Model Reactions for Bacterial Bioluminescence

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Summary Acid-catalysed addition of hydrogen peroxide to n-butyl vinyl ether gave a separable mixture of the hydroperoxides (**1**)—(**4**); peroxides (**1**)—(**3**) undergo chemiluminescent reactions in acetonitrile with 1,3,10-trimethylisoalloxazinium perchlorate in the presence of triethylamine, and the flavin analogue catalyses the decomposition of the peroxides.

AN enzyme-bound peroxide of reduced flavin mononucleotide (intermediate II, Scheme) has been established as an

$$\underset{\text{(I)}}{(\text{Enzyme} + \text{FMNH}_2)} \rightarrow \underset{\text{(II)}}{\text{Enzyme–FMNH}_2\text{–O}_2}$$

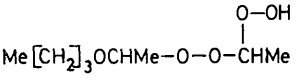
$$\text{(II)} + \text{R·CHO} \rightarrow \underset{\text{(IIa)}}{\text{Enzyme—FMNH·OOH–RCHO}}$$

$$\text{(IIa)} \rightarrow \text{Enzyme–E}^* + \text{R·CO}_2\text{H} + \text{H}_2\text{O}$$

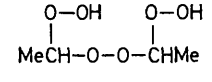
SCHEME

intermediate in the bioluminescent oxidation of long-chain aldehyde to fatty acid by bacterial luciferase.[1] The events which lead to light emission are not known, nor are the structures of intermediate (II) and the light-emitting molecule E. Reaction at the 4a or 10a positions of the flavin with aldehyde or with oxygen to form the corresponding peroxides has been suggested by several investigators.[2] Recently a lumiflavin 4a-hydroperoxide has been prepared and shown to have a similar u.v. spectrum to that of intermediate (II), and to give a chemiluminescent reaction with aldehydes.[3] However, the light-emitting species could not be identified nor was a quantum yield reported. We have now demonstrated that analogues of 10a-dihydroflavin peroxides, alternative models for intermediate (IIa), also give chemiluminescent reactions. The preliminary results are reported here, including tentative identification of the light-emitting chromophore and the quantum yields.

Treatment[4] of n-butyl vinyl ether (0·0125 mol) with hydrogen peroxide (35M, 0·019 mol) and sulphuric acid (0·019 mol) at 0 °C gave a mixture of the hydroperoxides (**1**)—(**4**). Separation was achieved by use of short-column chromatography[5] using silica gel (Merck GF 254) with chloroform as eluent. The structures were determined by n.m.r. spectroscopy, and the data obtained agree well with those of similar systems.[6]



(1) and (2) (diastereoisomers)



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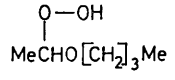
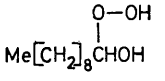


(4)



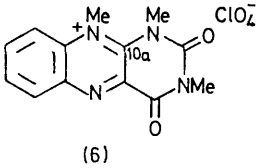
(5)

When a mixture of (**1**) and 1,3,10-trimethylisoalloxazinium perchlorate (**6**) in pure dry acetonitrile was treated with triethylamine, chemiluminescence was observed, easily visible to the non-dark-adapted eye. The emission had λ_{max} 530 nm with a spectrum corresponding to that of the fluorescence of the adduct[7] (**8**, R = MeO) and presumably that of (**8**, R = OH). The compound (**8**, R = OH) is thus assumed to be the emitting species. The u.v. and fluorescence spectra and previous work on adducts of compound (**6**)[7] all strongly suggest addition of the peroxides to position 10a. Maximum intensity was reached in much less than a second, decaying to zero in a few minutes. The decay was first order. I_{max} was found to be proportional to [(**6**)] and [Et_3N], whereas the rate constant, k, was dependent upon [Et_3N] only. Variation of [(**1**)] had no effect as long as [(**1**)]/[(**6**)] > 1. The reaction was unaffected by the presence of O_2. The peroxides (**2**) and (**3**) behaved similarly but (**4**) and (**5**) were virtually inactive (Table). The product in all cases was the spirohydantoin (**7**).[8] Decomposition of the adduct formed from H_2O_2 also gave (**7**) in a dark reaction.

