± 0.02	1.32 ± 0.01	$(3.3 \pm 0.2) \times 10^{-5}$	/
1[b]	RUB	0.69 ± 0.02	3.15 ± 0.05	$(8.1 \pm 0.5) \times 10^{-4}$	/
2[c]	RUB	2 ± 1	43 ± 1	$(1.0 \pm 0.2) \times 10^{-3}$	/
3[d]	RUB	/	/	/	$(1.5 \pm 0.9) \times 10^{-3}$
4[e]	RUB	/	/	/	$(3.9 \pm 0.6) \times 10^{-4}$
4[f]	RUB	/	/	/	$(1.3 \pm 0.5) \times 10^{-4}$

RUB = rubrene; PER = perylene.

[a] [**1**] = 1.0 mmol L^{-1}, in CH_2Cl_2 at 32.5 °C; [b] [**1**] = 1.0 mmol L^{-1}, in toluene at 25 °C; [c] [**2**] = 0.11 mmol L^{-1}, in CH_2Cl_2 at 25 °C; [d] [**3**] = 0.25 mmol L^{-1}, in toluene at 35 °C; [e] [**4**] = 3.0 μmol L^{-1}, in toluene at 25 °C; [f] [**4**] = 3.0 μmol L^{-1}, in ethyl acetate at 25 °C.

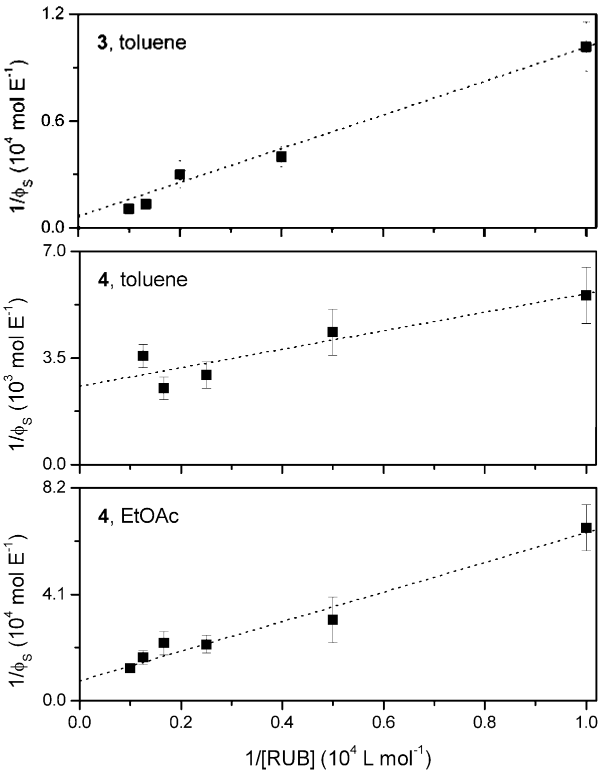


Figure 2. Double-reciprocal plots of the singlet quantum yields (Φ_S) and the activator concentration for the RUB-catalyzed decomposition of **3** and **4** (Φ_S data from Supporting Information, Tables S4–S6).

The quantum yields for the RUB-catalyzed decomposition of the 1,2-dioxetanone derivatives **3** and **4** are reported for the first time here and confirm the low values measured for peroxides **1** and **2** (Table 1). Although singlet quantum yields were obtained by two different methodologies, results can be compared because they were determined in conditions where each peroxide molecule interacts with the activator; i.e., Φ_S does not depend on the ACT concentration and correspond to the maximum quantum yields for the catalyzed reaction.

3. DISCUSSION

The measurement of the chemiexcitation quantum yields in the activated decomposition of cyclic peroxides is subject to a number of experimental variables such as (i) the concentration and purity of the peroxide, (ii) impurities in the reaction medium that might induce the dark decomposition of the peroxide, e.g., transition metal cations,[3] and (iii) the light emission calibration method. The difficult isolation and purification of α-peroxylactones, specially 3,3-dimethyl-1,2-dioxetanone (**2**), makes the exact determination of Φ_S extremely difficult, resulting in conflicting results reported throughout the years.[3,51−53]

