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Experimental Evidence of the Occurrence of Intramolecular Electron Transfer in Catalyzed 1,2-Dioxetane Decomposition

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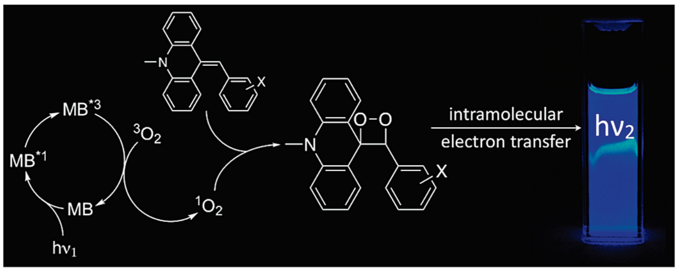

The activation parameters for the thermal decomposition of 13 acridinium-substituted 1,2-dioxetanes, bearing an aromatic moiety, were determined and their chemiluminescence emission quantum yields estimated, utilizing in situ photosensitized 1,2-dioxetane generation and observation of its thermal decomposition kinetics, without isolation of these highly unstable cyclic peroxides. Decomposition rate constants show linear free-energy correlation for electron-withdrawing substituents, with a Hammett reaction constant of $\rho = 1.3 \pm 0.1$, indicating the occurrence of an intramolecular electron transfer from the acridinium moiety to the 1,2-dioxetane ring, as postulated by the intramolecular chemically initiated electron exchange luminescence (CIEEL) mechanism. Emission quantum yield behavior can also be rationalized on the basis of the intramolecular CIEEL mechanism, additionally evidencing its occurrence in this transformation. Both relations constitute the first experimental evidence for the occurrence of the postulated intramolecular electron transfer in the catalyzed and induced decomposition of properly substituted 1,2-dioxetanes.

Introduction

Chemiluminescence is defined as light emission produced by a chemical reaction, where the electronically excited products generated can show direct light emission or transfer their excitation energy to suitable acceptors that are responsible for the emission.[1] The use of chemiluminescence in analytical applications has been extensively researched for decades, and there is a full spectrum of methods using 1,2-dioxetanes able to precisely detect, in very low concentrations, enzymes, reactive species, proteins, and nucleic acids, among other compounds of biological interest.[2,3]

The thermal, unimolecular decomposition of 1,2-dioxetanes substituted with an acridinium moiety in a spiro fashion (Scheme 1, **1**) is one of the few examples of this kind of reaction that shows high chemiluminescence quantum yields (Φ_{CL}), with Φ_{CL} values of up to 0.15 einstein mol^{-1}.[4] This observation is in contrast with the thermal decomposition of 1,2-dioxetanes substituted with alkyl or aryl groups, with Φ_{CL} values typically lower than 1×10^{-4} einstein mol^{-1}.[5] The mechanism proposed by Lee and Singer to

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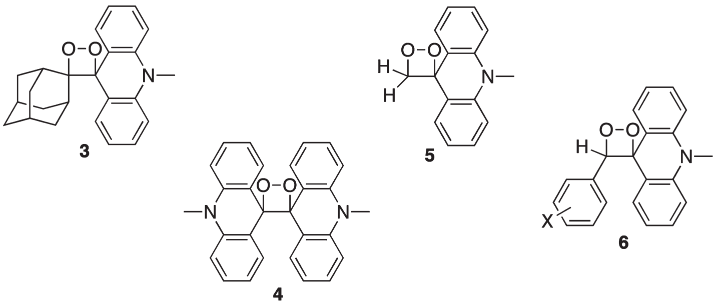
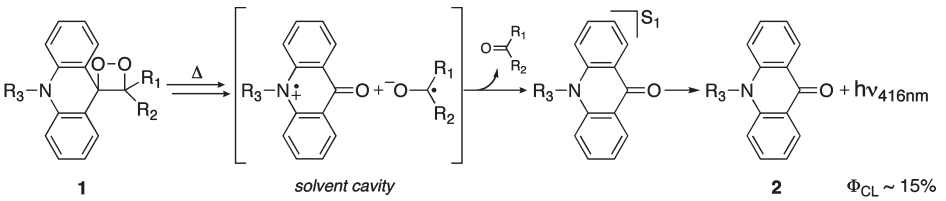


6	X
a	H
b	*p*-$N(CH_3)_2$
c	*p*-OH
d	*p*-OCH_3
e	*p*-CH_3
f	*m*-OH
g	*m*-OCH_3
h	*p*-Br
i	*m*-Cl
j	*m*-CN
k	*m*-NO_2
l	*p*-CN
m	*p*-NO_2

FIGURE 1. Structures of acridinium-derived 1,2-dioxetanes.

