# Possible Mechanism for Bacterial Bioluminescence and Luminol Chemiluminescence

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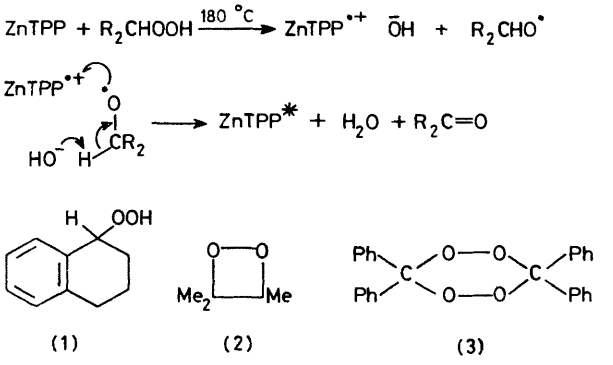
*Summary* The structures of the peroxides which react with flavinium salts with light emission, supported by a study of isotope effects, suggest an electron transfer mechanism with concomitant loss of a proton or H-atom; the mechanism may be applicable to the *in vivo* bacterial system and to luminol.

BACTERIAL bioluminescence[1] and the best known of the synthetic chemiluminescent compounds, luminol,[2] are among the outstanding problems in current research into the chemiluminescence of organic compounds. Extensive and elegant work in the laboratories of Hastings and White in particular has left it reasonably certain that they do not conform to the relatively well understood dioxetan[3] class of luminescent reaction. In contrast to the accepted mechanism for the classical luciferins and related model compounds,[4] no carbon–carbon bond is cleaved during the reaction. However, the loss of a hydrogen atom in the conversion of *e.g.* a long chain aldehyde into the corresponding carboxylic acid, in the bacterial system is central to any mechanistic consideration. In conjunction with recent developments in electron transfer chemiluminiescence[5,6] this provides the clue to a new and perhaps general mechanism.




SCHEME 1

The only previous example in the literature of a related mechanism is that suggested by Linschitz[7] for the reaction of primary or secondary hydroperoxides *e.g.* (**1**) with zinc tetraphenylporphin (ZnTPP, Scheme 1). It may be adapted to explain the excitation step in bacterial model systems,[8,9] (Scheme 2). There is an alternative pathway not considered by Linschitz involving the Russell mechanism† in which excited carbonyl products are first formed;[10] these subsequently transfer energy to an acceptor, in this case ZnTPP. However, this mechanism seems excluded by the relatively low quantum yields obtained by us using trimethyl dioxetan (**2**) and the peroxide (**3**) which are known to yield excited carbonyl products equally or more efficiently. Presumed electron transfer to a simple peracid and reaction with tertiary hydroperoxides under anaerobic conditions are similarly ineffective (Table 1). The metal is

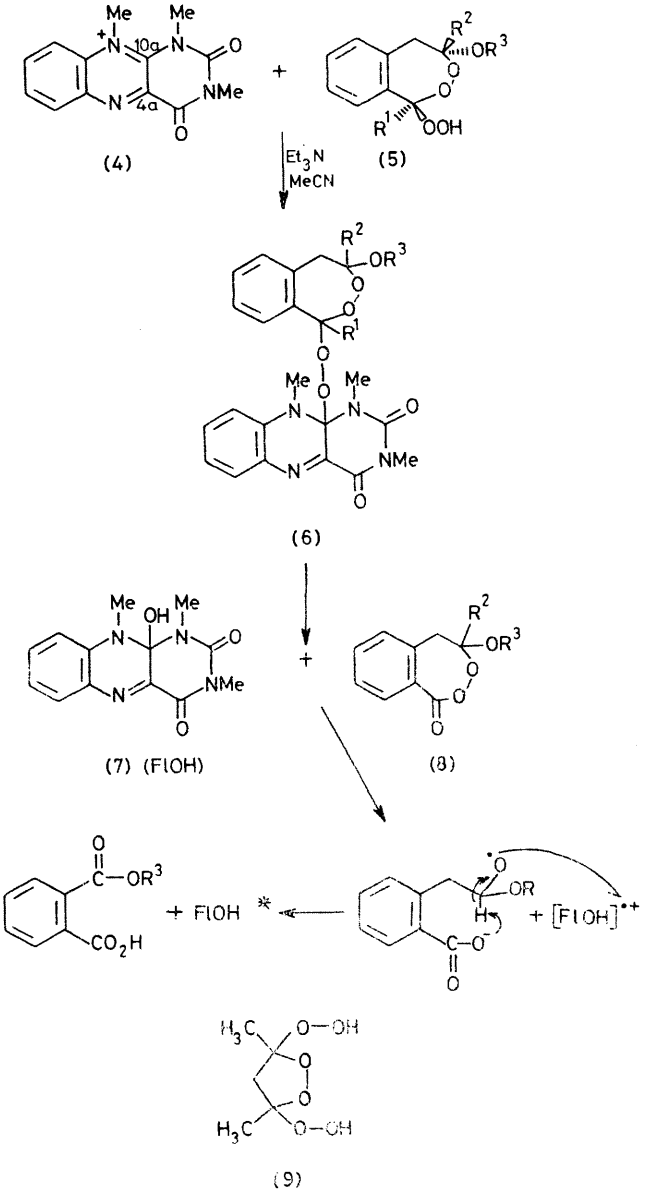
TABLE 1. Reaction of peroxides with ZnTPP

| Peroxide | (**1**) | (**2**) | (**3**) | Bu$^t$OOH | *m*CBP[b] |
|---|---|---|---|---|---|
| Relative light yields[a] | 100 | 5 | 0·9 | 0·8 | 0·05 |

