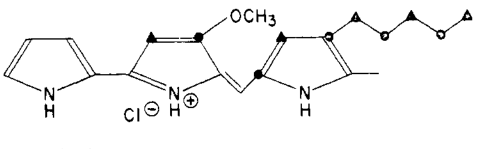

Figure 2. Labeling pattern of prodigiosin: ●, [1-^{13}C]acetate; ▲, [2-^{13}C]acetate.

role and the mutant-produced 3-methoxy-(5,2′)-bipyrrole-2-carboxaldehyde (III).[7b]

A sample of II, enriched with ^{13}C from CH_3–$^{13}COONa$, showed three tertiary ring carbons bearing label, including B-3. Degradation of II gave 2-methyl-3-amylpyrrole (IV)[12] enriched at positions 3 and 5 thus assigning C-3 and C-5 in II. A single resonance spectrum of *N,N′,N′′*-trideuterio II showed the upfield absorption of this pair as a doublet ($J \sim 4$ Hz) due to long-range coupling[13] with the C-4 proton; hence it was assigned to C-3.

Two further doublets ($J \sim 7$ Hz) were assigned to B-5 and C-2 on the basis of their long-range couplings. C-2 was assigned to the higher field absorption since it was noted that the chemical shifts of C-3, C-4, and C-5 were close to the region of typical pyrrole chemical shifts. The strongly deshielded absorption of the pair was assigned to B-5.

The two remaining tertiary carbons were assigned in the single resonance spectrum of perdeuterio II (ring positions A-3, A-5, B-4, and C-4 deuterated). The absorption at -44.72 ppm is a sharp doublet ($J \sim 7$ Hz) and is assigned to A-2 while the absorption at -68.78 ppm was a broad peak (B-2) due to coupling with 1′′-*H*.

Comparison of the B-ring ^{13}C chemical shifts with pyrrole[13–15] indicate large chemical shifts compared to the delocalized pyrrole structure. The α carbons B-2 and B-5 are shifted -28.54 and -29.99 ppm downfield from the α carbons in pyrrole while the free β position, B-4, undergoes a large diamagnetic shift ($+14.57$ ppm) due to the ortho methoxyl substituent.

The effect of alkyl groups on heterocyclic ring ^{13}C chemical shifts has been described previously.[14,16] However, the corresponding effects on alkyl ^{13}C shifts by the ring have hitherto not been reported. We have, in the course of this work, observed a marked upfield shift of ^{13}C resonance in alkyl groups at positions directly adjacent to the heterocyclic ring. Thus, as shown in Figure 1, ^{13}C resonance in the C-2 methyl group occurs at a substantially higher field ($+65.21$ ppm) than that of the alkyl methyl group at 5′ ($+63.49$ ppm) while the 1′ methylene resonance signal is found at a field higher than that of the methylenes 2′ or 3′.

Sodium [1-^{13}C]- and [2-^{13}C]acetate (Prochem Ltd., 62% ^{13}C) were fed in two separate experiments at 10 mmol/l. in a Bacto-peptone and glycerol medium in agar gel. The bacteria were harvested after 3 days and the prodigiosin extracted and purified by a modification of a known method.[17] The ^{13}C FT spectra given in Figures 1B and 1C show the pattern of primary incorporation from [1-^{13}C]- and [2-^{13}C]acetate, respectively. No convolution of the time domain data was performed on the spectra reported in Figure 1. The level of ^{13}C at any labeled center ($\sim$8% in Figure 1B and $\sim$11% in Figure 1C) is sufficient to differentiate clearly the labeled positions from Overhauser enhanced signals.

Figure 2 shows the proposed pattern of primary incorporation of acetate into prodigiosin. It is clear that the methylamylpyrrole moiety (ring C) is essentially constituted from an eight-carbon polyacetate chain. The origin of the two remaining carbons in ring C remains to be determined. The other prodigiosin analogs differ only in the nature and location of alkyl substitution in ring C. The above results suggest that these various ring C pyrrole moieties may also incorporate polyacetate chains.[18]

A significant finding is the lack of primary incorporation of acetate into ring A. This indicates a novel route to this pyrrole ring. Carbon-13 FT nmr studies of the biosynthetic origin of this ring and of the remaining carbons in prodigiosin are in progress and will be reported in due course.