SCHEME 1. Chemiluminescence from Acridinium-Derived 1,2-Dioxetanes



rationalize the chemiexcitation step in *N*-methylacridine-substituted 1,2-dioxetane (NMAD) chemiluminescence,[4] following the previous work of McCapra et al.,[6] postulates an intramolecular electron transfer from the lone electron pair of the acridinic nitrogen to the peroxidic ring, followed by O–O bond cleavage and formation of a pair of radical ions. The back electron transfer (BET) from the radical anion to the radical cation produces the carbonyl fragment *N*-methylacridone (**2**, $R_3 = CH_3$) in its singlet excited state, leading to light emission with high quantum yields (Scheme 1). This mechanistic approach was based on the proposal of Schuster et al. for the "chemically initiated electron exchange luminescence" (CIEEL) mechanism, initially formulated for the catalyzed decomposition of diphenoyl peroxide,[7] 1,2-dioxetanones,[8] and other cyclic and linear peroxides.[9] Later on, Schaap et al. developed a set of thermally stable phenoxy-substituted 1,2-dioxetanes,[10] whose fast decomposition can be triggered by a chemical or enzymatic reaction leading to high Φ_{CL} (~0.20 einstein mol^{-1}) and high singlet quantum yields ($\Phi_S \sim 0.50$ einstein mol^{-1}),[11] presumably through an intramolecular variation of the CIEEL sequence, as supposed for the decomposition of NMADs.[4]

Despite the early proposal of Lee and Singer,[4] there is still not any *conclusive experimental evidence* supporting the proposition that the CIEEL approach can be applied to rationalize chemiluminescence from NMADs (Scheme 1)[4] or from triggered phenoxy-substituted 1,2-dioxetanes.[11] One of the reasons for this lack of experimental data may be due to the difficult synthesis and purification of NMADs, a consequence of their very low thermal stability. The most stable 1,2-dioxetane of this type yet synthesized is the adamantyl derivate **3**, with a $\Delta G^{\ddagger}$ value for its thermal decomposition at 25 °C of 117.2 kJ mol^{-1}, corresponding to a $t_{1/2}$ value of ca. 10^7 s.[4] This stability is considered very high, especially in comparison to other derivatives of the same class, such as 1,2-dioxetane **4**, never isolated and postulated as an intermediate in the oxidation of lucigenin ($\Delta G^{\ddagger} = 73.4$ kJ mol^{-1}, $t_{1/2} \approx 6$ s),[12] the unsubstituted derivate **5** ($\Delta G^{\ddagger} = 83.3$ kJ mol^{-1}, $t_{1/2} \approx 40$ s),[4] and the phenyl-substituted derivate **6a** ($\Delta G^{\ddagger} = 82.5$ kJ mol^{-1}, $t_{1/2} \approx 33$ s) (Figure 1).[4]

1,2-Dioxetane synthesis is most conveniently performed by photooxygenation of electron-rich olefins at low temperatures, and purification can be achieved by low-temperature column chromatography; however, this process is normally susceptible to low isolated yields and contamination with decomposition products.[13] Due to these difficulties, we developed a dynamic process that circumvents the necessity to isolate and purify the unstable 1,2-dioxetanes, allowing us to obtain kinetic data for the thermal decomposition of those labile compounds *without* their isolation.[14] In this procedure, the cyclic peroxide is generated in situ from the reaction of the appropriate olefin

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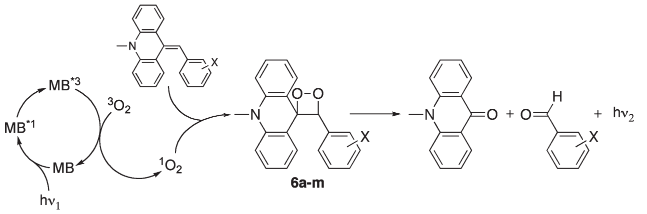
SCHEME 2. Methylene Blue (MB) Sensitized Photooxygenation of 9-Benzylidene-10-methylacridans, Leading to Intermediate 1,2-Dioxetane Formation and Its Unimolecular Decomposition, Accompanied by Chemiluminescence Emission



and singlet oxygen also generated in situ by photooxygenation sensitized by methylene blue, followed by the measurement of the light emission produced by the 1,2-dioxetane cleavage. This method allows the acquisition of kinetic data for the thermal decomposition of 1,2-dioxetanes with half-lives of 30 s or less, using just a simple commercial spectrofluorimeter.