The experimental procedure used in this work for the determination of the chemiexcitation efficiency includes thoroughly cleaning all of glassware with chelating agents to avoid transition-metal impurities and the use of the luminol secondary light standard method for light intensity calibration. By taking these measures, we reduce the dark decomposition of the peroxides and allow the comparison of Φ_S values of several chemiluminescent reactions without introducing errors related to light calibration, i.e., the luminol method for light intensity calibration is more frequently used than other methods, such as the radioactive Hastings−Weber standard[54,55] and the tetramethyl-1,2-dioxetane/9,10-dibromoanthracene method.[3,9,28] Furthermore, it has been reported that quantum yields determined by using the Hastings−Weber standard[54,55] have the tendency to show higher values by a factor of 2.5 than the ones determined by the luminol standard.[1,3,52] However, this discrepancy is not likely to cause the differences of orders of magnitude in the values pointed out here.

Compound **1** was purified by low-temperature recrystallization, and the value of Φ_S of its activated decomposition by RUB and PER was used as reference in the analysis of data obtained with peroxides **2**−**4**. The values of Φ_S^{CAT} determined, $(8.1 \pm 0.5) \times 10^{-4}$ einstein mol $^{-1}$ (RUB, toluene at 25 °C) and $(3.3 \pm 0.2) \times 10^{-5}$ einstein mol^{-1} (PER, DCM, 32 °C), are in good agreement with the value determined previously by Catalani and Wilson using PER as ACT [$\Phi_S = (2 \pm 1) \times 10^{-5}$ einstein mol^{-1}, DCM at 32 °C].[28] These values confirm that the chemiexcitation in the activated decomposition of **1** is extremely inefficient (Table 1).

However, the most important intermolecular CIEEL system is the activated decomposition of 1,2-dioxetanones, as these derivatives are model systems for firefly bioluminescence.[13,56−71] Peroxide **2** can only be isolated in solution and was carefully purified by low-temperature vacuum distillation. The identity of this highly unstable cyclic peroxide and its purity has been determined by low-temperature NMR spectroscopy, the ^{13}C NMR spectrum of **2** being reported for the first time by our group.[39,41] The concentration of the stock solutions of **2** was determined by iodometry utilizing spectrophotometric quantification of I_3^-,[72] which is reported to be more reliable than normal iodometric titration.

The quantum yield for the decomposition of derivative **2** by RUB was determined initially as $\Phi_S = 0.10 \pm 0.05$ einstein mol^{-1} (DCM at 24.5 °C, calibration made with TMD/DBA solutions in acetonitrile).[8] Subsequently, other research groups reported similar values for the quantum yields in the catalyzed decomposition of 1,2-dioxetanones.[29,73] Our redetermination of the Φ_S value for the rubrene-catalyzed decomposition of **2** under identical experimental conditions (DCM at 25 °C), except for the use of the luminol light calibration method, resulted in a value of $\Phi_S^{CAT} = (1.0 \pm 0.2) \times 10^{-3}$ einstein mol^{-1}, 2 orders of magnitude lower than the previously stated.[8] This difference might be related to differences in the purity of α-peroxylactone **2** and the accuracy of the determination of its concentration. However, the reaction system for the activated decomposition of **2** in the present work apparently contains less impurities due to the fact that the catalytic pathway contributed to a higher extend to the reaction than in the system previously studied.[8]

Therefore, to confirm the results with **2**, we have prepared the considerably more stable spiro-adamantyl-1,2-dioxetanone (**3**) and the hitherto unknown spiro-cyclopentyl-1,2-dioxetanone (**4**) and determined their chemiexcitation quantum yields. These peroxides can be handled and purified more conveniently because of their somewhat higher stability. Specifically, spiro-adamantyl derivative **3** can be purified by low-temperature recrystallization and therefore be obtained in high purity. Although **3** has been prepared before,[38] the study of its catalyzed decomposition is reported here for the first time. The methodology for quantum yield determination had to be changed for these 1,2-dioxetanone derivatives because the catalyzed pathway appears to be of less importance (see above). Therefore, the singlet quantum yields at infinite activator concentrations (Φ_S^{∞}) were determined for **3** and **4**, which can be compared to the singlet quantum yields of the catalyzed pathway (Φ_S^{CAT}) for peroxide derivatives **1** and **2**. In agreement with the yields obtained for derivative **2**, the quantum yields determined for the more stable derivatives **3** and **4** proved to be at least 2 orders of magnitude lower than those initially reported for this class of cyclic peroxides (Table 1).[8,29,73] Therefore, the generation of singlet-excited states in the RUB-activated chemiluminescent decompositions of **1**, **2**, **3**, and **4** is inefficient.