Acknowledgments. This work was supported by Grant No. RR 00356 from the Biotechnology Resources Branch of the National Institutes of Health and Grant No. AI-04798 of the National Institutes of Health. R. J. S. and H. H. W. would like to thank R. Holroyd, P. Jensen, P. Peverada, and J. Gage for valuable technical assistance, Dr. M. Bunting and Dr. N. Gerber for helpful discussions, Dr. J. Faller for use of his indor equipment, and (R. J. S.) the Old Dominion Foundation for a Mellon Fellowship at Yale, 1967–1969.

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[13] F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, 90, 3543 (1968).

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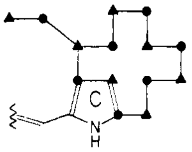

The Dioxetane-Sensitized Chemiluminescence of Lanthanide Chelates. A Chemical Source of "Monochromatic" Light

Sir:

We wish to report the observation of very narrow band chemiluminescence when trimethyldioxetane (I) is thermally decomposed in the presence of certain lanthanide chelates in solution. In particular, when europium tris(thenoyltrifluoroacetonate)-1,10-phenanthroline (II)[1] was used as the fluorescent acceptor, at

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[1] Prepared by the method of Bauer, *et al.* [H. Bauer, J. Blanc, and D. L. Ross, *J. Amer. Chem. Soc.*, 86, 5125 (1964)]: mp 246–248°; uv (95% ethanol) 340 (log ϵ 4.73), 265 (4.63), 230 nm (4.65). *Anal.* Calcd: C, 43.42; H, 2.02. Found: C, 43.68; H, 2.06.

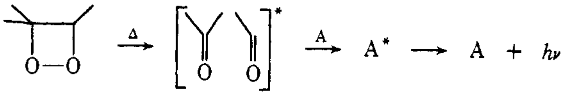
Table I. Light Yields

Concn of II,[a] $M \times 10^3$	Φ_{ch}[b] $\times 10^2$
0.01[c]	0.04
0.1	0.31
0.5	0.80
0.5[d]	1.24
1.0	1.09
1.0[d]	1.5

[a] Undegassed acetone solution containing an initial concentration of 0.001 *M* I except where otherwise noted. [b] Einsteins per mole of I decomposed. [c] Initial dioxetane concentration 0.004 *M*. [d] Degassed by three cycles of freeze-pump-thaw and sealed at $\sim 10^{-5}$ Torr.

least 80% of the light emitted appeared in a single band at 613 nm having a total width at half-height of about 5 nm (Figure 1). The very narrow band emission and moderate efficiency of the reaction (maximum quantum efficiency of light emission, $\Phi_{ch} \approx 0.015$ based on dioxetane decomposed) make it reasonable that such a system could be used as a chemical source of "monochromatic" light devised to generate a specific number of photons. In principle, the reaction could be extended to produce other spectral bands by the use of different metal complexes; for example, green light can be produced using terbium chelates.[2]

1,2-Dioxetanes (I) dissociate thermally to yield products in electronically excited states.[3] These species have been observed to transfer energy to stable fluorescent molecules[3] (eq 1) and also to molecules that can undergo "photochemical" changes.[4] The "photochemical" reactions induced by thermal decomposition of I all seem to involve triplet excited states.[4] By using as acceptors in reaction 1 lanthanide complexes which are highly efficient acceptors of n,π* lowest triplet energy[5] we have observed reasonably efficient chemically sensitized fluorescence.



(1)

Solutions of I and II in acetone were mixed at 0° and injected into a Pyrex cell at 50°. The light emitted was detected by an EMI 9558B photomultiplier, the output from which was recorded as a function of time. The total emission could then be calculated by graphical integration of the intensity *vs.* time curve. Alternatively, total emission could be measured directly by using the photomultiplier output to charge a capacitor and recording the final voltage drop across the capacitor. Absolute light yields were determined by comparison with the reaction of luminol with hydrogen peroxide and hemin in aqueous base, which has an emission quantum yield of 0.0125.[6]

In all cases, after constant temperature was established, the light intensity decreased exponentially with time with a rate constant of about 1.3×10^{-4} sec^{-1} at 50° independent of the concentration of II or the initial concentration of I.[7] The quantum yield of emission (Φ_{ch}, einsteins per mole of I decomposed) increased with increasing concentration of II in the range 10^{-5}–10^{-3} *M;* Φ_{ch} was independent of initial dioxetane concentration in the range 0.001–0.02 *M*. Degassing the solutions by three cycles of freeze–pump–thaw and sealing at 10^{-5} Torr resulted in a 1.5-fold increase in light yield.

