Mechanism of Schiff Base Chemiluminescence: Evidence for an Intermediate Dioxetan†

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Summary The decomposition of a derivative of a peroxide obtained by oxidation of a Schiff base shows that chemiluminescence occurs *via* an intermediate dioxetan.

The suggestion[1] that dioxetans should be effective chemiluminiscent compounds has been amply confirmed. Indeed, every dioxetan so far isolated has been shown to generate excited states efficiently on decomposition.[2] However, for

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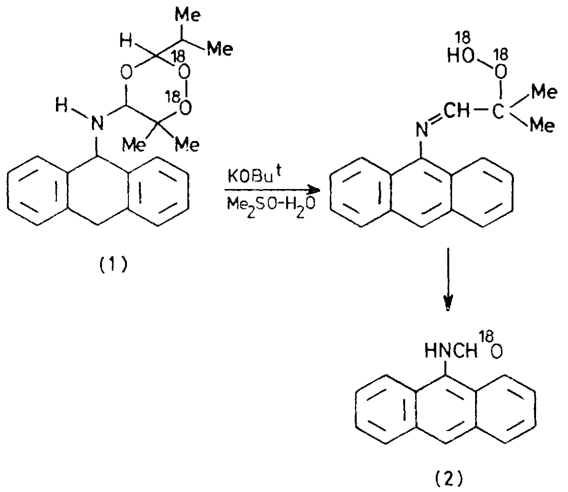
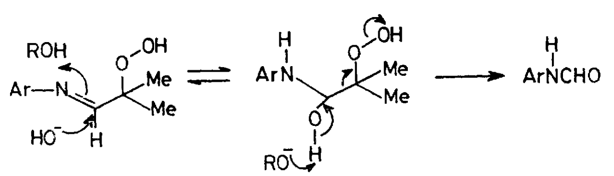
† No reprints are available.

those cases in which a dioxetan is inferred but not isolated during oxidation of a substrate by molecular oxygen, the situation is confusing. The most important examples in this category are those of the luciferins involved in bioluminescence. The use of $^{18}O_2$, $^{17}O_2$, and $H_2{}^{18}O$ in both chemical and biochemical experiments has provided evidence for a dioxetan mechanism in Cypridina[3] and firefly luciferin[4] (incorporation of $^{18}O_2$ into the CO_2 evolved) and against the dioxetan in Cypridina,[5] firefly,[6] and Renilla[7] luciferin (incorporation of $H_2{}^{18}O$). A large part of the confusion no doubt arises because the quantities used are necessarily small, the intermediate peroxide is unisolated, and collection of small quantities of CO_2 from aqueous solution or highly basic Me_2SO presents some problems.[4]

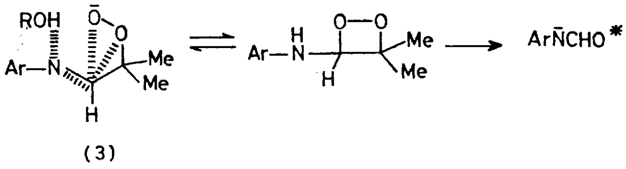
Although a dioxetan intermediate was demonstrated in the decomposition of indolyl hydroperoxides,[8] this evidence referred only to the ground state reaction since the quantum yield (a maximum detectable yield of excited states of 0·5%)[9] was too low to allow application of the results to the excited state pathway. The discovery[10] of the trioxan[11] (**1**) has allowed the investigation of the mechanism of Schiff base chemiluminescence in an unambiguous manner. We have recently shown[12] that the oxidation of Schiff bases can be a very efficient chemiluminescent reaction and the peroxide (**1**), an intermediate in the oxidation of the Schiff base derived from 9-aminoanthracene and isobutyraldehyde, is well suited to the study of the mechanism. The formation of the hydroperoxide from the trioxan is inferred from the labelling pattern and the fact that the parent Schiff base in the absence of additional aldehyde is autoxidised with a similar chemiluminescence efficiency. Related hydroperoxides have been isolated[8] following Schiff base oxidation. The trioxan was isolated in pure crystalline form with 25·5% enrichment in ^{18}O (Scheme 1) and gave a minimum quantum yield of 15% on decomposition by base under the conditions shown in the Table. The reaction is first order in (**1**) ($k = 0{\cdot}3\ s^{-1}$ in 95% Me_2SO) and it is clear from the total lack of incorporation of‡ $H_2{}^{18}O$ (30·8% enrichment, 10^{-2}% incorporation would have been detectable) that the linear route (Scheme 2) does not apply. The high and reproducible quantum yield allows the unequivocal statement that the cyclic route (Scheme 3) is a light yielding reaction. The 9-formamidoanthracene (**2**) was purified by crystallisation in each experiment and shown to exchange with water to a very small extent. Treatment of (**2**) with base in Me_2SO containing 5% $H_2{}^{18}O$ (v/v; 30·8% enrichment) for 40 min (twice the time normally allowed for reaction of the trioxan) showed an increase from 0·2 to 0·9% in the peak of mass number 223. This is an increase in ^{18}O content of 2·3% and represents, within experimental error (see Table), an insignificant contribution from exchange.