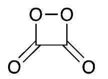
A compilation of the singlet quantum yields determined for several representative chemiluminescence and bioluminescence systems (Table 2) shows that *intermolecular* CIEEL systems, like the activated decomposition of **1**–**4**, tend to be much less efficient in generating singlet excited states than stated in the early reports.[8,9] A clear exception to this trend is the peroxyoxalate reaction, the only *intermolecular* CIEEL system with proven high quantum yields of up to $\Phi_S^{\infty} = 0.68$ einstein mol^{-1}, according to an earlier work of our research group.[30]

Several independent groups, including us, confirmed the high efficiency of the peroxyoxalate reaction using different oxalate esters and reaction conditions (Table 2).[30,74] This fact validates the use of the luminol light standard in the study of the catalyzed decomposition of peroxides **1**–**4** and reduces the uncertainty in the values of Φ_S to a factor of 2, i.e., the Φ_S of the peroxyoxalate reaction cannot be higher than 1.0 einstein mol^{-1}.[3] Moreover, the *intramolecular* CIEEL of protected aryloxy-1,2-dioxetanes **6**–**8** can be very efficient, as indicated in the literature using several peroxides and light calibration methods (Table 2). Again, the high quantum yield reported previously by us for the induced decomposition of derivative **6** validates the experimental light calibration method used in this work.

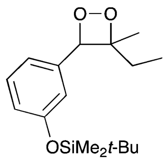
Table 2. Excited State Singlet Quantum Yields for Several Representative Chemiluminescent Systems

chemiluminescent system	$\Phi_S \times 100$ (einstein mol^{-1})	calibration method[a]	ref
Catalyzed Peroxide Decomposition—Intermolecular CIEEL			
diphenoyl peroxide (**1**)	10 ± 5	I	9
	$(2 \pm 1) \times 10^{-3}$	II	28
	$(3.3 \pm 0.2) \times 10^{-3}$	III	this work
	$(8.1 \pm 0.5) \times 10^{-2}$	III	this work
dimethyl-1,2-dioxetanone (**2**)	10 ± 5	I	8
	$(1.0 \pm 0.2) \times 10^{-1}$	III	this work
spiro-adamantyl-1,2-dioxetanone (**3**)	$(1.5 \pm 0.9) \times 10^{-1}$	III	this work
spiro-cyclopentyl-1,2-dioxetanone (**4**)	$(3.9 \pm 0.6) \times 10^{-2}$	III	this work
	$(1.3 \pm 0.5) \times 10^{-2}$	III	this work
Peroxyoxalate Reaction—Intermolecular CIEEL			
1,2-dioxetanedione (**5**) (peroxyoxalate HEI)	6.0 ± 0.8	III	30
	68 ± 5	III	30
	15.8	IV	74
Induced 1,2-Dioxetane Decomposition—Intramolecular CIEEL			
6	100 ± 30	III	26
7	27[b]	V	75
8	55 ± 7	II	76
	66 ± 9	II	76
	45	VI	18
	57	VI	18
Luciferin Bioluminescence—Intramolecular CIEEL			
firefly (*Photinus pyralis*) (**9**)	88 ± 22[c]	–[d]	77, 78
	41 ± 3[c]	VI	12
click beetle (*Pyrearinus termitilluminans*)	61 ± 2[c]	VII	62

[a] Calibration methods: I, through the triplet yield of a solution of tetramethyl-1,2-dioxetane, obtained with 9,10-dibromoanthracene; II, Hastings–Weber light standard (ref 54); III, modified method for the luminol standard (ref 30); IV, absolute calibration (ref 74); V, estimate based on the chemiluminescent decomposition of 3-adamantylidene-4-methoxy-4-(3-oxidophenyl)-1,2-dioxetane in TBAF/DMSO (ref 76); VI, luminol standard (refs 79–82); VII, determined by absolute calibration of the light detecting apparatus (ref 83). [b] Singlet yield not provided; value reported corresponds to the chemiluminescence yield. [c] Values for the bioluminescence yield. [d] Calibration method not specified.



