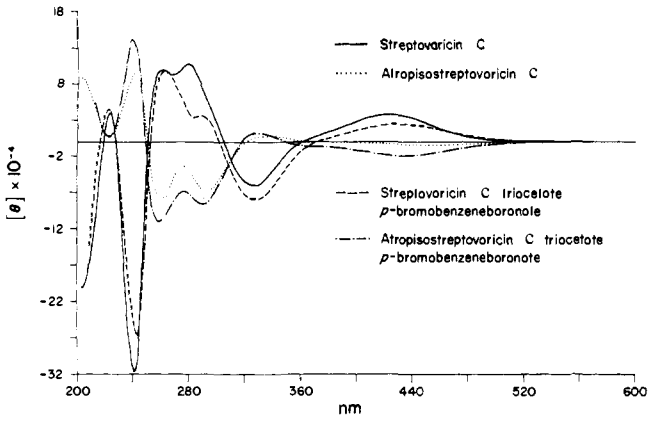

Figure 2. Circular dichroism curves for streptovaricin C (**1**), its atropisomer (**2**), streptovaricin C triacetate *p*-bromobenzeneboronate (**11**), and its atropisomer (**12**).

tion was carried out on the negative-rotating ("late," unnatural) isomer and the actual relative configuration of the natural streptovaricins must be that shown in Figure 1 (**1, 4, 7, 9, 11**).

To assign the absolute configuration of **12**, the two possible enantiomorphs (*i.e.*, **12** and its mirror image) were refined including the anomalous scattering contributions for the bromine and chlorine atoms. The enantiomorph **12** converged with a value of R_2 of 0.101, whereas the opposite enantiomorph converged with R_2 of 0.104, arguing that the absolute configuration is as shown for **12**; thus, that for the natural streptovaricin is that shown, *e.g.*, for **1** (6*R*, 7*R*, 8*R*, 9*R*, 10*S*, 11*S*, 12*R*, 13*S*, 14*R*, helicity P),[11] which agrees[12] with the helicity and absolute configurations at C-8 through C-14 of rifamycin B[13,14] and tolypomycin Y.[15]

To our knowledge these represent the first examples of the conversion of a naturally occurring compound to its atropisomer.

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(7) Streptovals C (**3**) and Fc (**6**) have no stable helicity since their atropisomeric forms, like those of streptovarone,[8b] interconvert at room temperature, a conversion demonstrated by the broadening of the methylenedioxy signals in the pmr spectra of streptovarone,[8b] **3** and **6**.
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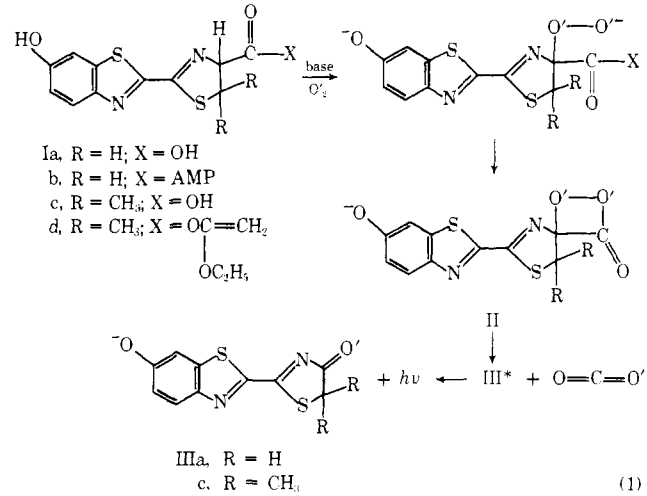
On the Mechanism of Firefly Luciferin Luminescence[1]

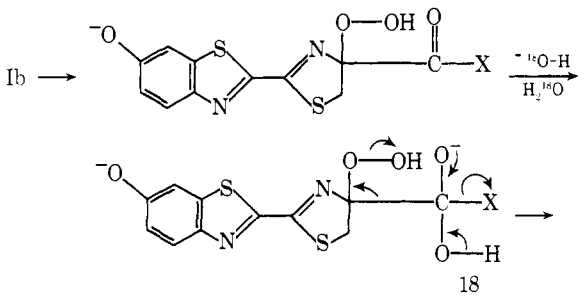
Sir:

The bioluminescence and the chemiluminescence of firefly luciferin (Ia)[2] are closely related processes in that both require oxygen,[3,4] both produce carbon dioxide[5,6] and lactam IIIa,[3,7] and both yield yellow-green or red light depending on the conditions.[3,8] On the basis of these facts, the identification of lactam III as the light emitter,[3,7] and analogy to other chemiluminescent reactions, the mechanism of eq 1 was proposed for both the chemi- and bioluminescence of firefly luciferin[3,9-11] (where X = any good leaving group). Since that time, 1,2-dioxetanes have been isolated,[12] and their chemistry has been elucidated;[13] they are, in fact, excellent sources of chemically produced excited states.[14]



(1)

Oxygen-18 studies of both the bio- and chemiluminescence of firefly luciferin (*via* the adenylate Ib) have been reported recently purporting to show that the carbon dioxide formed in the reactions was not labeled in bioluminescence (enzyme + $^{18}O_2$ in H_2O) and labeled to less than 10% in chemiluminescence (*tert*-butoxide + $^{18}O_2$ in DMSO).[15] In both reactions, it was further claimed that one oxygen atom of the carbon dioxide was derived from water.[15] The mechanism of eq 2 was proposed to account for these results.[15] In a related study, bioluminescence in the sea pansy led to <0.1 atom ^{18}O incorporation in the carbon dioxide

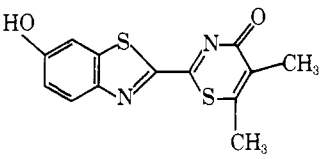


18

IIIa + $h\nu$ + O=C=O^{18} (2)

formed, and a similar mechanism was proposed.[16] These results have been cited as evidence that the dioxetanone mechanism (eq 1) is incorrect.[7b,13i,j,17]

We now report the results of an ^{18}O study of the chemiluminescence of a firefly luciferin analog that supports the original mechanism (eq 1). Dimethylluciferin (Ic) was chosen because the methyl groups block enolization in the product (III) and allow isolation of IIIc in analytically pure form.[18] Since the luciferyl adenylates have never been obtained analytically pure, we chose as starting material the ethoxyvinyl ester (Id),[19] which was prepared in a pure form from dimethylluciferin (Ic) and ethoxyacetylene.[20,21] Treatment of this "active" luciferin (5–20 mg) with potassium phenoxide and oxygen in DMSO produced carbon dioxide bound in the form of potassium phenyl carbonate, lactam IIIc (30%), recovered dimethylluciferin (12%), a rearrangement product (IV, 44%), and one minor product. Acidification of the carbonate under vacuum liberated the carbon dioxide (42%). Correcting the yields for the recovered luciferin and compound IV showed that the oxidation path produced lactam IIIc(68%) and carbon dioxide (95%).[22]



IV

When oxygen enriched in ^{18}O (92 atom %) was used for the reaction carried out in dried flasks equipped with rubber septa, 22% of the carbon dioxide formed contained an atom of ^{18}O (correcting the ^{18}O to 100%) and 60% of the lactam (IIIc) contained an atom of ^{18}O. Because of the use of both a base and an acid in the handling of the carbon dioxide, exchange of the oxygens with the oxygen atoms of adventitious water will occur. We have measured the extent of this "washing out" of the ^{18}O label in our system and found it to be 62% per cycle of dissolving carbon dioxide in potassium *tert*-butoxide–DMSO followed by acidification with 100% phosphoric acid. Since a single cycle is the minimum for reactions run under basic conditions, our raw value of 22%, on correction for the 62% loss per cycle, gives a value of 58% of ^{18}O labeling in the carbon dioxide formed in the chemiluminescence. When all operations were performed in a closed system using vacuum-line techniques, the directly measured ^{18}O incorporation in the carbon dioxide rose to 66% and the ^{18}O incorporation in lactam IIIc rose to 94%. The low ^{18}O incorporation reported by DeLuca, *et al.*,[15,16] is, in our opinion, a result of experimental difficulties in handling very small quantities of carbon dioxide.[23,24]