SCHEME 1



SCHEME 2



SCHEME 3

TABLE

% $H_2{}^{18}O$ in Me_2SO–$KOBu^t$ [a]	ϕ_R/% [b]	Incorporation of ^{18}O in (**2**)/%
0	18·4	99·7 ± 10
1	19·7	
2·5	18·4	
5·0	18·4	103·2 ± 2·5
5·0 ($H_2{}^{18}O$)	18·4	0·00 [using ^{16}O (**1**)]
10·0	14·5	

[a] Concentration of base $5{\cdot}0 \times 10^{-3}$ M. [b] Yield of excited states based on the yield of (**2**), typically >90% and the fluorescence quantum yield of the anion [λ_{max} (emission) 540 nm, ϕ_F 0·19].

Although our present experiments cannot resolve the conflicting results obtained in bioluminescence, they do raise the question of the source of the remarkable preference for internal peroxide attack. The high nucleophilicity of peroxide is well known and is usually ascribed to the α-effect.[13] However, at least one of the explanations for the effect cannot apply in the generation of four-membered rings. It has been suggested[14] that the $p\pi$–$p\pi$ repulsion, inevitably experienced in the ground state of the anion, is lost by progressive conformational staggering in the transition state. In four-membered rings there is very little opportunity for this energy minimising change. The transition state (**3**) suggested by calculations[15] of the

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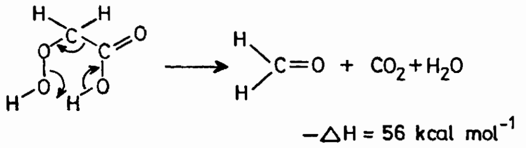
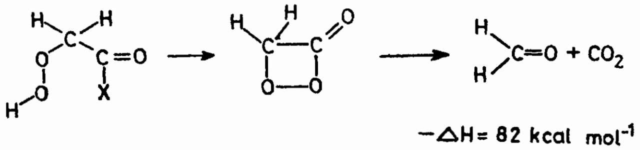
‡ The mass ratio 221 : 223 was determined using an AEI MS30 mass spectrometer with associated DS 50 computer. Multiple scanning and averaging was thus automatic. Tabulated results are also the mean of duplicate or triplicate experiments.

geometry of protonation of HO_2^-, combines the entropic advantage of the three-membered ring with the maximum use of the commonly accepted source of enhanced nucleophilicity in peroxides, the filled antibonding π orbital. The advantages of this pathway are likely to be retained in the enzymic reaction, and appear to operate in other model compounds[16] more closely related to the bioluminescent systems.



Scheme 4

It should also be noted that the shortest wavelength of light observed in the Renilla system[17] (340 nm) requires that the reaction produce a minimum of 85 kcal mol^{-1}.§ Although thermochemical data for the luciferins are not available, the statement[5,18] that the route in Scheme 4 will yield 100 kcal mol^{-1} must be treated circumspectly. Calculations¶ on the two model reactions (Schemes 4 and 5)



Scheme 5

seem to suggest that even with allowances for substitution and the addition of the activation energy, the loss of dioxetan strain energy may leave a considerable deficit for excited state formation.

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§ 1 calorie = 4·184 joule.

¶ The thermochemical calculations were made using data in S. W. Benson, 'Thermochemical Kinetics,' Wiley, New York, 1968. Scheme 4 is thermodynamically equivalent to removal of a proton by hydroxyl ion and regeneration of hydroxyl ion. Although energy changes as a result of differential solvation during this process may be expected to cancel, uncertainty as to solvation energy is inherent in all such calculations.

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