[a] ZnTPP and peroxide (both $10^{-4}$ M) in diphenyl ether at 180 °C. [b] *m*-Chloroperbenzoic acid.

an absolute requirement in this system, preventing direct comparison with the metal-free bacterial reaction. Nevertheless the mechanism as it pertains to the peroxide is apposite.

Our previous model studies in bacterial luminescence showed that peroxides with the part structure –CH–O–O–CH–O–OH were active in light emission. As in this work, we used the fluorescent 10a flavin adducts, since 4a adducts are not fluorescent in solution. However, enzyme-bound flavin, oxygenated at the 4a position is fluorescent,[11] and the mechanism to be discussed assumes formation of an excited enzyme-bound 4a pseudo base.[12]

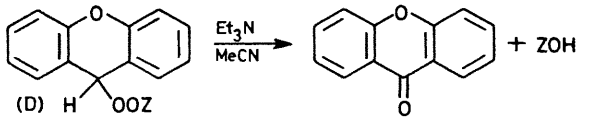



**a**; $R^1 = R^2 = H$, $R^3 = Et$
**b**; $R^1 = D$, $R^2 = H$, $R^3 = Et$
**c**; $R^1 = H$, $R^2 = D$, $R^3 = Et$
**d**; $R^1 = R^2 = H$, $R^3 = Et$
**e**; $R^1 = H$, $R^2 = Me$, $R^3 = OH$

SCHEME 2

† We thank Mr. Colin Kirby for preliminary experiments and synthesis of some of the peroxides of Table 1.

In order to probe the C–H bond breaking steps the peroxides (**5a—d**) were synthesised by ozonolysis[13] of the indenes derived from the appropriate indan-1-ones, followed by treatment with hydrogen peroxide and acid.‡ Reaction of these peroxides with the flavinium salt (**4**) in MeCN solution catalysed by $Et_3N$ gives the adduct (**6**) which decomposes by elimination as shown (Scheme 2). The quantum yield is moderate ($\phi_E = 0{\cdot}8\%$) and the pseudobase (**7**) is the proven emitter.[8] A related but dark elimination reaction occurs when 9-xanthyl hydroperoxide (**10**, Z = H, Scheme 3) is used, the convenient position of the




(10)

SCHEME 3

u.v. absorption of the product (xanthone) allowing monitoring of the reaction. The behaviour of this peroxide is typical of simple primary or secondary hydroperoxides in that light emission is absent. More stable adducts are formed from tertiary hydroperoxides, *e.g.* (**9**), but as might be expected these generate no light. The data of Table 2 place the flavin pseudobase as a leaving group of intermediate character.

TABLE 2. Rate of formation of xanthone[a] (Scheme 3)

| -OZ | $k$(rel)/s$^{-1}$ | $k_H/k_D$ |
|---|---|---|
| OH .. .. | 1·0 | 7·9 ± 0·3 |
| OBu$^t$ .. .. | 3·1 | 7·7 ± 0·6 |
| O-10a Flavin .. | 7·1 × 10$^2$ | 4·2 ± 0·3 |
| $O_2C{\cdot}[CH_2]_8{\cdot}Me$ .. | 2·6 × 10$^3$ | 4·0 ± 0·4 |
| $O_2C{\cdot}C_6H_4{\cdot}Cl$ .. | 1·4 × 10$^4$ | 3·8 ± 0·3 |

[a] Addition of peroxide to xanthylium perchlorate or the flavinium salt at 25 °C in MeCN with equal concentrations of peroxide, the salt, and $Et_3N$ (typically 2·6 × 10$^{-4}$ M).