5



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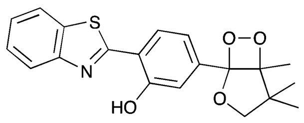

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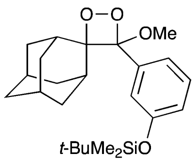

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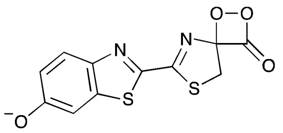

9

4. CONCLUSION

We provide unequivocal experimental evidence that the chemiexcitation in the activated decomposition of 1,2-dioxetanones (α-peroxylactones) is highly inefficient. These data are specially important as they include 3,3-dimethyl-1,2-dioxetanone (**2**), the model-compound used to propose the CIEEL mechanism. The firefly bioluminescence is an example for highly efficient *intramolecular* CIEEL transformation, which includes a 1,2-dioxetanone as HEI.[13,77,78] Conversely, the peroxyoxalate reaction is the only example of an efficient *intermolecular* CIEEL-active system,[84] where a 1,2-dioxetanone (1,2-dioxetanedione)[1] is assumed to be the HEI. These data show that the intermolecular activated decomposition of 1,2-dioxetanones is a poor model for the efficient chemical generation of electronically excited states, and the factors that determine the chemiexcitation efficiency have still to be clarified.

5. EXPERIMENTAL SECTION

Materials. Toluene and dichloromethane (DCM) for the kinetic chemiluminescence assays were stirred overnight over EDTA, filtered, distilled, and then redistilled from metallic sodium (toluene) or P_2O_5 (DCM). Ethyl acetate (EtOAc) was kept over $CaCl_2$ during 24 h, filtered, mechanically stirred with NaOH pellets (40 g of NaOH/L of EtOAc), filtered, and distilled from P_2O_5 under inert atmosphere. Rubrene (RUB), perylene (PER), and 9,10-diphenylanthracene (DPA) were used as received. Diphenoyl peroxide (**1**), 3,3-dimethyl-1,2-dioxetanone (**2**), spiro-adamantyl-1,2-dioxetanone (**3**), and spiro-cyclopentyl-1,2-dioxetanone (**4**), as well as their precursors, were prepared and characterized as described elsewhere.[39] Peroxide stock solutions were stored in vials at low temperature (−80 °C) and kept at −78 °C during the experiments. All glassware, including pipettes and microsyringes, were treated with EDTA solutions to remove traces of metal ions.

UV−Vis Spectrophotometry. UV−vis spectra were obtained on a spectrophotometer with a cell holder thermostated at 25.0 ± 0.5 °C by a water-circulating bath. Peroxide concentration was determined by an iodometric assay, using an absorption cuvette with 3.0 mL of a 0.05 mol L^{-1} potassium iodide solution in 0.1 mol L^{-1} HOAc/OAc^- buffer (pH = 3.8), containing 10 μL of a 1 mg mL^{-1} aqueous solution of HRP-VI (hydrogen-peroxidase oxidoreductase, EC 1.11.1.7, type VI-A, from horseradish) and 10 μL of peroxide solution diluted in MeOH, in order to obtain an absorbance between 0.5 and 0.8 at 353 nm ($\varepsilon_{353} = 2.55 \times 10^4$ L mol^{-1} cm^{-1} for I_3^-).

Light Emission and Fluorescence Spectroscopy. Fluorescence spectra and chemiluminescence emission kinetic assays were obtained on a fluorescence spectrometer, with a cell holder coupled to a magnetic stirrer and thermostated by a water-circulating bath. Also, a tube luminometer was used for low-intensity emissions obtained in the decomposition of peroxide **4**.

Kinetic Chemiluminescence Assays. All peroxides were directly added to the quartz cuvette or to the luminometer glass tube already charged with solvent (for thermolysis assays) or with the activator solution (for catalyzed decomposition assays). Peroxide stock solutions were kept at −78 °C and were transferred by microsyringes previously cooled in a dry ice chamber to avoid thermal peroxide decomposition. Microsyringes were washed with freshly distilled DCM and dried under an argon flux between the measurements. After peroxide addition, the chemiluminescence light intensity decay was registered for at least three half-lives; the equipment baseline was discounted for all kinetic assays. The light emission intensity, registered in arbitrary units (au s^{-1}), was transformed into absolute light units (einstein s^{-1}) through calibration of the photomultiplier tube (PMT) with the luminol standard (see below). Light emission time profiles were fitted with a first-order exponential decay equation, to determine the initial emission intensities (I_0, in einstein s^{-1}) and the observed rate constants (k_{obs}, in s^{-1}) (eq 6).