The maximum chemiluminescence efficiency Φ_{cl} we have measured for the reaction of eq 1d is 9%; corrected for the 12% of luciferin Ic recovered and the 44% yield of IV, this value becomes 20%. This value is effectively constant over the concentration range 2.7×10^{-6} to 6.9×10^{-5} *M*, proving that energy transfer is not involved and that the excited lactam is produced directly. The fluorescence efficiency (Φ_{fl}) of lactam IIIc is 60%. Substituting the above values into the general equation $\phi_{cl} = \phi_r\phi_{es}\phi_{fl}$ gives $\phi_r\phi_{es} = 0.33$. That is, 33% of the luciferin ester molecules that undergo oxidation produce excited states of III; since the yield of carbon dioxide is 95%, these results mean that carbon dioxide is a product of the pathway leading to excited states. Our ^{18}O data are consistent with this pathway involving dioxetanones (compound II).

An oxygen-18 tracer study of *Cypridina* bioluminescence (in which carbon dioxide is a product) has yielded labeling data consistent with the dioxetanone pathway.[25] In view of the close structural relationships between Cypridina, Renilla (sea pansy),[26] Aequorin,[27] and other coelenterate luciferins,[28] it seems certain that subsequent ^{18}O studies will show that all of these reactions proceed *via* the thermal cleavage of dioxetanones (as in eq 1) and that the dioxetane mechanism will be found to be a general one for both bioluminescence and chemiluminescence.[29]

Acknowledgment. We thank Dr. Peter D. Wildes for the fluorescence quantum yield and other measurements, Professor F. H. Johnson for a preprint of ref 27, and the U.S. Public Health Service for its financial support (Grant GM19488).

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(23) Insufficient data were presented to prove that on the scale of operations chosen (0.7 μl of CO_2 produced in 6.5 ml of glycylglycine buffer at pH 7.8;[15] 0.9 μl of CO_2 in 3.7 ml of phosphate buffer at pH 7.2)[16] that (1) the carbon dioxide could be recovered in reasonable amounts from the aqueous phase, in competition with exchange, and (2) the carbon dioxide produced from the luciferin was not swamped by atmospheric carbon dioxide. Also, control runs in which bubbles of carbon dioxide are passed through solutions (either aqueous or DMSO + *tert*-butoxide) are not close to the processes occurring in bio- or chemiluminescence in which carbon dioxide is produced in true solution—molecule by molecule. See also ref 3 and "Chemiluminescence and Bioluminescence," M. J. Cormier, D. M. Hercules, and J. Lee, Ed., Plenum Press, New York, N.Y., 1973, pp 358–359.
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Electrogenerated Chemiluminescence. XXI. Energy Transfer from an Exciplex to a Rare Earth Chelate

Sir:

Intermolecular energy transfer from the lowest triplet (n, π^*) state of a carbonyl compound (*e.g.*, benzophenone) to the ligand of a rare earth chelate, which in turn intramolecularly transfers its energy to the central metal ion with subsequent narrow band emission from the metal ion, has been reported by several investigators.[1-5] For example, El-Sayed and Bhaumik[2,3] showed this "inter-intra" molecular energy transfer from photoexcited benzophenone to a Eu(III) chelate, while Wildes and White[4] described sensitized chemiluminescence of lanthanide chelates by energy transfer from the excited species generated by dissociation of dioxetane. We report here the observation of intermolecular energy transfer from an excited charge-transfer complex (an exciplex or heteroexcimer) directly to a europium chelate. The exciplex was produced by the electron transfer reaction of electrogenerated radical ions[6] under conditions where the triplet states cannot be formed and to our knowledge is the first reported example of intermolecular exciplex-sensitized luminescence.