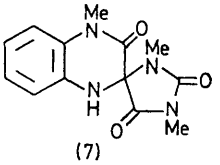
TABLE. Reaction of the peroxides (**1**)—(**5**) with (**6**) in MeCN–Et_3N

Hydroperoxide	ϕ[a]	ϕ_E[b]	k/s^{-1}[c]
(**1**)	$2{\cdot}1 \times 10^{-5}$	$1{\cdot}9 \times 10^{-4}$	$3{\cdot}2 \times 10^{-2}$
(**2**)	$0{\cdot}5 \times 10^{-5}$	$0{\cdot}45 \times 10^{-4}$	$6{\cdot}0 \times 10^{-2}$
(**3**)	$1{\cdot}7 \times 10^{-5}$	$1{\cdot}5 \times 10^{-4}$	$6{\cdot}8 \times 10^{-2}$
(**4**)	$<2{\cdot}0 \times 10^{-7}$	$<2{\cdot}0 \times 10^{-6}$	—
(**5**)	0	0	—

[a] Corrected for photomultiplier response, luminol as standard. [b] Population of excited state taking ϕ(fluorescence) for (**8**, R = MeO) as 0·11 and assuming yield of emitter is quantitative. [c] [(**6**)] $5{\cdot}8 \times 10^{-4}$ M; [Et_3N] $1{\cdot}4 \times 10^{-2}$ M at 22·4 °C.



(6)



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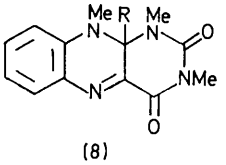

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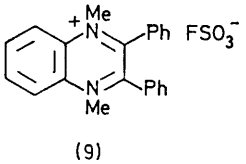

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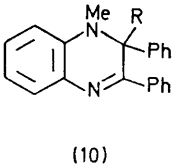
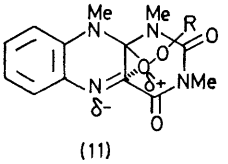


(10)



(11)

Adducts of (**6**) with MeOH and n-butyl hydroperoxide[7,9,10] reacted very slowly with the peroxides (**1**)—(**3**) and with a lower quantum yield owing to the competing decomposition[6]

of the peroxides by Et_3N. The n-butylperoxy adduct (**8**, R = Bu^n–O–O–) reacted more quickly than the methoxy adduct (**8**, R = MeO) suggesting, in agreement with the other evidence, that the chemiluminescent reaction involved decomposition of adducts formed from the peroxides (**1**)—(**3**) at the free 10a position of (**6**). The changing u.v. spectra also supported this interpretation.[7,9] There are several competing routes to the spirohydantoin[7,11] and the structure of the peroxides used to form the adducts may affect the relative participation of light and dark pathways.†

A variety of positions for attachment of oxygen in flavin-catalysed oxidation has been proposed[7,9,10,12] and although addition at the 10a position[7,11] is most likely in the present case, we do not exclude the involvement of the 4a position *in vivo*. Indeed, the reaction appears to be capable of extension to other simpler model systems. On the addition of the peroxides (**1**)—(**3**) to 1-methyl-2,3-diphenylquinoxalinium fluorosulphonate (**9**) and treatment with Et_3N as before, light was emitted with a much greater quantum yield (ϕ *ca.* $1{\cdot}6 \times 10^{-4}$). The product was the corresponding ψ-base (**10**, R = OH). The fluorescence spectra of (**9**) and (**10**, R = OH) both resemble the chemiluminescence spectrum (λ_{max} 515 nm) and the synthesis of other model compounds is required before we can unambiguously identify the emitter in this class of compounds. This reaction produces a very bright light, and since neither reaction conditions nor the fluorescence yields of potential emitters have been optimised, this series seems likely to number among the more efficient chemiluminescent reactions.[13]

We suggest that the flavin nucleus enhances the rate of O–O bond cleavage in these reactions and in flavin-catalysed oxygenations generally by formation of a quasi-per-epoxide[14] (**11**). The possibility is indicated by differences in the u.v. spectra and particularly in the fluorescence spectra of (**8**, R = MeO) and (**8**, R = Bu^n–O–O–). Specific interaction of the peroxide with the chromophore is apparent in this comparison.

Much work remains to be done before a decision between the various possible mechanisms can be made, but it is already clear that these models and the bacterial system itself cannot be easily related to other better understood bioluminescent reactions.[15] We are investigating a variety of similar reactions in order to identify the underlying mechanism of chemiluminescence.

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† 1,4-Bishydroperoxy-1,4-dihydro-2,3-benzodioxin and (**6**) react with $\phi_E = 4{\cdot}4 \times 10^{-3}$, similar in efficiency to models for the luciferins (ref. 15).

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