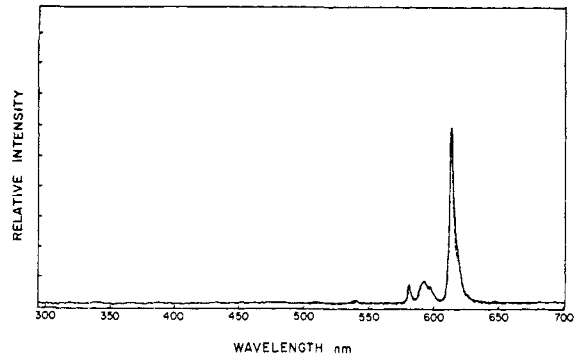


Figure 1. Chemiluminescent (———) and fluorescent (- - -) emission of II in acetone recorded using a Hitachi MPF-2A spectrophotofluorometer: chemiluminescence solution, 5×10^{-3} *M* II and 0.004 *M* I (initial concentration); $T = 50°$; fluorescence solution, 1×10^{-3} *M* II, $\lambda_{excitation} = 380$ nm.

The results, summarized in Table I, are consistent with the reaction scheme described by eq 2–6, where C =

$$\mathrm{I} \xrightarrow{k_1} \alpha\mathrm{C}^* + (1 - \alpha)\mathrm{C} \qquad (2)$$

$$\mathrm{C}^* + \mathrm{II} \xrightarrow{k_2} \mathrm{C} + \mathrm{II}^* \qquad (3)$$

$$\mathrm{C}^* \xrightarrow{k_3} \mathrm{C} \qquad (4)$$

$$\mathrm{II}^* \xrightarrow{k_4} \mathrm{II} + h\nu \qquad (5)$$

$$\mathrm{II}^* \xrightarrow{k_5} \mathrm{II} \qquad (6)$$

dioxetane decomposition product; α = fraction of dioxetanes which yield an excited state product on decomposition.

For a chemiluminescent reaction of this type the quantum yield Φ_{ch} can be expressed as the product of three efficiencies: Φ_{fl}, the fluorescence efficiency of the acceptor (europium complex II); α, the efficiency of excited product formation from the decomposition of the dioxetane I; and Φ_{et}, the efficiency of energy transfer from the initially formed excited product to the fluorescent acceptor. From eq 2–6

$$\Phi_{ch} = \Phi_{fl}\alpha\Phi_{et} = \Phi_{fl}\alpha\frac{k_2[\mathrm{II}]}{k_2[\mathrm{II}] + k_3}$$

and

$$\frac{1}{\Phi_{ch}} = \frac{1}{\alpha\Phi_{fl}}\left(1 + \frac{k_3}{k_2}\frac{1}{[\mathrm{II}]}\right)$$

A plot of $1/\Phi_{ch}$ *vs.* $1/[\mathrm{II}]$ should therefore give a straight line with slope = $k_3/k_2\alpha\Phi_{fl}$, intercept = $1/\alpha\Phi_{fl}$, and slope/intercept = k_3/k_2. A plot of $1/\Phi_{ch}$ *vs.* $1/[\mathrm{II}]$ does give a good straight line with slope ≈ 0.03 and intercept ≈ 65; thus the maximum emission yield, $\alpha\Phi_{fl}$, ≈ 0.015. The quantum yield of fluorescence of II is approximately

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[7] Clean, dry systems and pure solvents must be used for reproducible rates and light yields.

0.11 in acetone solution;[8,9] therefore, about 14% of the dioxetane molecules which decompose lead to excitation of the europium complex to its fluorescent excited state. However, both the rate and yield of the reaction were found to be sensitive to solvent impurities[7] so the yield values in Table I are minimum values and the figure for $k_1 = 1.3 \times 10^{-4}$ sec^{-1} at 50° is a maximum value. Quenching of C* by II without excitation of II is also a possibility.

In earlier experiments involving energy transfer from I to various molecules that subsequently underwent a "photochemical" change, the excited state generation was calculated to be about 0.5–4%.[4b] The energy required for the "photochemical" reactions (~55–70 kcal) was considerably higher than the ~47 kcal/mol excitation of the europium compounds. The excitation energy available from C* is not known but possibly the lower value previously obtained was a result of the greater energy requirement.

