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THERMAL GENERATION OF ELECTRONIC EXCITATION WITH HYPERENERGETIC MOLECULES

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Abstract- The synthesis of a large variety of hyperenergetic molecules such as the 1,2-dioxetanes, α-peroxylactones, 3-imino-1,2-dioxetanes, benzo-1,4-dioxin dioxetanes, bisdioxetanes, α-pyrone endoperoxides and cyclic peroxalates are presented. The utilization of these unique substances for the thermal generation of electronically excited states is reviewed, with special reference to the total excitation yield (ϕ^{T+S}) and spin state selectivities ($\phi^{T}/\phi S$). The physical and chemical methods for the determination of excitation yields are discussed and scrutinized for their reliability and effectiveness. Mechanistic problems such as energy transfer, heavy atom effects, electron exchange, etc. are analyzed. The biological implications of such "high energy" molecules is emphasized.

INTRODUCTION

In a chemiluminescent reaction chemical energy is converted into electronic energy (Ref. 1). As summarized in the chemiluminescence mechanism (eqn. 1), the ground state reactant R_o acquires sufficient thermal energy on heating

$$\begin{aligned} R_o + \Delta &\longrightarrow (\neq) \\ (\neq) &\longrightarrow P^* \\ P^* &\longrightarrow P_o + h\nu \end{aligned} \qquad (1)$$

to decompose into the activated complex (≠). Subsequently the activated complex transforms into the electronically excited product P*. The latter rids itself of the excitation energy by exhibiting luminescence, i.e. fluorescence when singlet excited product $^{S}P^*$ and phosphorescence when triplet excited product $^{T}P^*$ intervene. This is to be contrasted with the usual thermal decomposition reactions (exothermic), in which the absorbed heat is utilized to change bonds in the molecules involved, but the excess energy is degraded into heat. Thus, as shown in eqn. 2, the ground state reactant R_o leads again to the activated complex (≠) on heating, but the (≠) leads to a vibrationally excited

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$$R_o + \Delta \longrightarrow (\neq)$$
$$(\neq) \longrightarrow P^{\neq} \qquad (2)$$
$$P^{\neq} \longrightarrow P_o + \Delta$$

product molecule $P^{\neq}$, which disposes of its excess energy by evolving heat. The latter process (eqn. 2) is common, the former (eqn. 1) is rare.

Besides thermal activation, molecules can be induced to react on irradiation with light of the appropriate wavelength. Typically, as illustrated in eqn. 3,

$$R_o + h\nu \longrightarrow R^*$$
$$R^* \longrightarrow P^{\neq} \qquad (3)$$
$$P^{\neq} \longrightarrow P_o + \Delta$$

the ground state reactant molecule R_o absorbs a photon to produce an electronically excited reactant R*, which utilizes the acquired electronic energy to promote a chemical reaction, affording the vibrationally excited product molecule $P^{\neq}$. The latter evolves heat. Therefore, the chemiluminescent process shows traits which are common to both modes of promoting chemical reactions, i.e. by means of heat or light. Specifically, in the chemi-energized mode, heat is utilized to produce the electronically excited state, while in the photo-energized mode light is used. Irrespective of its past history, the excited state intermediate exhibits its presence either through photophysical changes (luminescence, energy transfer, etc.) or photochemical changes (fragmentations, isomerizations, rearrangements, cycloaddition, etc.

The three processes in eqns. 1-3 are illustrated in form of energy level diagrams in Fig. 1. Clearly, it is the favorable exothermicity of the chemi-

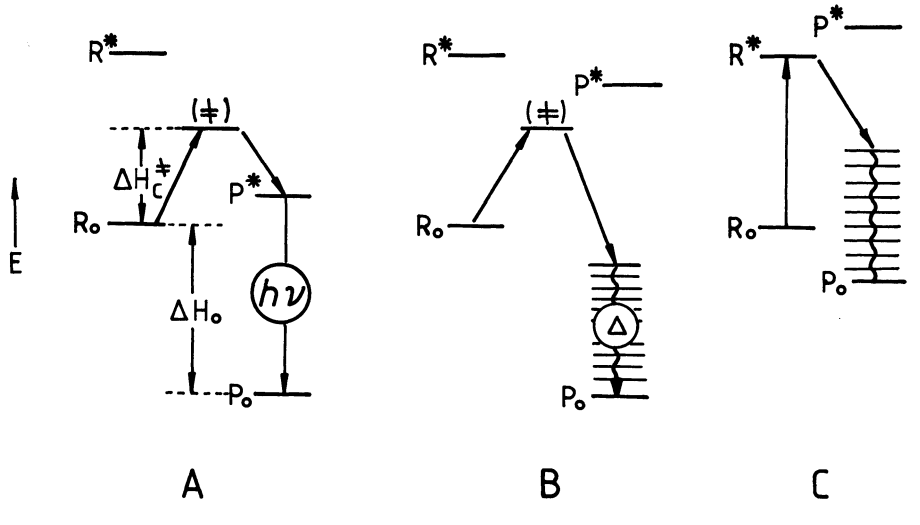
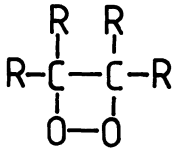


Fig. 1. Energy level diagrams of chemi-energized luminescent reaction (A), exothermic thermal reaction (B), photo-energized photochemical reaction (C).

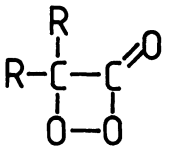
energized process which leads to electronically excited product P* rather than vibrationally excited product $P^{\neq}$. Thus, one of the most important conditions that a molecule decomposes on heating into electronically excited product is that the sum of its activation enthalpy ($\Delta H^{\neq}$) and reaction enthalpy ($\Delta H°$) be greater than the excitation energy (E*) of the electronically excited product (eqn. 4). We designate such a molecule as hyperenergetic.

$$\Delta H^{\neq} + (-\Delta H_o) \geq E^* \qquad (4)$$

An important class of such "high energy" molecules are the 1,2-dioxetanes (1) and their derivatives, namely the dioxetanones (2), also designated as α-



(1)



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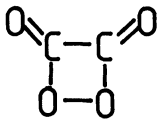


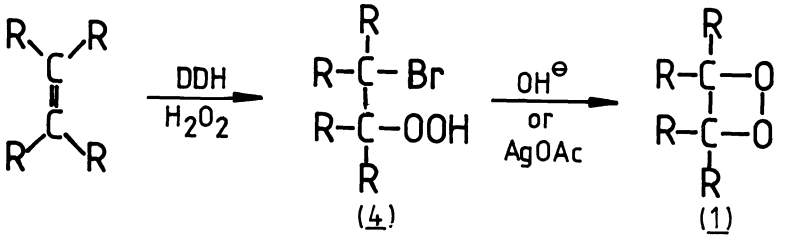
(3)

peroxylactones, and the 1,2-dioxetanedione (3), Ref. 2. Of these, the first fully characterized example was the trimethyl-1,2-dioxetane (1a), discovered by Kopecky and Mumford (Ref. 3), which marked the beginning of this exciting area of chemistry. Shortly after, we reported (Ref. 4) the preparation, isolation and characterization of the first stable α-peroxylactone, specifically the t-butyl derivative (2a). These labile materials had previously been postulated as the active intermediates in luciferin bioluminescence, responsible for the light emission by living organisms, e.g. the firefly (Ref. 5). The 1,2-dioxetanedione (3), or carbon dioxide dimer, has been actively investigated by Rauhut et al. (Ref. 6) for commercial exploitation as emergency light. While this industrial application of chemiluminescence has come to fruition, it is still dubious whether the postulated 1,2-dioxetanedione (3) indeed intervenes in the perhydrolysis of aryl oxalates. At least to date no spectroscopic nor chemical evidence has been reported, other than the light emission in the presence of fluorescers, that confirm the existence of the cyclic structure. Since our work has been exclusively concerned with the 1,2-dioxetanes (1) and α-peroxylactones (2), we limit the present discussion to our own experiences on these two systems.

