Table I. Effect of Sensitizer on Product Distribution of 2,3-Diphenylindene

run	solvent	sensitizer	product, % 1a	product, % 2a	product, % 5a
1	acetone	eosin B		88^{a}	
2	acetone	RB^{b}		$93^{c,d}$	
3	methanol	RB^{b}	$55^{c,e}$		
4	acetone	$ZnTPP^{f}$		83^{a}	
5	Freon-11	TPP			77^{c}
6	acetone	$ZnPc^{g}$	80		20^{h}
7	acetone-d_6	MB^{i}			100^{j}
8	acetone-d_6	HP^{k}	5	39	45^{l}
9	methanol–acetone (3:7)	TPP	$99^{a,m}$		

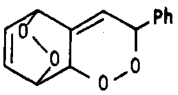
[a] Yield determined by LC. [b] Rose bengal. [c] Isolated yield. [d] Reference 3. [e] Reference 2. [f] Zinc tetraphenylporphine. [g] Zinc phthalocyanine–tetrakis-*N*-alkylsulfonamide. [h] Ratio by NMR. [i] Methylene blue chloride. [j] Only product in NMR. The exact yield was not determined. [k] Hematoporphyrin. [l] Yield by NMR relative to an internal standard. [m] As diketone, after pyrolysis.

ditions. Since the suggested intermediate **3** may either rearrange or add a second $^1O_2$ molecule, conditions leading to a high steady state $^1O_2$ concentration would be expected to trap **3** more effectively and increase the amount of **5** relative to **2**. Thus, high lamp intensity promotes the formation of **5** over **1** and **2**. Similarly, the solvents Freon-11 and acetone-d_6 also favor the formation of **5**. In these solvents, $^1O_2$ has an unusually long lifetime,[8,9] and would thus have a high relative steady-state concentration.

The effect of dye is less certain. One effect would certainly be on the efficiency of light absorption and of $^1O_2$ production; another might be to catalyze the rearrangement of triene endoperoxide **3** to **4**.[10] However, no explanation has yet been developed that is completely satisfactory.

The conditions employed in the preparation of **5a** were applied to the preparation of other diendoperoxides. The following have been isolated (recrystallized yield in parentheses): **5a** (77%), **5b** (82%), and **5c** (62%) from photooxidation in Freon-11, acetone-d_6, and Freon-11, respectively.[5] For comparison, Rose Bengal in acetone at −78 °C gave diepoxyendoperoxides **2a** (93%) and **2b** (78%) as the photooxidation products.[3]

The applicability of these reactions to a nonindene system was tested on *trans*-stilbene. The diendoperoxide was formed in high yield, and an effect of reaction conditions as profound as with the indenes was found. Matsumoto had previously reported the preparation of *trans*-stilbene diendoperoxide (**6**).[4,11]



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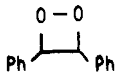

7

Under the reported conditions,[11] at room temperature in CCl_4 using TPP as the sensitizer, the yield of **6** was 16% based on reacted starting material. The other major product, benzaldehyde, was produced in 80% yield. However, at −78 °C in acetone-d_6 using TPP, **6** was produced in 76% yield (by LC analysis). Compound **6** was the *only* product detected in the NMR. Neither benzaldehyde nor its likely precursor, dioxetane **7**,[12] was present. Further, the reaction was much more rapid at −78 °C than at room temperature, as was reported for the indenes.[3] Dye bleaching was a problem, however, with TPP and, to a lesser extent, with Rose Bengal. The analogues of **2** were not formed from stilbene under any conditions investigated. The kinetics and mechanism of these reactions are under current investigation.

References and Notes

(1) Paper 31: H. S. Ryang and C. S. Foote, *J. Am. Chem. Soc.*, in press. Supported by National Science Foundation Grant No. CHE 77-21560. Bruker NMR was provided by National Science Foundation Grant No. CHE76-05926.

(2) P. A. Burns and C. S. Foote, *J. Am. Chem. Soc.*, **96**, 4339 (1974).

(3) P. A. Burns and C. S. Foote, *J. Org. Chem.*, **41**, 908 (1976); P. A. Burns, C. S. Foote, and S. Mazur, *J. Org. Chem.*, **41**, 899 (1976).

(4) This reaction is similar to the reaction of certain styrene derivatives: (a) M. Matsumoto and K. Kuroda, *Tetrahedron Lett.*, 1607 (1979); (b) D. Lerdal and C. S. Foote, *ibid.*, 3227 (1978); (c) C. S. Foote, S. Mazur, P. A. Burns, and D. Lerdal, *J. Am. Chem. Soc.*, **95**, 586 (1973).