It was recently reported that a similar compound, *cis*-1,2-diethoxydioxetane, yields on thermal decomposition the excited product with 100% efficiency, *i.e.* $\alpha = 1$, based on observation of the sensitized fluorescence of dibromoanthracene and diphenylanthracene.[10] We found that *cis*-1,2-diethoxydioxetane[11] yielded less than 1% as much light as trimethyldioxetane when thermally decomposed in the presence of II. This may be due in part to a higher value of k_3 for excited ethyl formate[10] than for the product of decomposition of I. The possibility of contamination of the diethoxydioxetane sample also cannot be discounted.

Acknowledgment. We thank the Public Health Service for its financial support (Research Grant No. 5 RO 1 NS-07868 from the National Institute of Neurological Diseases and Stroke).

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Raman Spectrum of the 11-Cis Isomer of Retinaldehyde

Sir:

Complementing the series of Raman spectra[1] of the more common isomers of retinal, we report the results on the 11-cis isomer,[2] which is the chromophore of rhodopsin in the dark-adapted retina.[3] The spectra of 3.5 m*M* solutions in ethanol, carbon tetrachloride, and EPA[4] were uniquely characteristic of 11-cis retinal, and indicated its stability against krypton-ion-laser irradiation at 17,600 cm^{-1} (yellow) or 15,455 cm^{-1} (red). Uv absorption measurements confirmed this observation. The spectrum in CCl_4 is shown in Figure 1, where it is compared with the spectra of *trans-* and 13-*cis*-retinals. The strength of the Raman emissions is due to the resonance enhancement phenomenon, as discussed previously.[1,5] The detectable lines correspond to vibrations of the conjugated carbon skeleton or to modes strongly coupled to it. The most intense line (at 1577 cm^{-1} for the 11-cis isomer) is identified as the ethylenic mode. Its frequency, $\bar{\nu}(C{=}C)$, is sensitive to the conjugation (degree of delocalization) of the π electrons, as shown by solvent studies which indicate a linear decrease of $\bar{\nu}(C{=}C)$ with increasing wavelength of the lowest singlet $\pi^* \leftarrow \pi$ transition[6] (which, in turn, is accepted as an indicator of degree of conjugation).[7] Table I shows that for CCl_4 solutions such a simple relation does *not* hold from isomer to isomer: the values of λ_{max} and $\epsilon(\lambda_{max})$ both indicate that the π conjugation, strongest in the trans isomer, is markedly perturbed in the 11-cis isomer,[4] yet $\bar{\nu}(C{=}C)$ for the 11-cis isomer is very close to the respective value in *trans*-retinal. The same situation holds true in the two other solvents. This result is a consequence of the fact that vibrational frequencies are determined not only by the force constants (through which $\bar{\nu}(C{=}C)$ depends on conjugation) but also by molecular geometry. Changes in the latter by cis isomerization at the 11–12 double bond have a stronger influence on $\bar{\nu}(C{=}C)$ than the corresponding decreased conjugation. Table I also shows that the intensity of the "ethylenic mode," when measured against the CCl_4 standard, is not proportional to $\epsilon^2(\lambda_{max})$. This indicates, in agreement with results for *trans*-retinal,[6] that the formula for the Raman excitation spectrum contains terms other than the ones contributed by the lowest $^1(\pi\pi^*)$ state. These other terms, which vary from isomer to isomer, probably are cross-products of $^1(\pi\pi^*)$ terms and some $^3(\pi\pi^*)$ terms, the latter being more sensitive to isomeric configuration than those contributed by the singlet state.

Table I. Comparison of the First $^1(\pi^*\pi)$ Transition λ_{max} (nm) with the Ethylenic Mode Frequency, $\bar{\nu}(C{=}C)$ (cm^{-1}), the Molar Extinction ϵ at λ_{max}, and the Calibrated Intensity of the Ethylenic Mode $I(C{=}C)/I(CCl_4)$ Relative to ϵ^2

Isomer	λ_{max}, nm	$\nu(C{=}C)$,[a] cm^{-1}	$\epsilon \times 10^{-3}$	$\epsilon^{-2}I(C{=}C)/I(CCl_4)$
Trans	382	1578	47.6	4×10^{-10}
13-Cis	377	1584	38.8	6.6×10^{-10}
11-Cis	376	1577	26.4	19×10^{-10}

[a] $\nu(C{=}C)$ is the frequency and $I(C{=}C)/I(CCl_4)$ is the calibrated intensity of the ethylenic mode. ϵ is the molar extinction at λ_{max}.

Table II lists the major Raman transitions and their plausible assignments. As discussed in earlier work,[1,8] the chain modes of the isoprenoid polyene can be grouped in bands, in analogy with phonons in a unidimensional crystal. The frequency spread within an indi-

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