SYNTHETIC ASPECTS

1,2-Dioxetanes.

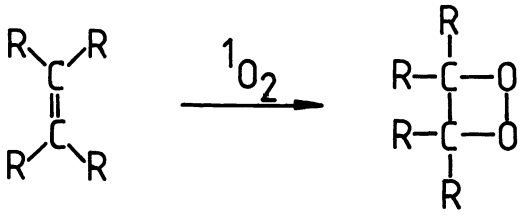
As already stated in the Introduction, the very first four-membered ring cyclic peroxide to be characterized was trimethyl-1,2-dioxetane (1a), prepared according to the general sequence shown in eqn. 5 (Ref. 7). DDH stands for



(4) (1) (5)

1,3-dibromo-5,5-dimethylhydantoin, concentrated hydrogen peroxide (CAUTION!) is used, and as cyclizing agents either base or silver salts are employed, the former for secondary and the latter for tertiary β-bromohydroperoxides (4).

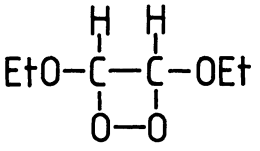
Alternatively, 1,2-dioxetanes (1) can be prepared via singlet oxygenation of suitable olefin substrates (eqn. 6), as first demonstrated by Bartlett and Schaap (Ref. 8) for the 3,4-diethoxy-1,2-dioxetane (1b). For alkyl- or aryl-



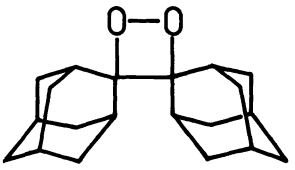
(6)

(1)

substituted olefins complicating side reactions are the ene-reaction

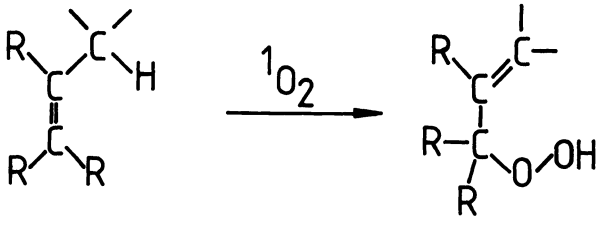


(1b)



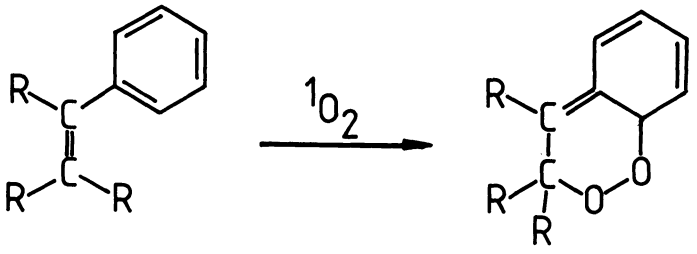
(1c)

(eqn. 7) and the (2+4)-cycloaddition involving the aryl substituent



(7)

(eqn. 8), Ref. 9. The ene-reaction (eqn. 7) can be suppressed when the allylic hydrogens are at bridge-head positions. An illustrative example is diada-

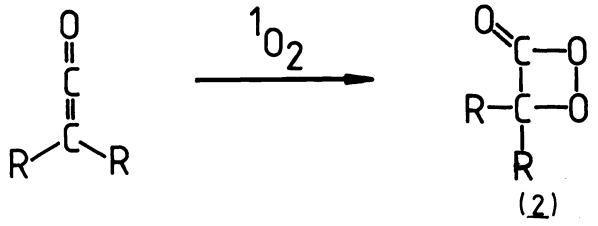


(8)

mantylidene, affording the 1,2-dioxetane (1c) quantitatively on singlet oxygenation (Ref. 10).

α-Peroxylactones

The most direct route to α-peroxylactones (2) is via singlet oxygenation of ketenes (Ref. 11), as shown in eqn. 9. The limitations are, of course, that

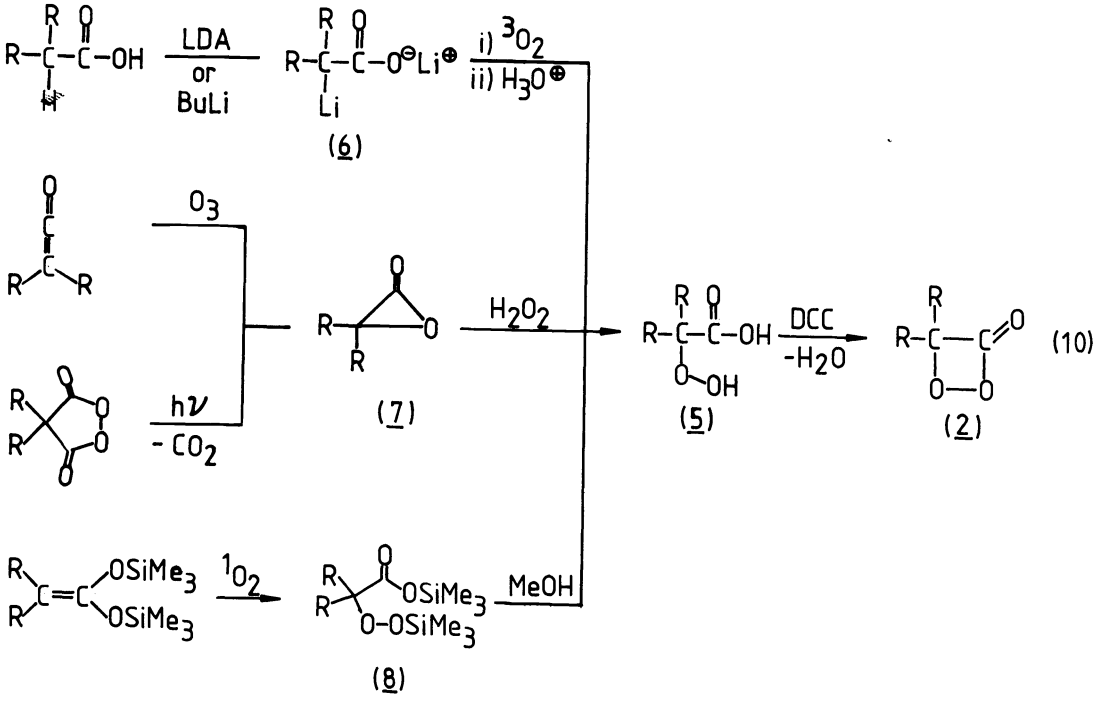


(9)

(2)

stable ketenes, such as the electronically deactivated bis(trifluoromethyl)-ketene or the sterically hindered di-tert-butylketene, are unreactive towards $^1O_2$, while the reactive ketenes readily dimerize. Yet, this direct preparative method enabled the synthesis of dimethyl- and diphenyl-α-peroxylactones (Ref. 11).

