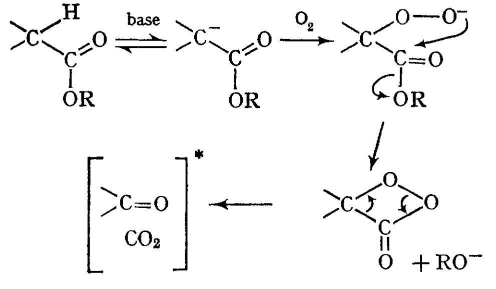
The Chemiluminescence of a Firefly Luciferin Analogue

By FRANK MCCAPRA,* Y. C. CHANG, and V. P. FRANCOIS
(*The Chemical Laboratory, University of Sussex, Brighton*, BN1 9QJ)

ALL of the well investigated bioluminescent organisms[1] produce light by the reaction of oxygen with a substrate (called luciferin) forming some fluorescent product in an excited state. We have now obtained evidence for a possible route to light emission in the North American firefly. This route is of the pattern indicated by us in our investigations[2,3] into the very different luciferins in the sea pansy, *Renilla reniformis*,[4] and the crustacean *Cypridina hilgendorfii*.[5]

The suggested mechanism[6] for this particular class of chemiluminescent reaction involves the formation of a peroxide in a position adjacent to a group capable of adding the resulting anion to form a reactive four-membered peroxide intermediate. This group can be an imine[2,7] or, of wider application, a carboxylate derivative such that the leaving group is a weaker base than the entering peroxy-anion (see Scheme). The carbonyl derivative formed by the decomposition of this intermediate will appear in an electronically excited state.



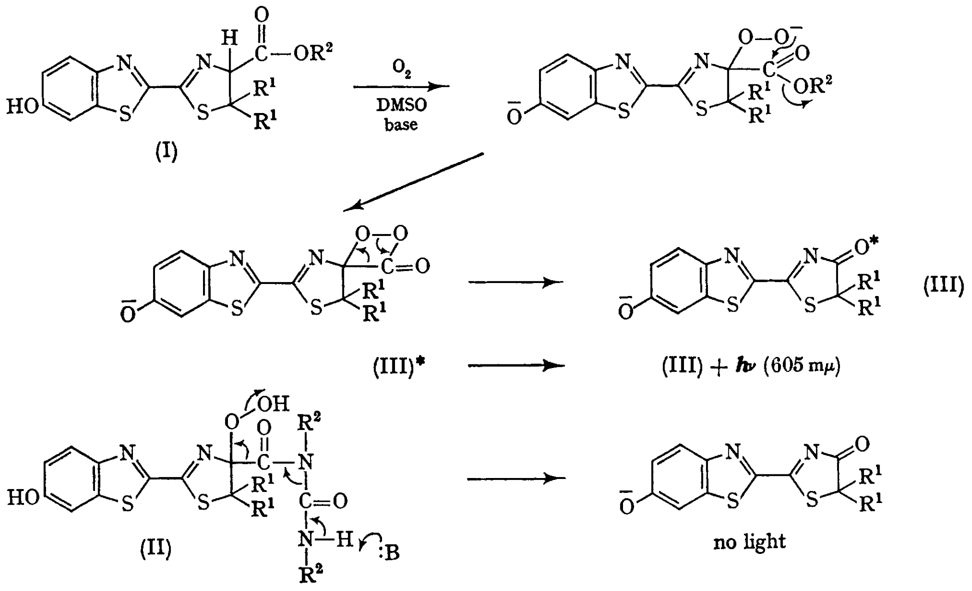
SCHEME

Applying this concept to firefly luciferin (Ia; $R^1=R^2=H$) it was obvious to us that in the natural system, the formation of a phosphate served to increase the acidity of the adjacent proton and provide the required leaving group. With luciferin itself the easy oxidation to dehydroluciferin[8] confuses the essential process, and we synthesized (Ib; $R^1=Me$, $R^2=Ph$) to prevent this unwanted side reaction.

In our attempts to synthesize the phenyl ester (Ib) we have inadvertently prepared, in good yield (II; R^2=cyclohexyl) the product of acyl migration in the intermediate DCC complex. Synthesis of the phenyl ester was in fact achieved by using trifluoroacetic anhydride. However, the smooth oxidation of (II) (as shown) in dimethyl sulphoxide with guanidine carbonate as base, to (III), provided a ready source of the suspected emitter, and confirms the suggested mechanism, since oxidation occurred *without chemiluminescence*. It is fully in accord with our previous work that a peroxide anion will not displace a urea from the carbonyl group.

The phenyl ester (Ib) on the other hand, in dimethyl sulphoxide with guanidine carbonate, gave a *brilliant red chemiluminescence*, easily visible in full sunlight. The emission spectrum with maximum at 605 mμ was exactly superimposable on the spectrum of the fluorescence of (III), isolated from the reaction mixture and shown to be identical with a synthetic sample.

We have established the structures of the compounds given by the usual physical measurements, and will comment on the interesting and easily explained mass spectra given by them, in our full paper. There is now little doubt that the suggestions made by us in 1964[6] have an effective predictive value in this type of strong chemiluminescence and we will report shortly on the chemiluminescence of other new systems.[9]



(*Received, November 1st,* 1967; *Com.* 1182

[1] M. J. Cormier and J. R. Totter, *Ann. Rev. Biochem.*, 1964, **33**, 431.
[2] F. McCapra and Y. C. Chang, *Chem. Comm.*, 1966, 522.
[3] F. McCapra and Y. C. Chang, *Chem. Comm.*, 1967, 1011.
[4] M. J. Cormier and C. G. Eckroade, *Biochim. Biophys. Acta*, 1962, **64**, 340.
[5] Y. Kishi, T. Goto, Y. Hirata, O. Shimomura, and F. H. Johnson, *Tetrahedron Letters*, 1966, 3427.
[6] F. McCapra and D. G. Richardson, *Tetrahedron Letters*, 1964, 3167; F. McCapra, D. G. Richardson, and Y. C. Chang, *Photochem. and Photobiol.*, 1965, **4**, 1111.
[7] E. H. White and M. J. C. Harding, *Photochem. and Photobiol.*, 1965, **4**, 1129.
[8] E. H. White, F. McCapra, and G. F. Field, *J. Amer. Chem. Soc.*, 1963, **85**, 337.
[9] F. McCapra and R. Hann, to be published.