Kinetic studies on the thermal decomposition of a series of NMADs, obtained by photooxygenation of various substituted 9-benzylidene-10-methylacridans using this in situ methodology, permitted the determination of activation parameters for those 1,2-dioxetanes. The results obtained provide substantial experimental evidence for the occurrence of an electron transfer to the peroxidic ring in the initial step of the 1,2-dioxetane decomposition. Therefore, these data show, for the first time, experimental evidence for the occurrence of an initial intramolecular electron transfer in the chemiexcitation step of intramolecularly catalyzed 1,2-dioxetane decomposition, as proposed in the intramolecular version of the CIEEL mechanism, thought to operate in NMADs as well as triggered phenoxy-substituted 1,2-dioxetane decomposition.[1,4,16,17,19]

Results

The thermal decomposition of the NMADs **6a–m** (Figure 1) produced, as expected, two carbonyl compounds: *N*-methylacridone (**2**, $R_3 = CH_3$) and the corresponding substituted aromatic aldehyde. These fragments were identified by TLC analysis of the spent reaction mixture, after irradiation with light and kinetic data acquisition.

The formation of the carbonyl compounds is, together with the observation of chemiluminescence emission, good evidence for the formation and identity of the 1,2-dioxetanes, which were not isolated in this work due to their instability. However, even without isolation or purification, kinetic data from the thermal decomposition of the 1,2-dioxetanes **6a–m**, using the novel in situ generation and measurement method, could be obtained with very high reproducibility, clean first-order kinetics always being observed (Scheme 2, Figure 5 in the Experimental Section).

The determination of the absolute chemiluminescence quantum yields for the decomposition of 1,2-dioxetanes **6a–m** could not be performed in a straightforward manner, because of the difficult assessment of some parameters intrinsic to the in situ method used. However, the relative quantum yields could be estimated with a good degree of confidence for all 1,2-dioxetanes, normalizing the numeric values of the integrated emission intensity vs time plots for each derivative with the integrated emission of **6a**, the strongest emitter in the series (I_{int}, Table 1). This approach is valid if the yield of 1,2-dioxetane formation does not vary significantly with the electronic character of the substituent on the aromatic ring, which may be assumed to be true, as the chemical yield of 1,2-dioxetane formation from adequate olefins by photooxygenation is normally high (80–100%).[15] Furthermore, it should be pointed out that addition of the corresponding aldehyde to the reaction medium does not lead to the reduction of the emission quantum yields; proving that aldehydes, in moderate concentrations, do not significantly quench the electronically excited state of the *N*-methylacridone, conflicting with observations made in previous literature works.[3,25b]

Using the method of isothermal kinetics, the activation parameters for 1,2-dioxetanes **6a–m** were determined by linear fitting of the Eyring plots for the k_{obs} values obtained from the emission intensity decay curves (Figure 2). Good linear correlations were obtained in these plots in most of the cases, specially with 1,2-dioxetanes with high chemiluminescence quantum yields, enabling the precise determination of the activation parameters for the chemiluminescent decomposition of the NMADs **6a–m** (Table 1).

The studied NMADs **6a–m** show similar thermal stabilities at 25 °C, with $\Delta G^{\ddagger}$ values that differ only about 2–3 kJ mol^{-1} (Table 1). For the derivate **6a,** a $\Delta G^{\ddagger}$ value of 82.6 kJ mol^{-1} was obtained by our in situ methodology; this value is very similar to $\Delta G^{\ddagger} = 82.5$ kJ mol^{-1}, determined previously for the thermal decomposition of the *isolated* 1,2-dioxetane **6a,**[4] thus confirming the validity and reliability of this new kinetic methodology. The most stable NMADs, **6d**,**e,** are still ca. 35 kJ mol^{-1} less stable than the adamantyl derivative **3** ($\Delta G^{\ddagger} = 117.2$ kJ mol^{-1}).[4] The least stable of them, **6j**, is ca. 5 kJ mol^{-1} more stable than the highly unstable lucigenin-derived 1,2-dioxetane **4** ($\Delta G^{\ddagger} = 73.4$ kJ mol^{-1}),[12] placing the NMADs obtained in this work in the lower half of the thermal stability scale for these acridinium-substituted 1,2-dioxetanes.

Direct comparisons between the experimental k_{obs} values for the NMADs **6a–m** cannot be performed, since they were not all determined at the same temperature. Therefore, the thermal decomposition rate constants at 25 °C for the 1,2-dioxetanes **6a–m** were calculated by using their experimental free activation energies (k, Table 1). The calculated values proved to be very similar to the experimental k_{obs} values measured at 25 °C for some of the derivates (see the Supporting Information, Table S1).