A more general synthetic route, especially at the preparative scale, is the cyclodehydration of α-hydroperoxy acids (5), as illustrated in eqn. 10. Di-



cyclohexylcarbodiimide (DCC) has proven itself as most effective cyclodehydrating agent, in view of its great reactivity even at subambient temperatures, its inert nature towards the extremely labile and sensitive α-peroxylactone product, and the fact that the inert dicyclohexylurea precipitates during the cyclization step (Ref. 12). The more elaborate task is the preparation of the acid- and base-sensitive α-hydroperoxy acids (5), which serve as precursors to the α-peroxylactones (2). Three general routes for the preparation of the essential α-hydroperoxy acids (5) are exhibited in eqn. 10. These involve low temperature (-78°C) oxygenation of α-lithiocarboxylates (6), perhydrolysis of α-lactones (7), and desilylation of α-silylperoxyesters (8), conveniently prepared via singlet oxygenation of ketene acetals.

In the subsequent discussion, it is assumed that the 1,2-dioxetanes (1) and α-peroxylactones (2) that are utilized herein can be prepared via the above synthetic methods. We will, therefore, not enter into details concerning their preparation. Before considering some mechanistic problems, we shall review the important methods for determining the excitation yields of these interesting molecules.

ANALYTICAL ASPECTS

Singlet Yields

The most direct method is the quantitation of the fluorescence emission observed in the chemiluminescent decomposition of the hyperenergetic molecule. This method is specific for chemi-energized singlet states because most electronically excited molecules do not phosphoresce due to rapid deactivation of the triplet states in solution and in the presence of molecular oxygen. These are the usual conditions for dioxetane chemiluminescence.

In such cases the direct chemiluminescence quantum yield (ϕ_K^{DC}) is given by eqn. 11, where ϕ^S is the singlet excitation yield of the chemi-energized pro-

$$\phi_K^{DC} = \phi^S \cdot \phi_K^{fl} = I_K^{DC}/k[D] \qquad (11)$$

cess and ϕ_K^{fl} is the fluorescence yield of the chemi-energized singlet state excited carbonyl product $^SK^*$. The quantum yield ϕ^{DC} is determined experimentally for the system under study by measuring the rate of photon production, given by I_K^{DC} the direct chemiluminescence intensity, versus the rate of dioxetane decomposition (k[D]), as shown in eqn. 11. The experimental details are well described and shall not be reiterated here, except to say that it is critical to carry out reliable standardization of the light yield (Ref. 13). Furthermore, it is essential that the structure of the singlet excited carbonyl product be known through spectrofluorimetry, so that the fluorescence quantum yield (ϕ^{fl}) can be assigned. Frequently it is required to measure ϕ_K^{fl} experimentally if it is not reported in the literature under the conditions of the chemi-energization. Once ϕ^{DC} has been measured and ϕ_K^{fl} is available either from the literature or by experiment, it is a simple matter to calculate the desired ϕ^S parameter.

Frequently it is not possible to know the structure of the excited singlet state carbonyl product, e.g. the direct chemiluminescence is too weak to record a fluorescence spectrum or the product only very weakly fluoresces. In that case the direct chemiluminescence technique to determine ϕ^S is not applicable. To circumvent such problems, one resorts to energy transfer chemiluminescence. In other words, the chemi-energization process is performed in the presence of an efficient fluorescer (Fl) which accepts the singlet excitation energy of $^SK^*$. The chemi-energized $^SFl^*$ then efficiently emits its excitation energy, thereby enhancing the light output.

A typical fluorescer for singlet state determinations is 9,10-diphenylanthracene (DPA), Ref. 14, for which the enhanced chemiluminescence yield (ϕ_{DPA})is given by eqn. 12, where ϕ_{ET}^{SS} is the singlet-singlet energy transfer efficiency. As before, ϕ_{DPA}^{EC} is measured experimentally by determining the enhanced chemi-

$$\phi_{DPA}^{EC} = \phi^S \cdot \phi_{ET}^{SS} \cdot \phi_{DPA}^{fl} = I_{DPA\infty}^{EC}/k[D] \qquad (12)$$

luminescence intensity, but at infinite DPA concentration (extrapolated from a double reciprocal plot of the emission intensity and DPA concentration) and the rate of dioxetane decomposition (k[D]), as shown in eqn. 12. At infinite DPA concentration the energy transfer efficiency ϕ_{ET}^{SS} is 100%. Since the DPA fluorescence yield (ϕ_{DPA}^{fl}) is known, with the help of eqn. 12 the singlet excitation yield is readily computed. A few selected ϕ^S values of some new dioxetanes, together with tetramethyl-1,2-dioxetane (1d), dimethyl-1,2-dioxetanone (2a), are listed in Table 1 for comparison.

Triplet Yields

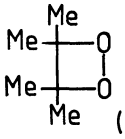
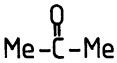
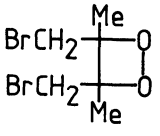
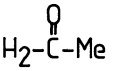
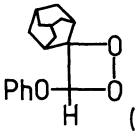
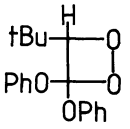
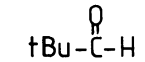
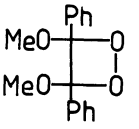
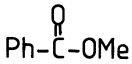
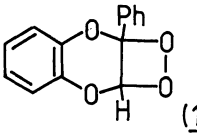
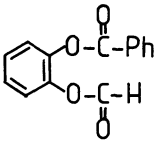
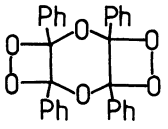
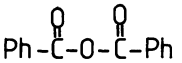
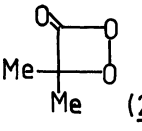
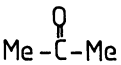
Triplet excitation yields have been notoriously difficult to determine because triplet states do not usually emit light, i.e. phosphoresce, either directly or energized via energy transfer. However, in the latter case the energy transfer chemiluminescence with 9,10-dibromoanthracene (DBA) has proven useful for the determination of triplet excitation yields (ϕ^T). The heavy bromine atoms enhance the spin-forbidden triplet-singlet energy transfer (eqn. 13) through spin-orbit coupling (Ref. 15).