Comparison of the peroxides (**5a—d**) with the simpler sort (Tables 2 and 3) implies that elimination is the rate determining step in these cases also. By analogy with other examples of electron transfer luminescence,[5] the

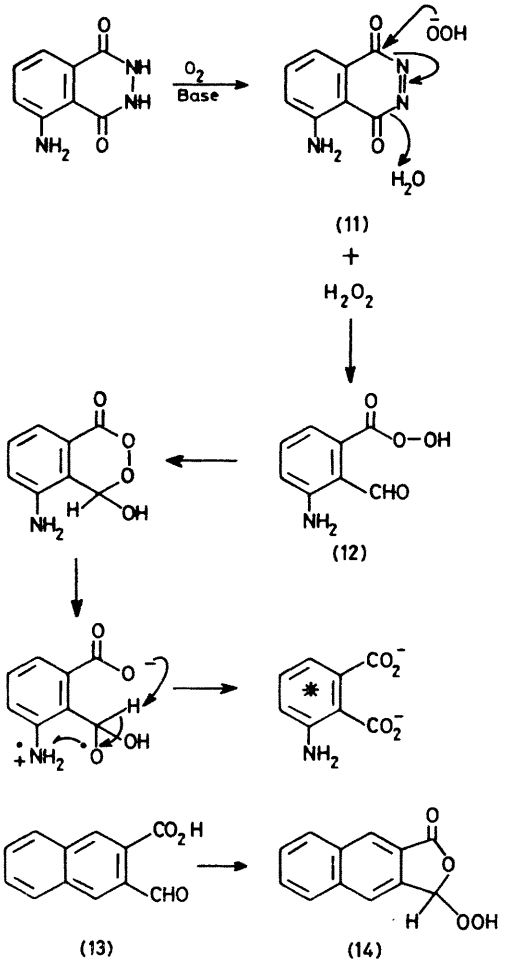
TABLE 3. Reaction of (**6**)[a] with $Et_3N$

| | Peroxide (**5**) R$^1$ | R$^2$ | $k_H/k_D$[b] | $\phi_H/\phi_D$ |
|---|---|---|---|---|
| (**b**) | D | H | 6·8 ± 0·3 | 1·24 ± 0·14 |
| (**c**) | H | D | 1·03 ± 0·03 | 1·44 ± 0·12 |
| (**d**) | D | D | 6·7 ± 0·3 | 2·10 ± 0·13 |
| (**e**) | H | Me | — | No light |

[a] Formed *in situ* from [(**4**)], 4·9 × 10$^{-5}$ M; [(**5**)], 3·1 × 10$^{-4}$ M; [$Et_3N$], 2·9 × 10$^{-4}$ M, at 26 °C in MeCN. [b] With (**5a**), $k$ = 0·40 s$^{-1}$; all rates were followed by observing decay of light emission.

resulting perester (**8**) is a likely electron acceptor. However, the excitation step itself appears to involve breakage of the second C–H bond in that a *product* isotope effect is observed, *i.e.* $\phi_H/\phi_D$, Table 3. This isotope effect can be explained by the fact that in electron transfer luminiscence, a diffusion of the radical anion from the radical cation followed by unproductive reactions is competitive with the light yielding transfer. Replacement of the H atom, *i.e.* R$^2$, by Me results in decomposition of the adduct (**6**) at the expected rate but without light emission, in confirmation of the isotope effect.

The new and significant feature of the mechanism is that although excitation is accomplished by electron transfer, it is only possible from an alkoxy-radical if a proton is removed. This breakage of a C–H bond, an unlikely light-yielding step in itself, is thus coupled to the theoretically more acceptable electron transfer mechanism.[14] The alternative direction of cleavage of the O–O bond (giving a carboxyl radical and alkoxy anion) followed by hydrogen *atom* abstraction would result in a ketyl radical ($R_2C{=}O)\cdot^-$, an excellent electron donor. Distinction between these variations is not possible with the available evidence.




SCHEME 4

A rather remarkable coincidence is the seemingly similar double kinetic isotope effect recently found by Hastings *et al.*[15] for the bacterial luciferase reaction, using aldehydes such as $Me[CH_2]_8{\cdot}CDO$. Whether this reflects a stoicheiometry involving two molecules each of aldehyde and oxygen is not known at present, but the electron transfer mechanism requires (from the evidence presented here) that the adduct be oxidised by a peroxide linkage of

‡ Satisfactory analyses and spectra were obtained for all new compounds isolated. The stereochemistry shown follows from a comparison of the n.m.r. spectrum with published spectra (K. Tori and M. Ohtsuru, *Chem. Comm.*, 1966, 886).

greater potential than that of a simple alkyl peroxide. Formation of an intermediate perester from two molecules of aldehyde satisfies this requirement. An alternative is that the enzyme alters the redox potential of the 1:1 adduct.[16]

With the intermediate diazaquinone (**11**)[17] as a starting point, a similar mechanism[18] for luminol and perhaps hydrazides in general[2] may be written. Carboxylic acid aldehydes [such as (**13**)] are by-products, suggesting that addition of $H_2O_2$ and $H_2O$ are competitive.[18,19] White *et al.*[20] have suggested that the efficiencies obtained from variously substituted hydrazides best fit an unspecified electron transfer mechanism. We are attempting to provide direct evidence for our hypothesis (Scheme 4), but the peracid (**12**) is understandably difficult to prepare. All attempts to add $H_2O_2$ to compounds such as (**13**) have given only the non-chemiluminescent isomer (**14**).[21] Nevertheless we feel that further investigation with the mechanism depicted here as a model will lead to an acceptable solution of both of these intriguing problems.

(*Received, 29th September* 1978; *Com.* 1043.)

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