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TABLE 1. Activation Parameters, Thermal Decomposition Rate Constants (k), and Integrated Normalized Chemiluminescence Quantum Yields (I_{int}) for the NMADs 6a−m

1,2-dioxetane	R	σ	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	$\Delta G^{\ddagger}$ (kJ mol^{-1})[a]	10^2k (s^{-1})[b]	I_{int} (au)[c]
6b	*p*-N(CH$_3$)$_2$	−0.83	61 ± 3	−63 ± 4	79 ± 5	7.5 ± 0.5	0.03
6c	*p*-OH	−0.37	51.1 ± 0.8	−101 ± 2	81 ± 3	3.6 ± 0.1	0.17
6d	*p*-OCH$_3$	−0.27	74.1 ± 0.8	−29.3 ± 0.4	83 ± 1	1.90 ± 0.02	0.48
6e	*p*-CH$_3$	−0.17	77.5 ± 0.4	−18.5 ± 0.1	83.0 ± 0.4	1.80 ± 0.01	0.69
6a	H	0.00	76.2 ± 0.4	−21.5 ± 0.1	82.6 ± 0.4	2.09 ± 0.01	1.00
6f	*m*-OH	+0.12	70.4 ± 0.8	−32.3 ± 0.5	80 ± 1	5.85 ± 0.07	0.06
6g	*m*-OCH$_3$	+0.12	76.6 ± 0.8	−17.8 ± 0.2	82 ± 1	2.76 ± 0.03	0.66
6h	*p*-Br	+0.23	72.4 ± 0.4	−28.9 ± 0.2	81.1 ± 0.4	3.91 ± 0.02	0.31
6i	*m*-Cl	+0.37	74.5 ± 0.8	−19.6 ± 0.2	80.4 ± 0.5	5.14 ± 0.03	0.17
6j	*m*-CN	+0.56	57.8 ± 0.4	−68.2 ± 0.2	78.1 ± 0.5	12.9 ± 0.1	0.05
6k	*m*-NO$_2$	+0.71	71 ± 4	−25 ± 3	79.0 ± 5.0	10.8 ± 0.7	0.01
6l	*p*-CN	+0.66	62.8 ± 0.4	−54.8 ± 0.8	79 ± 1	8.4 ± 0.1	0.04
6m	*p*-NO$_2$	+0.78	80.0 ± 0.4	−3.5 ± 0.2	81.0 ± 0.5	4.00 ± 0.02	0.02

[a] At 298 K. [b] Calculated from the $\Delta G^{\ddagger}$ values, at 298 K. [c] Estimated from the experimental emission intensity decay curves obtained at 298 K, using numerical integration and normalized with respect to the integrated emission of **6a**.

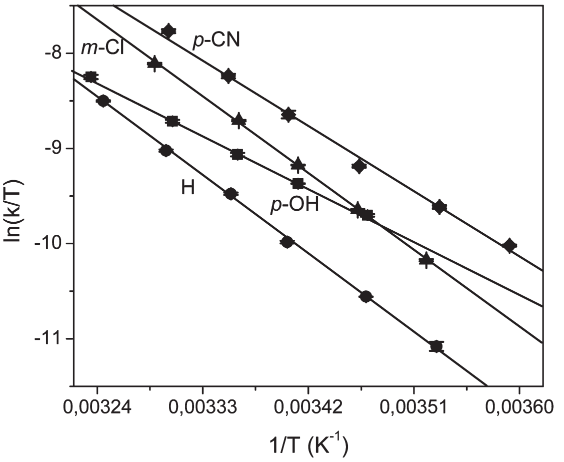

FIGURE 2. Eyring plots for the determination of activation parameters in the unimolecular decomposition of the NMADs **6a** (H), **6c** (*p*-OH), **6i** (*m*-Cl) and **6l** (*p*-CN).

Discussion

Mechanistic Aspects of NMAD Chemiluminescence. The decomposition mechanism of the NMADs **6a−m** can be formulated in analogy to the intramolecular CIEEL mechanism proposed for the decomposition of "silyl-triggered" 3-phenoxyl-substituted 1,2-dioxetanes.[16−18] The reaction is initiated by deprotection of the silyl group and formation of a phenolate anion, followed by an intramolecular electron transfer (ET) from the phenolate to the cyclic peroxide, leading to the formation of a biradical anion (Scheme 3). This biradical anion undergoes C−C bond cleavage, forming either a radical pair (Scheme 3, path A) or another biradical anion (Scheme 3, path B). Consequently, annihilation of those radical or biradical species may occur by inter- or intramolecular back electron transfer (BET) processes, which can lead to the formation of the corresponding phenolate anion in its singlet excited state, responsible for the observed light emission.[16−18]

The high singlet quantum yields ($\Phi_S \approx 1.0$ einstein mol^{-1})[16] obtained in the induced decomposition of "silyl-triggered" 3-phenoxyl-substituted 1,2-dioxetanes, in contrast to the much lower yields observed in intermolecular CIEEL systems ($\Phi_S \approx 10^{-4}$ einstein mol^{-1}),[19] indicate that the whole CIEEL sequence should occur in a totally intramolecular fashion (Scheme 3, path B).[1] In contrast to this observation, studies on the solvent cavity effect on the quantum yields for these transformations pointed to the occurrence of an intermolecular BET step (Scheme 3, path A).[20] Apart from this still unresolved question, it has to be mentioned that there is still not any direct experimental evidence on the reaction steps postulated, although these appear to be reasonable on the basis of the intermolecular CIEEL mechanism.[1,11,16,17]