$$^TK^* + DBA_o \rightarrow K_o + {}^SDBA^* \qquad (13)$$

The enhanced chemiluminescence yield for DBA (ϕ_{DBA}^{EC}), analogous to DPA, is given by eqn. 14, where ϕ_{ET}^{TS} is the triplet-singlet energy transfer efficiency

$$\phi_{DBA}^{EC} = \phi^T \cdot \phi_{ET}^{TS} \cdot \phi_{DBA}^{fl} = I_{DBA\infty}^{EC}/k[D] \qquad (14)$$

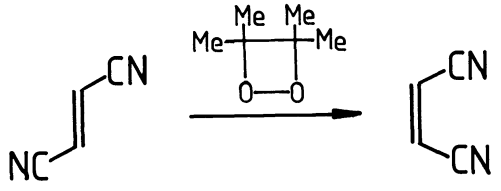
TABLE 1. Singlet and triplet excitation yields of 1,2-dioxetanes via DPA and DBA enhanced chemiluminescence.[a]

Dioxetane	Excited Product[b]	ϕ^S (%)	Ref.	ϕ^T (%)	Ref.	ϕ^{T+S} (%)	ϕ^T/ϕ^S
 (1d)		0.04 ±0.01	22	31 ±8	22	31	700
 (1e)		0.0057 ±0.0007	24	0.0071 ±0.0006	24	0.013	1.2
 (1f)		1.2 ±0.1	25	5.0 ±0.3	25	6	4
 (1g)		0.031 ±0.004	26	0.095 ±0.003	26	0.13	3
 (1h)		0.020 ±0.001	25	12.0 ±0.6	25	12	600
 (1i)		0.00082 ±0.00008	27	11 ±1	27	11	10,000
 (1j)		0.10 ±0.01	21	22 ±2	21	22	220
 (2a)		0.050 ±0.002	13[c]	1.5	23[d]	1.6	30

a. Hastings scintillation cocktail was used as light standard.
b. n,π* excitation
c. Direct fluorescence
d. Direct phosphorescence.

(which is 0.25±0.05 at infinite DBA concentration, Ref. 16) and ϕ^{fl}_{DBA} the fluorescence yield of DBA. The ϕ^{EC}_{DBA} quantity is experimentally determined by quantitating the enhanced chemiluminescence intensity of DBA (I^{EC}_{DBA}) at infinite DBA concentration (extrapolated from a double reciprocal plot of I^{EC}_{DBA} versus ϕ^{fl}_{DBA}) and the rate of dioxetane decomposition ($k_d[D]$). Since ϕ^{TS}_{ET} is known and ϕ^{TS}_{ET} is 0.25±0.05 under these conditions, the desired ϕ^{T} parameter can readily be assessed. A few typical ϕ^{T} values of some new dioxetanes, together with (1d) and (2a), are listed in Table 1 for comparison.

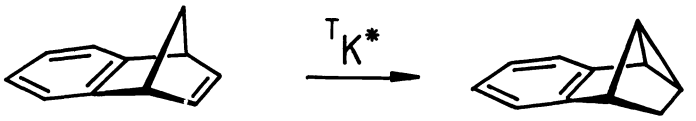
An alternative method for determining triplet yields of chemi-energized processes is chemical titration. The excited triplet state carbonyl product transfers its excitation energy selectively to a suitable acceptor which then undergoes a spin-state specific photochemical transformation. The first application of this novel method has been the cis-trans isomerization of fumaronitrile, chemi-energized by TMD (eqn. 15), Ref. 17. Subsequently, other



(15)

triplet state titrants have been developed, including stilbene isomerization (Ref. 18), oxetane formation (Ref. 19), etc.

Recently we have employed the triplet state-specific photo-rearrangement of benzonorbornadiene (BND) into benzotricyclonorbornene (BTN) (eqn. 16) for the



(16)

BND BTN

titration of chemi-energized triplet carbonyl products derived from 1,2-dioxetanes (Ref. 20). The results are summarized in Table 2. The advantage of this method is that BND is readily available, the BTN is sufficiently thermally stable for VPC analysis, the photo-isomerization yield is high (ca. 50%), and the photo-isomerization triplet state-specific. The most serious disadvantage is that the triplet state of BND is high (ca. 70 kcal/mole) which limits this method for the titration of chemi-energized triplet state carbonyl products with triplet energies greater than 70 kcal/mole. Clearly, it seems advisable to develop additional chemical titration methods for the determination of singlet and triplet products.

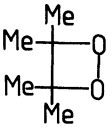
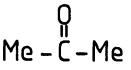
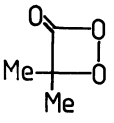
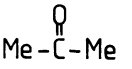
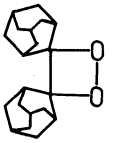
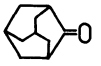
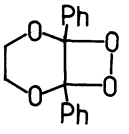
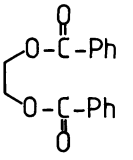
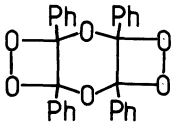
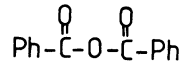
MECHANISTIC ASPECTS

In the following we take up some typical mechanistic problems concerning the thermal decomposition of 1,2-dioxetanes which have preoccupied us intensively during the last decade. The prime motivation was to understand the chemi-energization process, in which chemical energy is set free as electronic energy as witnessed through the phenomenon of light emission. Much has been learned, yet more remains still obscure.

Relative excitation efficiencies of 1,2-dioxetanes versus α-peroxylactones

In Fig. 2, we contrast the energetics of the thermolysis of tetramethyl-1,2-dioxetanes (1d) versus dimethyl α-peroxylactone (2a) in terms of a heat of formation (ΔH_f) diagram. The relative heats of reaction (ΔH_r) and heats of formation of the diradicals (ΔH_f^{DR}) were estimated from thermochemical calculations (Ref.28), the activation enthalpies ($\Delta H^{\neq}$) were determined experimentally by means of isothermal kinetics (Ref. 2), and the singlet and triplet n,π^* excitation energies of the chemi-energized carbonyl products come from photochemical data (Ref. 29).

TABLE 2. Triplet excitation yields of 1,2-dioxetanes via benzonorbornadiene titration.

Dioxetane		Excited Carbonyl Product	ϕ^T(%)
	(1d)		31±2[a]
	(2a)		1.1±0.1[a]
	(1c)		1.6±0.1[a]
	(1i)		21±2[a]
	(1j)		22±1[b]

a. Ref. 20
b. Ref. 21.

Both hyperenergetic reactants generate electronically excited (n,π*) acetone, but their leaving groups are distinct, i.e. CO_2 for the dimethyl α-peroxylactone (2a) and CH_3COCH_3 for tetramethyl-1,2-dioxetane (1d). In the former case also electronically excited CO_2 could be formed; however, as the right-hand part of Fig. 2 reveals, (2a) is not energy sufficient to chemi-energize CO_2. Furthermore, we observe in Fig. 1 that the decarboxylation process of the α-peroxylactone (2a) is by ca. 22 kcal/mole (ΔH_r values are 91 and 69 kcal/mole, respectively) more exothermic than the 1,2-dioxetane (1d) and requires ca. 4 kcal/mole ($\Delta H^{\neq}$ values are 21 and 25 kcal/mole, respectively) less activation energy. Consequently, the acetone n,π* singlet state lies 28 versus 10 kcal/mole below the activated complexes of (2a) and (1d), respectively, while the n,π* triplet state lies 34 versus 16 kcal/mole below, respectively. This is to say that the $(2a)^{\neq} \rightarrow CH_3COCH_3^* + CO_2$ step is more than double more energetic than the $(1d)^{\neq} \rightarrow CH_3COCH_3^* + CH_3COCH_3$ step. The differentiation in the relative exothermicities is still more dramatic for the respective diradical intermediates, if these are bona fide reaction intermediates.