(5) In a typical preparation, 400 mg of 2,3-diphenylindene was dissolved in 40 mL of Freon-11 containing 4×10^{-5} M TPP (saturated). Photooxidation was done at −78 °C in a half-silvered Dewar flask. The light from a 650-W tungsten lamp at 120 V was filtered through a solution of 0.1 g of $K_2Cr_2O_7$ in 50 mL of H_2O (50% cutoff at 510 nm). Oxygen was slowly bubbled into the solution. The reaction was run until complete by TLC (~1 h). Solvent removal gave crystals which were recrystallized from dichloromethane–hexane to yield 77% white microneedles.

(6) (a) The unsymmetrical triplet at δ 6.97 is covered under aromatic signals. However, it could be resolved by decoupling at δ 5.25 and subtracting the decoupled spectrum from the fully coupled. (b) N. R. Easton, Jr., F. A. L. Anet, P. A. Burns, and C. S. Foote, *J. Am. Chem. Soc.*, **96**, 3945 (1974).

(7) C. S. Strouse, J. D. Boyd, L. Goldsmith, and C. S. Foote, unpublished work.

(8) The rate of disappearance of limonene (>510-nm filtered tungsten light, TPP sensitizer) was estimated to be ~14 times greater in acetone-d_6 than in acetone. However, an earlier report gave the $^1O_2$ lifetime in both acetone-d_6 and acetone as 26 μs.[9]

(9) P. B. Merkel and D. R. Kearns, *J. Am. Chem. Soc.*, **94**, 7244 (1972); C. A. Long and D. R. Kearns, *ibid.*, **97**, 2018 (1975).

(10) For example, by dye-sensitized rearrangement of **3**, either by energy or electron transfer; the latter mechanism is currently preferred because we have very recently found a very smooth cobalt-catalyzed rearrangement of the same type (J. D. Boyd and C. S. Foote, unpublished work). No evidence for ground-state sensitizer–substrate association has been found.

(11) M. Matsumoto, S. Dobashi, and K. Kondo, *Tetrahedron Lett.*, 2329 (1977); M. Matsumoto, S. Dobashi, and K. Kuroda, *ibid.*, 3361 (1977); M. Matsumoto, S. Dobashi, and K. Kondo, *Bull. Chem. Soc., Jpn.*, **51**, 185 (1978); M. Matsumoto and K. Kondo, *Tetrahedron Lett.*, 3935 (1975).

(12) P. D. Bartlett, M. E. Landis, and M. J. Shapiro, *J. Org. Chem.*, **42**, 1661 (1977).

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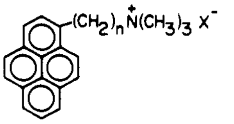
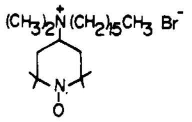
Spectroscopic Studies on Small Aggregates of Amphipathic Molecules in Aqueous Solution

Sir:

Recently we described the fluorescence quenching of several cationic pyrenyl derivatives (**1**-*n* where *n* = methylene chain length) by the surfactant nitroxyl radical **2** (Q) in aqueous solution over a concentration range below and above the critical micelle concentration (cmc = 4.6×10^{-4} M) of the latter.[1] At a total quencher concentration of $[Q_T] <$ cmc, there is negligible *diffusional* quenching ($\Phi_0/\Phi \leq 1.1$) of **1**-1 and **1**-5 in contrast to the moderate *static* quenching ($\Phi_0/\Phi \leq 5$) of **1**-11.[2] The latter result shows the tendency of amphipathic materials to associate into premicellar aggregates in aqueous solution. We now report observations on this important, but relatively unexplored, phenomenon using fluorophor **3** (F).



1-n



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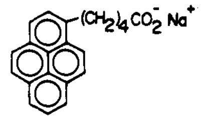

3

Fluorescence Quenching of Sodium 5-(1-Pyrenyl)pentanoate (3). The Stern–Volmer plot of the fluorescence quenching of

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Table I. Correlation of ESR Spin Intensity Data with Fluorescence Quenching Data for Several Candidate Aggregate Structures