Using the aforementioned mechanistic outlines, the chemiluminescent decomposition of the NMADs **6a−m** can also be rationalized. The first, rate-limiting step is an endothermic electron transfer (ET) from the lone pair of the nitrogen atom of the acridinium moiety to the peroxidic ring (Scheme 4). This intramolecular ET (ET$_{intra}$) is supposed to occur simultaneously to the cleavage of the O−O bond, thereby avoiding the highly favorable exothermic back electron transfer, which would reset the process to the initial state. Two biradical species can be formed in this process (**7a**,**b**), differing only in the position of the negative charge and the unpaired electrons. It should be noted that structures **7a**,**b** are distinct species; they do not represent resonance structures, since there is no conjugation between the two oxygen atoms (Scheme 4). However, conjugation does occurs in the transition state of the C−C bond cleavage (Scheme 5) of the next reaction step. Consequently, independently of the intermediate formed initially, **7a** or **7b**, C−C bond cleavage can result in the formation of both of the following species, an acridinium radical cation/aldehyde radical anion pair or an acridinium biradical zwitterion; the latter is accompanied by the formation of a neutral benzaldehyde derivative (Scheme 4).

If the acridinium biradical zwitterion is generated, an intramolecular back electron transfer (BET$_{intra}$) may directly form the *N*-methylacridone in the singlet excited state, probably in high quantum yields. Alternatively, C−C bond cleavage and BET can occur as a concerted process, as the acridinium biradical zwitterion might be considered a canonical structure of the *N*-methylacridone in the singlet excited state (Scheme 4).[1] The other possibility is an intermolecular back electron transfer (BET$_{inter}$), from the aldehyde radical anion to the acridinium radical cation, a pathway that probably leads to low yields for excited state formation, because

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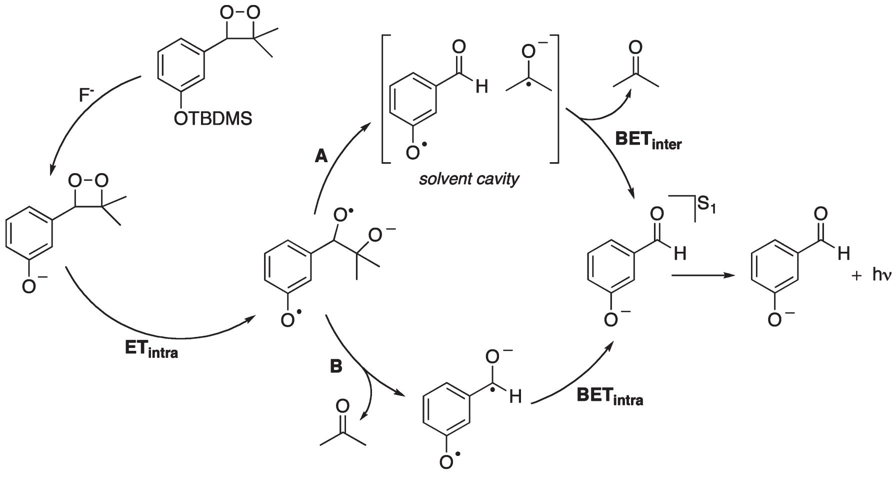
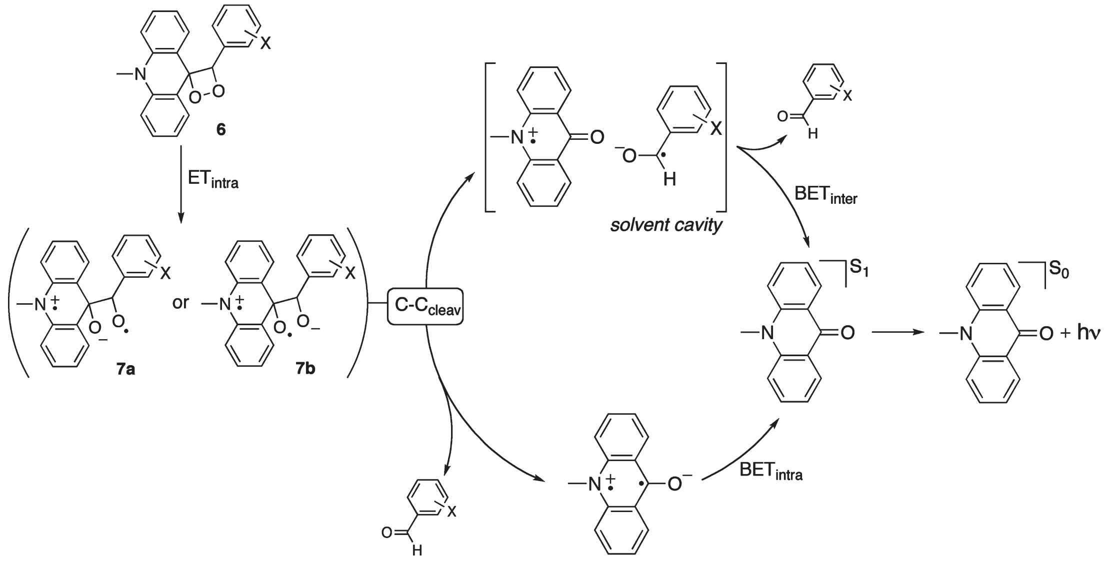
SCHEME 3. **Intramolecular CIEEL Mechanism in the Induced Decomposition of "Silyl-Triggered" 3-Phenoxyl-Substituted 1,2-Dioxetanes**[a]