On the basis of the relative energetics for these two systems we would anticipate that the α-peroxylactone (2a) should have the greater propensity to chemi-energize the singlet and triplet n,π* excited states of acetone. The excitation yields in Table 1 clearly contradict this expectation. For example, the total excitation efficiency (ϕ^{T+S}) is only ca. 1.6% for (2a) but ca. 31%

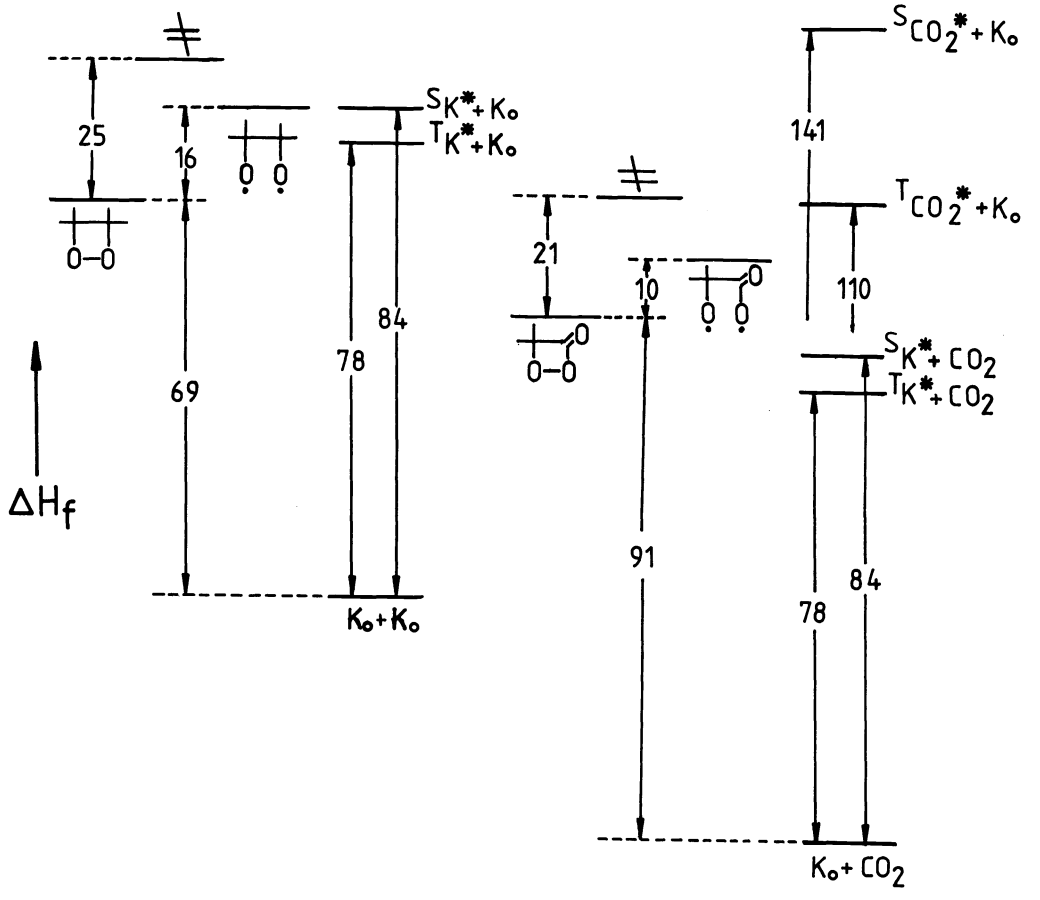


Fig.2. Heat of formation (ΔH_f) diagram for the thermolysis of tetramethyl-1,2-dioxetane (left) and dimethyl α-peroxylactone (right), their activated complexes (≠) and their diradical intermediates.

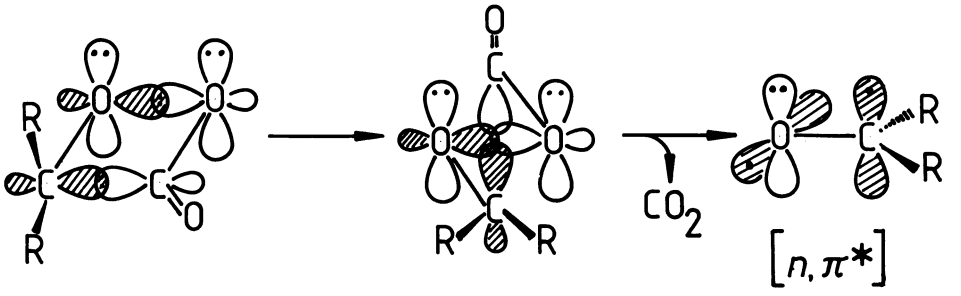
for (1d). Thus, the more energetic α-peroxylactone (2a) is ca. 20-fold less effective in chemi-energizing electronically excited acetone than the 1,2-dioxetane (1d).

The spin state selection, however, can be rationalized in terms of the relative energetics of these two substrates. For example (Table 1), the triplet/singlet excitation yield ratio (ϕ^T/ϕ^S) is ca. 700 for (1d) and ca. 30 for (2a) Inspection of Fig. 2 makes it quite evident that the 1,2-dioxetane (1d) should discriminate between singlet and triplet excited acetone much more effectively than the α-peroxylactone (2a) in view of the exothermicity of the latter. However, the situation is again more complex than anticipated. The discrimination resides completely in the triplet excitation yields (ϕ^T). Thus, the singlet excitation yields (ϕ^S) for the two substrates are essentially the same, i.e. ϕ^S values are ca. 0.04±0.01% (1d) and ca. 0.050±0.002% (2a). On the other hand, the triplet yields (ϕ^T) are ca. 20-fold smaller for (2a), i.e. the ϕ^T values are 31% (1d) and 1.5% (2a). Again this does not follow common sense expectation on the basis of the relative energetics involved, because the differentiation should be more pronounced for the singlet excitation rather than the triplet excitation process. We are tempted to conclude that the re-

lative exothermicities do not dictate the total excitation yields (ϕ^{T+S}) nor the spin state selections (ϕ^T/ϕ^S) in the chemi-energization of acetone by the 1,2-dioxetane (1d) and α-peroxylactone (2a). Of course, it must be stressed that the "high energy" molecule must be energy sufficient so that chemi-energization is feasible in the first place; but once this energy balance condition is fulfilled, other factors determine the efficiency and spin state selection.