In electrogenerated chemiluminescence (ecl) and radical ion chemiluminescence (cl) reactions, excited states are formed by an energetic electron transfer reaction; frequently these are excited states of the acceptor (A) or donor (D) species themselves

$$A\cdot^- + D\cdot^+ \longrightarrow A^* + D \text{ or } A + D^* \quad (1)$$

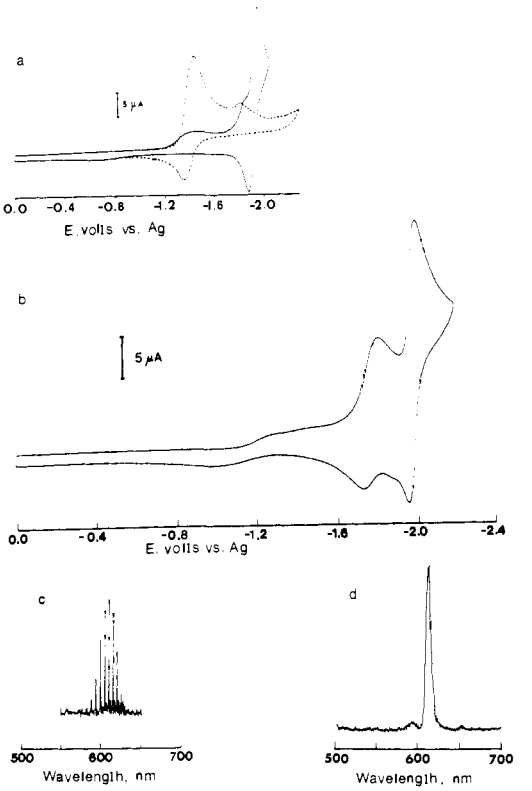

Figure 1. (a) Cyclic voltammogram of 1 m*M* Eu(DBM)$_3$ · pip and 0.1 *M* TBAP acetonitrile at a platinum disk electrode with a scan rate of 200 mV/sec. Dotted line is reduction of free ligand, DBMH; (b) cyclic voltammogram of TPTA-benzophenone with Eu(DBM)$_3$ · pip in acetonitrile. Scan rate was 200 mV/sec at the platinum disk electrode. Solution contained 1.2 m*M* TPTA, 1.1 m*M* benzophenone, 1.6 m*M* Eu(DBM)$_3$ · pip, and 0.1 *M* TBAP; (c) ecl spectrum obtained from solution in (b) with pulse duration, 1 sec; (d) fluorescence spectrum of Eu(DBM)$_3$ · pip in acetonitrile, with excitation at 421 nm.

In many cases, however, longer wavelength (red-shifted from the excited singlet A or D peaks by about 6000 cm^{-1}), structureless, emission is also observed and this has been identified as originating from an exciplex directly formed in the radical ion reaction.[6-10]

$$A\cdot^- + D^+ \longrightarrow {}^1(A^-D^+)^* \quad (2)$$

$$^1(A^-D^+)^* \longrightarrow A + D + h\nu \quad (3)$$

In some cases, where the energy of the radical ion reaction is less than that necessary to form excited singlet or triplet states of A and D, *e.g.*, for the case of the reaction of tri-*p*-tolylamine (TPTA) radical cation and either benzophenone (BP) or dibenzoylmethane (DBMH) radical anion, only exciplex emission is observed. If the europium chelate Eu(DBM)$_3$ · piperidine or Eu(DNM)$_3$ · piperidine (where DBM is dibenzoylmethide and DNM is dinaphthoylmethide) is added to the TPTA-BP or TPTA-DBMH ecl systems, emission characteristic of Eu(III) is observed, and we describe experiments below which demonstrate that this emission is a result of energy transfer from the exciplex.

Experimental techniques in the ecl studies followed previous practice;[6,9,10] details on chelate preparation, solvent purification, and apparatus are available.[11] Spectroscopic and electrochemical data for the Eu chelates as well as TPTA, BP, and DBMH are given in Table I. Consider an ecl experiment with a solution containing 1.2 m*M* TPTA, 1.1 m*M* BP, 1.6 m*M* Eu(DBM)$_3$ · piperidine and 0.1 *M* tetra-*n*-butylammonium perchlorate (TBAP) in acetonitrile at a Pt electrode (Figure 1). A cyclic voltammogram of the chelate (Figure 1a) is characterized by a reversible one-electron reduction wave at −1.94 V; the small waves at −1.43 and