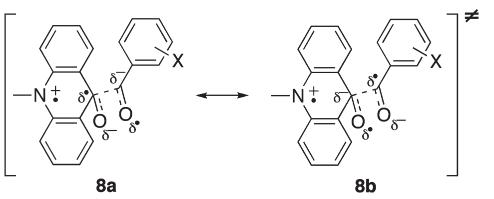


[a]TBDMS = *tert*-butyldimethylsilyl.

SCHEME 4. **Possible Chemiexcitation Pathways for the Decomposition of the NMADs**



SCHEME 5. **Structure of the Transition State for the C−C Bond Cleavage Step**



of its intermolecular nature.[1] The low chemiexcitation quantum yields of CIEEL systems with an initial intermolecular ET step ($\Phi_S \approx 10^{-4}$ einstein mol^{-1})[19] and the much higher yields observed for systems where an initial intramolecular ET process is supposed to occur ($\Phi_S \approx 1.0$ einstein mol^{-1})[1,16] suggest that the decomposition of NMADs, showing high emission quantum yields, should occur by an entirely intramolecular process, including the back electron transfer step (Scheme 4, BET$_{\text{intra}}$).

Activation Parameters for NMADs Decomposition. The activation parameters determined for the decomposition of 1,2-dioxetanes **6** show that activation enthalpy and entropy values do not vary in a systematic way with the electronic properties of the substituents (Table 1). Activation entropies are negative in all cases, which might be due to the necessity of a specific conformation of the acridinium moiety for the occurrence on the intramolecular electron transfer.[4,25b] However, the free activation energies, although showing little variation, appear to vary systematically for electron-withdrawing substituents, with generally lower values for more strongly electron attracting substituents (Table 1). However, also the good electron donor substituted derivatives **6b**,**c** show $\Delta G^{\ddagger}$ values lower than that for the unsubstituted derivative **6a**. The rate constant values calculated from the activation parameters at 25 °C show variations of almost 1 order of magnitude, being lowest for **6a** (X = H) and showing the highest values for good electron acceptors as well as donating substituted derivatives (Table 1).

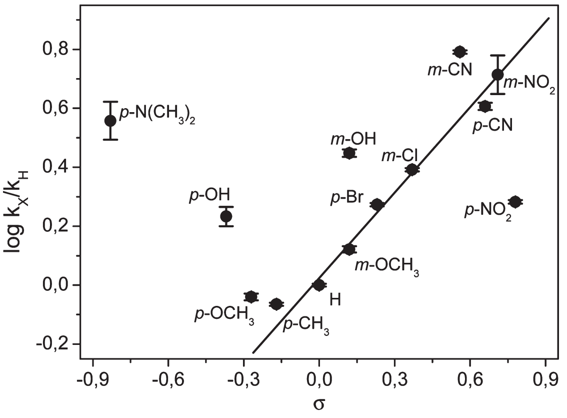


FIGURE 3. Linear free-energy correlation of the rate constants (k_X/k_H) of the emission intensity decay in the decomposition of the NMADs **6a**−**m** with the Hammett substituent constants (σ). Values for the substituents p-N(CH$_3$)$_2$, p-OH, p-OCH$_3$, m-OH, m-CN, and p-NO$_2$ were not included in the correlation.

Linear Free-Energy Correlations for NMADs Chemiluminescence. The linear free-energy correlation of the rate constants (k_X/k_H) for the thermal decomposition of the NMADs with the Hammett substituent constants (σ) shows a good linear dependence for moderately electron withdrawing substituents, with $\rho = 1.3 \pm 0.1$ (Figure 3). The positive sign of the parameter ρ indicates the formation of a partial negative charge in the transition state of the rate-determining step: namely, the intramolecular electron transfer from the acridine nitrogen to the dioxetane ring (ET$_{\text{intra}}$, Scheme 4). Additionally, a ρ value close to 1.0 indicates a transition state structure similar to that of the benzoic acid anion, implying that the most probable structure of the intermediate, formed after initial electron transfer and O−O bond cleavage, is of type **7b** (Scheme 4). This can be implied since the distance of the negative charge to the stabilizing substituent, in this case, is similar to that in the corresponding conjugated base of benzoic acid. The result obtained in the linear free-energy correlation with electron-withdrawing substituted derivatives constitutes the first experimental evidence for the occurrence of an intramolecular electron transfer in the catalyzed decomposition of properly substituted 1,2-dioxetanes, such as the NMADs studied in the present work (Figure 3).