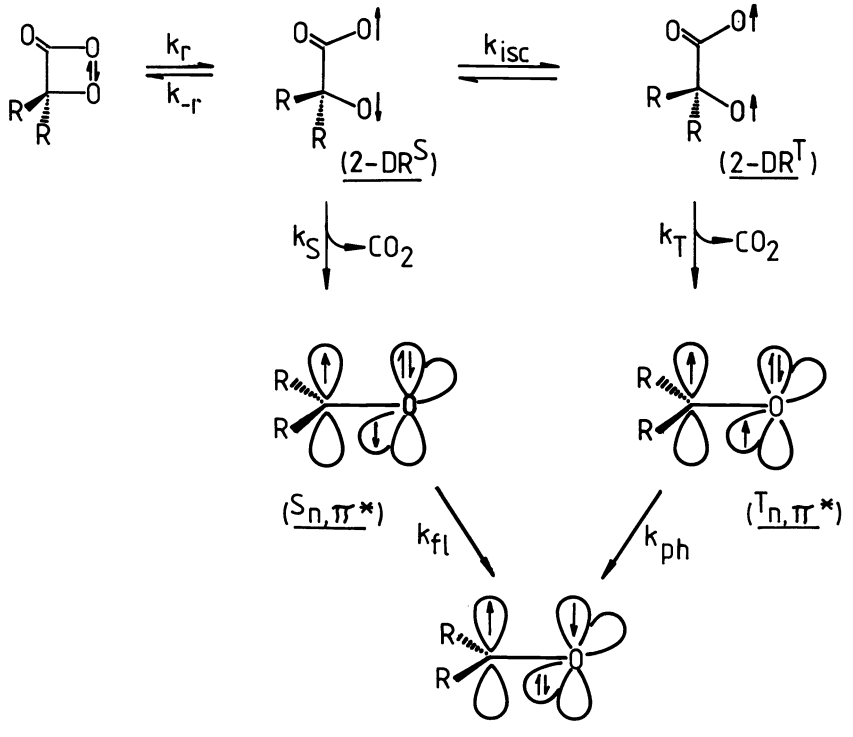
Concerted versus diradical path

One way to reconcile the contrasting behavior of 1,2-dioxetanes (1) versus α-peroxylactones (2) would be to postulate different mechanisms of decomposition, e.g. a concerted versus a diradical path. The two possibilities are illustrated for α-peroxylactones (2) in eqn. 17 (concerted) and eqn. 18 (step-



(17)

wise) in terms of the orbitals that are involved. In the concerted path (eqn. 17) the planar α-peroxylactone presumably first puckers, thereby alligning

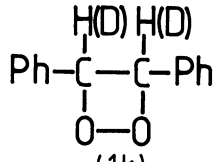


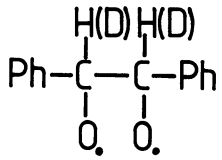
(18)

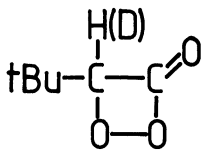
the orbitals optimally to generate an n,π* excited state of the carbonyl product. Alternatively, in the stepwise decomposition the singlet diradical (2-DR^S) is first formed, which either decarboxylates via step k_S to generate a singlet n,π* excited state product or suffers intersystem-crossing via step k_{isc} to afford the triplet diradical (2-DR^T). The latter diradical fragments into the triplet n,π* excited state product. Photo-deactivation leads to the ground state product, accompanied by fluorescence (common) or phosphorescence (rare), respectively from the singlet or triplet n,π* excited state. If the condition $k_T > k_{isc} > k_S$ applies, then the ϕ^T/ϕ^S ratio will be high, thus favoring triplet excitation. In the concerted path the ϕ^T/ϕ^S should be governed by the efficiency of spin-orbit coupling in the puckered transition state. The concerted mechanism was first suggested by Turro (Ref. 30) and the diradical mechanism by Richardson (Ref. 28). For the simpler 1,2-dioxetanes (1) the question of concerted versus stepwise decomposition is still being debated; but the majority of experimental facts point to the diradical mechanism. We now present our attempts to make such mechanistic distinctions.

Isotope effects

It was shown (Ref. 31) that the secondary isotope effect for 3,4-diphenyl-1,2-dioxetane (1k) was unity. This suggested that the diradical (1k-DR) is formed


(1k)


(1k-DR)

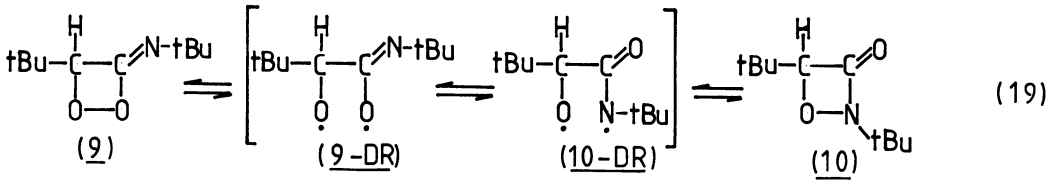

(2b)

in the rate determining step since otherwise carbon-carbon scission should have reflected a secondary isotope effect.

We examined the secondary isotope effect for the α-peroxylactone (2b) and found $k_H/k_D = 1.06 \pm 0.03$ (Ref. 31). Unfortunately, on the basis of this experimental value it is difficult to make a clear-cut distinction between concerted and stepwise decomposition. It is of interest to mention that the triplet excitation yield was ca. 5-fold greater for the deuterated α-peroxylactone (2b), but the singlet yields were the same within experimental error. Apparently deuteration does help intersystem-crossing; however, whether this manifests itself in the puckered activated complex (concerted) or in the diradical intermediate (stepwise) is difficult to differentiate.

3-Imino-1,2-dioxetane.

As a probe for diradical intermediates we prepared the 3-imino-1,2-dioxetane (9), via photosensitized singlet oxygenation of ketenimines (Ref. 32). If the diradical (9-DR) intervenes (eqn. 19), it was argued that through its valence


(9) (9-DR) (10-DR) (10) (19)

isomer (10-DR) it might recyclize into the 1,2-oxazetidin-3-one (10). Although the latter are known, stable heterocycles (Ref. 33) with well defined carbonyl bands at 1780-1800 cm^{-1} in the infrared, the only products were those of fragmentation. Again, this result is inconclusive as far as the mechanism of dioxetane decomposition is concerned, because either the diradical (9-DR) is not formed or if it is formed, it fragments faster than valence-isomerizing to diradical (10-DR).

Heavy atom effects

Since 1,2-dioxetanes selectively chemi-energize triplet states (ϕ^T/ϕ^S large, Table 1), a spin-forbidden reaction is involved in this process in which a ground state hyperenergetic molecules decomposes thermally into a triplet state excited product (eqn. 20). In such intersystem-crossing reactions spin-

orbit coupling is one of the principal mechanisms of action, which is promo-

$$S_{R_o} \xrightarrow{\Delta} T_{P^*} \qquad (20)$$

ted through "heavy atom" perturbations (Ref. 34). It was, therefore, of interest to explore the "heavy atom" effect in the thermal decomposition of 1,2-dioxetanes. For this purpose we decided to determine the activation parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$) and excitation parameters (ϕ^{T+S} and ϕ^{T}/ϕ^{S}) of dioxetane (1e) and compare them with those of (1d). The excitation parameters are summarized in Table 1 (Ref. 24) and the activation parameters are within the experimental error the same for dioxetanes (1d) and (1e), Ref. 35.