$$I_t = I_0 e^{-k_{obs}t} \tag{6}$$

Chemiluminescence Quantum Yields (Φ_{CL}) and Singlet Excited State Formation Quantum Yields (Φ_S). The chemiluminescence quantum yields (Φ_{CL}) for the ACT-catalyzed decomposition of diphenoyl peroxide or 1,2-dioxetanone were determined from the total amount of light (Q_{EM}) emitted during a kinetic assay, obtained by integration of the time profile associated with the decay of chemiluminescence intensity (AUC). This total amount of light emission in arbitrary units was transformed into absolute units (einstein), using the luminol calibration factor (f_{lum}) and the PMT's wavelength sensibility factor (f_{PMT}), and then related to the number of moles of organic peroxide (n_{O-O}) used as limiting reagent to obtain the Φ_{CL} in einstein mol^{-1} (eq 7). The luminol calibration factor (f_{lum}) for the PMT of each detection apparatus was determined by using a modified version[30] of the luminol standard,[79] consisting of the hemin-catalyzed oxidation of luminol by hydrogen peroxide in buffered aqueous medium with a quantum yield of $\Phi_{lum} = (1.14 \pm 0.06)\ 10^{-2}$ einstein mol^{-1},[80] which is independent of the initial luminol concentration (10^{-9} to 2×10^{-3} mol L^{-1}).[81] The wavelength sensibility factor (f_{PMT}) is obtained from specifications of the PMT manufacturer.

$$\Phi_{CL} = \frac{Q_{EM} f_{lum} f_{PMT}}{n_{O-O}} \tag{7}$$

The quantum yields for singlet excited state formation (Φ_S) in a given experimental condition were obtained from the chemiluminescence quantum yields (Φ_{CL}) by considering the fluorescence quantum yield of the ACT (Φ_{FL})[30,85] (eq 8).

$$\Phi_S = \frac{\Phi_{CL}}{\Phi_{FL}} \tag{8}$$

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Kinetic data and chemiluminescence parameters for the activated decomposition of peroxides **1**−**4** (Tables S1−S6) and time profiles for the emission intensity decay (Figures S1−S3). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Fax: +55 (11) 3091-3815. E-mail: wjbaader@iq.usp.br.

Present Address

[‡]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, SP, Brazil.

Notes

The authors declare no competing financial interest.

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■ DEDICATION

[†]This article is dedicated to Prof. Waldemar Adam on the occasion of his 75th anniversary.

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(41) From ref 39, 3,3-dimethyl-1,2-dioxetanone (**2**): ^{1}H NMR ($CDCl_3$, 500 MHz, −20 °C) δ (ppm) = 1.80 (s, 6H, two CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz, −20 °C) δ (ppm) = 22.1 (CH_3), 99.0 ($C(CH_3)_2$), 169.8 (C=O).

(42) From ref 39, spiro-adamantyl-1,2-dioxetanone (**3**): R_f = 0.7 (SiO_2, Hex/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 500 MHz, −38 °C) δ (ppm) = 1.71−1.97 (m, 10H, adamantane-H), 2.07−2.10 (m, 2H, adamantane-H), 2.61 (bs, 2H, adamantane-H); ^{13}C NMR ($CDCl_3$, 125 MHz, −38 °C): δ (ppm) = 25.2, 25.3, 31.7, 33.0, 33.5, 35.3, 104.6, 169.3 (C=O).

(43) From ref 39, spiro-cyclopentyl-1,2-dioxetanone (**4**): ^{1}H NMR ($CDCl_3$, 500 MHz, −40 °C) δ (ppm) = 1.89−1.94 (m, 4H), 2.00−2.01 (m, 2H), 2.12−2.15 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz, −40 °C) δ (ppm) = 23.4 (C3 and C4), 38.7 (C2 and C5), both with low intensities. Absence of 220.6 ppm, for C=O of the peroxide decomposition product, cyclopentanone. See ref 39 for further details on the characterization of **4**.

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