Although the results discussed above indicate the preferential formation of **7b** in the initial electron transfer step, any of these radical species can lead to both of the possible C−C bond cleavage products; therefore, these results do not give insight into this part of the chemiexcitation mechanism. The correlation of the normalized chemiluminescence quantum yields (I_{int}) with the Hammett substituent constants (σ) shows that, for NMADs bearing electron-withdrawing substituents, the quantum yield decreases with an increase in the electron-accepting ability of the substituent (Figure 4). Assuming efficient excited state formation in the intramolecular process (BET$_{\text{intra}}$), justified by the fact that the acridinium biradical zwitterion can be considered as a charge-separated structure of the electronically excited N-methylacridone, the variation of the electron acceptor quality of the substituent is not expected to exert any influence on the efficiency of excited state formation, as there are always the same species participating in the back electron transfer step (Scheme 4).

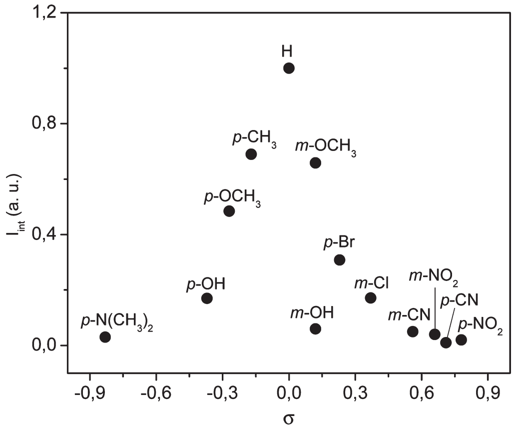


FIGURE 4. Correlation of the normalized quantum yields of light emission (I_{int}) for the NMADs **6a**−**m** and the Hammett substituent constants (σ).

However, if it is assumed that both sets of structures (the radical ion pair and the acridinium biradical zwitterion) can be formed after C−C bond cleavage in a "competitive" manner, it is possible to explain the decrease of the chemiluminescence quantum yields by the stabilization effect of the aldehyde radical anion for more strongly electron withdrawing substituents. This stabilization effect would decrease the portion of the reaction occurring through the completely intramolecular, high excitation quantum yield, pathway (via an acridinium biradical zwitterion). Therefore, the observation that the chemiluminescence quantum yields decrease with more strongly electron withdrawing substituents can be rationalized by the competition of the inter- and intramolecular BET steps, supposing that only the BET$_{\text{intra}}$ leads to efficient excited state formation (Scheme 4).

Chemiexcitation Pathways in NMADs Chemiluminescence. The experimental facts can also be rationalized on the basis of the exclusive occurrence of the intermolecular BET. In this case, the efficiency of excited state formation will be determined by the energy liberated in the BET step from the aldehyde radical anion to the acridinium radical cation, as discussed elsewhere for the peroxyoxalate reaction.[21] As electron-withdrawing substituents stabilize the benzaldehyde derivative, less energy will be available for chemiexcitation in the decomposition of NMADs bearing these substituents, explaining the experimentally observed lower quantum yields (Figure 4). In contrast, if the only BET process operating would be the intramolecular one, the observed quantum yield behavior can be also rationalized on the basis of energy considerations. The principal intermediate **7b** (and also **7a**, although to a lesser extent) will have a lower energy content for the derivatives bearing electron-withdrawing substituents. Therefore, less energy will be liberated in the combined C−C bond cleavage and intramolecular BET processes, the lower quantum yields being due to energy reasons.[21] In this argument, C−C bond cleavage and BET are formulated as concerted processes, as the acridinium biradical zwitterion is considered a canonical structure of the N-methylacridone in the singlet excited state.[1]

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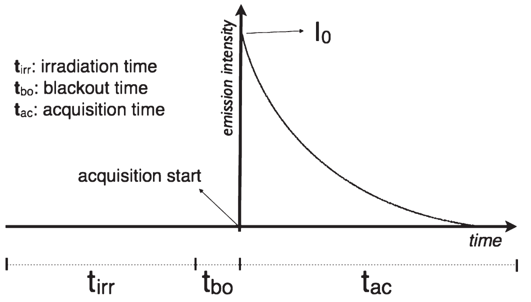

FIGURE 5. Example of an experimental emission intensity decay curve obtained from in situ generation of a NMAD by methylene blue sensitized photooxygenation of a substituted 9-benzylidene-10-methylacridan.