In the case of a concerted fragmentation, in which both the oxygen-oxygen and carbon-carbon bonds are being disengaged in the activated complex, we would expect that the "heavy atom" would promote this intersystem-crossing step through spin-orbit coupling. The $\Delta H^{\neq}$ term should have been lower for the dibromo derivative (1e) than for (1d). This expectation is not borne out in our data. Either the perturbation is too small to be sensed by such kinetic effects, or the decomposition mechanism is not concerted.

The situation is different as far as the excitation parameters are concerned (Table 1). We observe a dramatic reduction in the triplet excitation yield (ca. 4000-fold) and also to an appreciable extent in the singlet excitation yield (ca. 7-fold). In fact, the bromine substituents extinguish the ability of dioxetane (1e) to chemi-energize electronic excitation compared to (1d) since ϕ^{T+S} are ca. 0.013% versus 31%, respectively. Also surprising is the finding that there is no spin state selection for (1e) compared to (1d) since ϕ^{T}/ϕ^{S} are ca. 1 versus 700, respectively.

Thus, the dibromo-1,2-dioxetane (1e) behaves very differently in its excitation properties than the 1,2-dioxetane (1d), although both exhibit the same kinetic behavior. Assuming that a diradical mechanism obtains, since the activation parameters speak against concerted decomposition, the bromine substituent can be rationalized in terms of the "heavy atom" effect, as illustrated in Fig. 3.

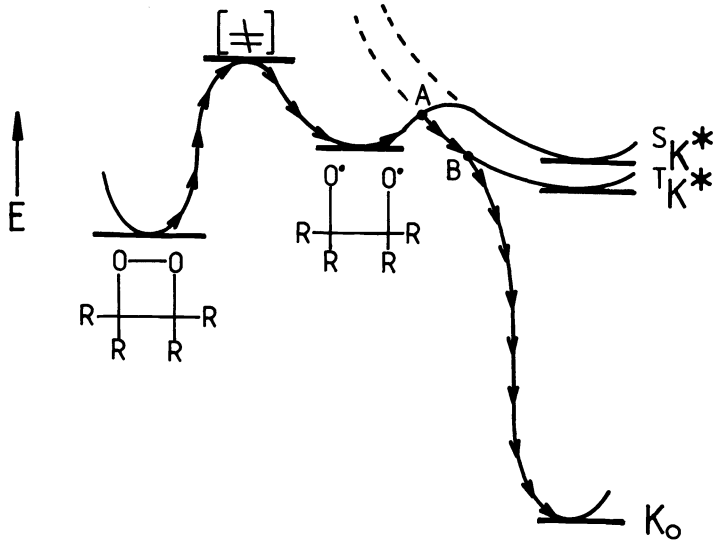
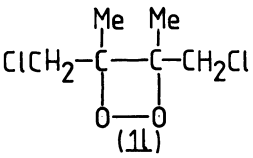


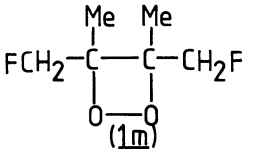
Fig. 3 Heavy atom effect by bromine substituent on the decomposition of 1,2-dioxetanes.

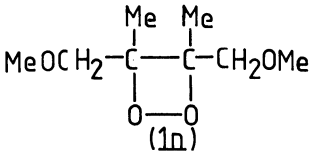
As the oxygen-oxygen bond is broken, a singlet diradical intermediate is formed first which then proceeds to fragment into electronically excited product. Through effective spin-orbit coupling by the heavy bromine atom, most of the energy is channeled onto the triplet energy surface (Fig. 3, Intersection A). Thus, the ϕ^{S} value is decreased for (1e) compared to (1d). However,

once on the triplet excited energy surface, again through the "heavy atom" effect, most of the energy is diverted along the singlet ground state energy surface (Fig. 3, Intersection B). Consequently, the bromine atom through its spin-orbit coupling efficiency provides a by-pass not only for the singlet excited state product $^{S}K^{*}$, but also for the triplet excited state product $^{T}K^{*}$, leading to ground state product.

The worrisome point about this mechanistic interpretation of low excitation yield of the dibromo-1,2-dioxetane (1e) versus (1d) is that the "heavy atom" effect is much too large (Ref. 34). Alternatively, this may have nothing to do with the spin-orbit coupling propensity of bromine, but rather with its electron-withdrawing properties. To sort this out, we plan to examine the excitation parameters of the dichloro-, difluoro-, and dimethoxy-1,2-dioxetanes (1ℓ-n). So far it has been difficult to prepare these dioxetanes.

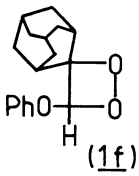

(1ℓ)

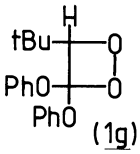

(1m)

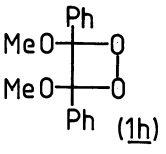

(1n)

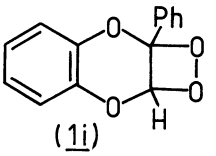
Hetero-atom substitution

To test the effect of electron-withdrawing substituents more directly, we examined the hetero-atom substituted 1,2-dioxetanes (1f-i). Here the alkoxy or


(1f)


(1g)


(1h)

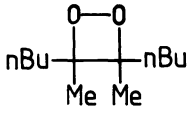

(1i)

aryloxy substituent is bound to the α-carbon of the peroxide linkage and the effect should be more pronounced. Furthermore, such oxygen substitution, especially in the 1,2-dioxetane (1g), was intended to simulate the effect of the carbonyl oxygen atom in the α-peroxylactones. Of course, this rests on the premise that electron withdrawal is responsible for the substantially lower excitation yields of the α-peroxylactone (2a) versus the 1,2-dioxetane (1d). We chose the unsymmetrically substituted derivatives (1f) and (1g) versus the symmetrically substituted (in terms of oxygen) derivatives (1h) and (1i) and assessed their excitation parameters. Of course, our initial efforts attempted to utilize a series in which the other substituents were structurally as similar as possible; but unfortunately synthetic difficulties detained us. The results are summarized in Table 1.

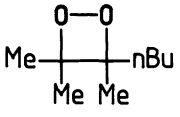
The data show that the unsymmetrical derivatives (1f) and (1g), especially the latter, show considerably lower ϕ^{T+S} values, e.g. ca. 50-fold lower for the diphenoxy-substituted (1g) compared with the monophenoxy-substituted (1f). It is of interest to note that the ϕ^{T}/ϕ^{S} ratios are about the same for (1f) and (1g), but both much lower (ca. 200-fold) than for the 1,2-dioxetane (1d). Thus, these aryloxy-substituted 1,2-dioxetanes reflect the general differences in the excitation parameters between the α-peroxylactones (2) and 1,2-dioxetanes (1). On the other hand, the symmetrically substituted 1,2-dioxetanes (1h) and (1i) exhibit much higher total excitation efficiencies (ϕ^{T+S} ca. 12%) and large spin state selectivities ($\phi^{T}/\phi^{S} > 100$). These resemble more closely the 1,2-dioxetanes (1) in their excitation behavior.

Although to this date we have not been able to answer with certainty the question whether a concerted or stepwise mechanism obtains in the thermal decomposition of dioxetanes, our experimental evidence points preferentially to the intervention of diradical intermediates. The observation that symmetrically substituted derivatives show i) greater excitation yields (ϕ^{T+S}) and ii) larger spin state selectivities (ϕ^{T}/ϕ^{S}) merit further mechanistic scrutiny. Examination of the excitation parameters on reported dioxetanes suggest that this empirical trend is quite general, but of course there are many exceptions. A few examples are shown below. The symmetrical derivatives (1o) and (1r) are

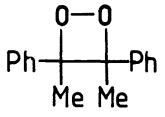
considerably more efficient in chemi-energizing electronically excited states



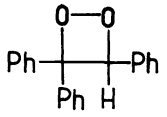
(1o)



(1p)



(1r)



(1s)

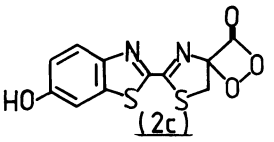
% ϕ^{T+S}	25	0.5	30	0.5

than the unsymmetrical derivatives (1p) and (1s). Whether in the case of the unsymmetrical 1,2-dioxetanes exciplex formation is a possible cause for the low excitation yields is worth further investigation.

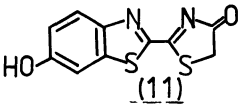
BIOLOGICAL ASPECTS

Bioluminescence

Whatever the reason, α-peroxylactones (2) are inefficient thermal sources for electronic excitation. Yet, in the firefly luciferin bioluminescence, which exhibits nearly quantitative yields of singlet excited states (Ref. 5), the α-peroxylactone (2c) was established as bona fide reaction intermediate, res-

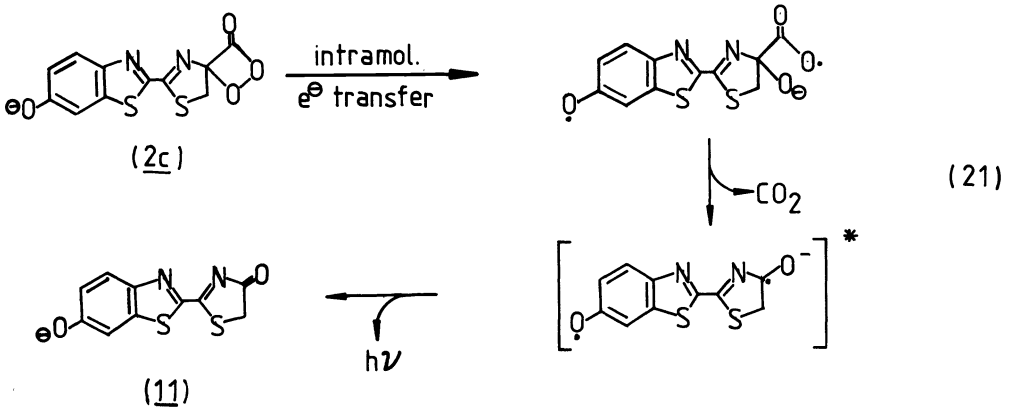


(2c)



(11)

ponsible for the yellow light emitted by the singlet excited state of the oxyluciferin (11). Two facts are striking about this bioluminescent system, i.e. the high excitation yield (ca. 100%) and that singlet excited states are bioenergized selectively. Both facts are to be contrasted with the model α-peroxylactone (2a), for which the excitation yield is low (ca. 1%) and that triplet excited states are chemi-energized selectively. Of course, the mystery behind this contrasting behavior of the biological system is intramolecular electron exchange, as illustrated in eqn. 21. The electron-rich benzenethiazolyl moiety

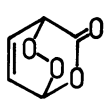


(21)

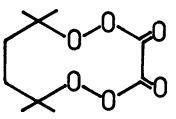
transfers an electron to the α-peroxylactone group and decarboxylation leads to the electronically excited oxyluciferin, followed by light emission (Ref. 37). The phenolate ion of (2c) is critical for this intramolecular electron exchange because in unionized form only feable bioluminescence is observed (Ref. 38). Model studies on intermolecular electron exchange between easily oxidized polycyclic aromatics and simple α-peroxylactones (Ref. 39) have helped in clarifying this mechanistic problem.

The electron exchange chemiluminescence mechanism appears to be quite general, provided the cyclic peroxide is energy sufficient. For example, we have shown recently that the α-pyrone endoperoxide (12), Ref. 40, and the cyclic peroxa-

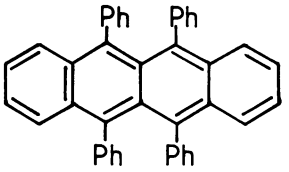
late (13), Ref. 41, release chemiluminescence when treated with easily oxidized aromatic hydrocarbons such as rubrene (14). However, compared to the



(12)



(13)

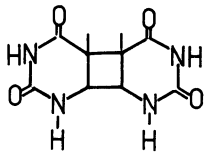


(14)

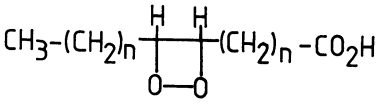
α-peroxylactones (2), the efficiency of electron exchange chemiluminescence is very low.

Interaction of dioxetanes with cells

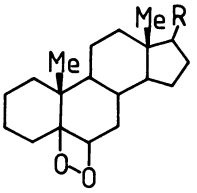
Since dioxetane (1d) releases a high density of triplet excited acetone (Table 1) and since such chemi-energized triplet acetone has led to thymine dimers (15) in cell-free DNA (Ref. 42), we expected that exposure of living



(15)



(1t)



(1u)

cells to (1d) could result in such photodamage of the genetic material of the cell. Furthermore, since enzyme-generated electronic excitation has been demonstrated in metabolic processes (Ref. 43), such photodamaging effects could have a natural origin. Finally, the connection between the photodamaging effects of UV-radiation on skin and its ultimate cause of skin cancer appears also established (Ref. 44). Consequently, we investigated the interaction of the 1,2-dioxetane (1d) with several cell lines, to explore the efficiency of such hyperenergetic substances in promoting cell mutations. Such mutations, if demonstrable, could provide evidence for the search of photodamaging interaction between living cells and "high energy" substances.

Preliminary studies revealed (Ref. 45) that dioxetane (1d) was absorbed by the cells and that it caused moderate mutation frequencies (ca. 10-fold greater than acetone). However, the simple dioxetane (1d) was too toxic and led mainly to cell death. It will be necessary to design dioxetanes derived from biomolecules, e.g. the fatty acid+steroid derived derivatives (1t) and (1u), respectively, whose toxicity might be sufficiently moderated to probe further into this fascinating problem.

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