In conclusion from this part, it can be stated that the experimentally observed tendency of the singlet excitation quantum yields can be explained on the basis of the intramolecular CIEEL mechanism, being involved an intra- or intermolecular BET. In turn, this fact also contributes to the validation of the proposed CIEEL sequence for the decomposition of the NMADs.

From the Hammett plot, it can be observed that two NMADs clearly do not fit into the linear free-energy correlation: the electron-donor-substituted derivatives **6b** (*p*-N(CH_3)$_2$) and **6c** (*p*-OH) (Figure 3). This behavior can be rationalized by the fact that in these cases the aromatic group itself constitutes a very good electron donor and, therefore, this group can transfer an electron to the dioxetane ring.[22] This parallel reaction is expected to lead to an increase in the observed decomposition rate constant for **6b**,**c**. This effect may also contribute to the slightly higher rate constants obtained for the methoxy- and methyl-substituted derivatives **6d**,**e** (Figure 4). Nevertheless, the rate constant value for the *m*-OH derivate **6f**, considerably higher than would be expected from its σ value (+0.12), cannot be explained by the occurrence of this parallel reaction. However, it is known that also *m*-phenoxy-substituted 1,2-dioxetanes can undergo decomposition from an intramolecular electron transfer from the phenolate to the dioxetanic ring;[11b,20b] a corresponding effect may be taking place in the present case.

This direct electron transfer from electron-donor substituents to the dioxetane ring suggested above can also explain the decrease in the chemiluminescence quantum yields for NMADs containing such groups (Figure 4). This process will afford radical species of the substituted benzaldehyde which,[23] by an intra- or intermolecular back electron transfer, will lead to the production of the singlet excited state of the aldehyde derivative. Since the fluorescence quantum yields of substituted benzaldehyde derivatives are extremely low (ca. 10^{-5}),[24] the expected chemiluminescence quantum yields would also be low for those NMADs bearing electron-donor substituents, which is in agreement with the experimental observations.

Conclusions

This work provides, for the first time, unequivocal experimental evidence for the occurrence of an intramolecular electron transfer from a electron-donor group (*N*-methylacridinium) to the peroxidic ring, in the intramolecularly catalyzed decomposition of 1,2-dioxetanes. This experimental evidence can be extended to the analogous case of phenoxyl-substituted 1,2-dioxetanes, widely utilized in bioanalytical applications, making it of general importance for an understanding of the mechanisms of efficient electronic excited state formation. Furthermore, the influence of the electronic properties of substituents on the emission quantum yields of the derivatives could be rationalized on the basis of the intramolecular CIEEL mechanism, validating its application to this case.

Experimental Section

Acetonitrile (VWR, 99.9%) was distilled twice from CaH_2 under an inert atmosphere before use. The preparation of 9-diethylphosphono-*N*-methylacridan and the 9-benzylidene-10-methylacridans substituted with various aryl groups, synthesized by the Horner−Wadsworth−Emmons (HWE) method, is described elsewhere.[3,25] The detailed procedures and analytical data for all compounds are given in the Supporting Information.

The kinetic measurements were performed in a cuvette containing 2 mL of a methylene blue (14.4 μmol L^{-1}, Merck, $>95\%$) solution and the olefin (97.5 μmol L^{-1}) in acetonitrile. This solvent has two important characteristics related to the in situ singlet oxygen generation: high oxygen solubility (1.9 mmol L^{-1} at 293 K and 0.21 atm of O_2)[26a] and long singlet oxygen lifetime (30 μs).[26b] Air-equilibrated solutions ($[O_2] \approx$ 1.9 mmol L^{-1}) were utilized in all the experiments performed. The cuvette was placed in the cell holder of the spectrofluorimeter with magnetic stirring and allowed to reach the desired temperature. The solution was then irradiated with light of wavelength 655 nm for 100 s, after which the excitation shutter was closed and the emission shutter opened to initiate the measurement of the integrated (whole spectral range) light emission (Figure 5). The emission intensity was always shown to be maximum at acquisition time 0 and showed a first-order decay, which was followed during at least 4 half-lives. The observed rate constants (k_{obs}) were obtained by fitting the intensity versus time curves using a monoexponential decay function. In all cases, rate constants were shown to be independent of the irradiation time, as expected for the case of unimolecular 1,2-dioxetane decomposition.

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Supporting Information Available: Text and a table giving the detailed synthesis of the substituted 9-benzylidene-10-methylacridan and observed rate constants at different temperatures for the *N*-methylacridan-substituted 1,2-dioxetanes **6a**−**m**. This material is available free of charge via the Internet at http://pubs.acs.org.

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