$[Q_T] \times 10^5$ M[a]	% spin loss	$[Q_a] \times 10^5$ M[b]	predicted range[c] in Φ_0/Φ for F_mQ_n[d] F_2Q_2	FQ_2	F_2Q_3	F_3Q_4	F_3Q_5	$(\Phi_0/\Phi)_{obsd}$[e]
2.0	92 ± 2	1.80-1.88	2.50-2.68	1.43-1.46	1.67-1.72	1.81-1.89	1.56-1.60	1.59
3.0	89 ± 2	2.61-1.73	7.69-11.1	1.77-1.84	2.38-2.54	2.94-3.16	2.09-2.20	2.64
4.0	88 ± 2	3.44-3.60	*f*	2.34-2.50	4.25-5.00	7.14-10.0	3.21-3.57	5.90
5.0	77 ± 2	3.75-3.85	*f*	2.67-2.93	6.00-8.10	15.8-27.3	4.00-4.76	10.2
6.0	68 ± 2	3.96-4.20	*f*	2.94-3.33	8.33-15.0	*f*	4.81-6.25	17.2
7.0	62 ± 2	4.20-4.48	*f*	3.33-3.95	15.0-225	*f*	6.25-9.62	26.9

[a] Total concentration of surfactant nitroxyl radical **2.** All were at $[F_T] = 3.0 \times 10^{-5}$ M. [b] Concentration of **2** in F_mQ_n aggregate structures based on $[Q_T] - [Q] = [Q_a]$. [c] Calculated from the relationships $\Phi_0/\Phi = [F_T]/[F] = \{[F_T]/([F_T] - m[F_mQ_n])\} = \{[F_T]/([F_T] - m[Q_a]/n)\}$. [d] Charges and counterions have been omitted from these structures. [e] From steady-state fluorescence quenching experiment. [f] All fluorophor is in aggregate.

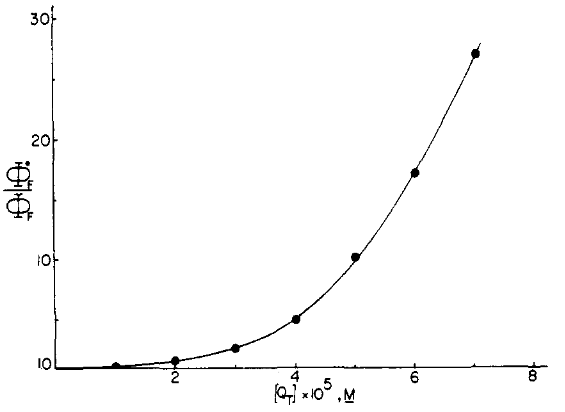

Figure 1. Fluorescence quenching of F at $[F_T] = 3.0 \times 10^{-5}$ M by Q under nondegassed conditions at pH 8.0. The data were corrected for small changes in absorbance with added Q (<10%) at the excitation wavelength (313 nm). Emission was monitored at 395 nm.

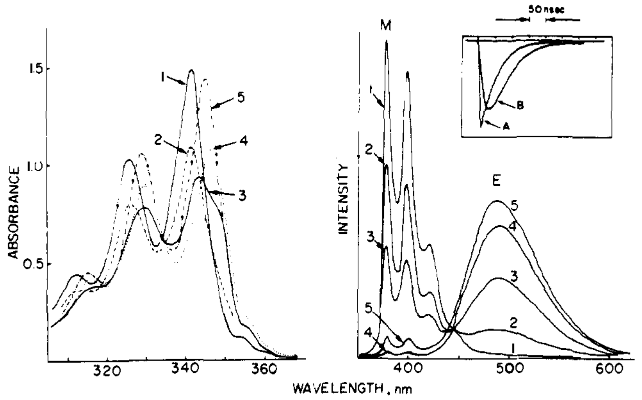

Figure 2. Left: absorption spectra of F at $[F_T] = 3.0 \times 10^{-5}$ M at [CTAC] = (1) 0, (2) 2.5×10^{-5}, (3) 5.0×10^{-5}, (4) $(0.5\text{-}3.0) \times 10^{-3}$, (5) 4.0×10^{-3} M. Right: monomer (M) and excimer (E) fluorescences from F at $[F_T] = 3.0 \times 10^{-5}$ M at the [CTAC]s given above; at the excitation wavelength of 335 nm, the absorbance change with added CTAC was <10%. Inset: excimer fluorescence decays following pulsed nitrogen laser excitation (337 nm) of nondegassed aqueous solutions of $[F_T] = 3.0 \times 10^{-5}$ M in the presence of (A) [CTAC] = 5.0×10^{-5} M and (B) [CTAC] = 5.0×10^{-3} M. Excimer fluorescence was monitored at 500 nm. All were at pH 8.0.

an aqueous solution of total fluorophor concentration $[F_T] = 3.0 \times 10^{-5}$ M by Q is shown in Figure 1. Note that very efficient quenching is observed at $[Q_T] \ll$ cmc. A static mechanism is implied by the invariant fluorescence lifetime ($\tau_f = 101 \pm 2$ ns) between $0 \leq [Q_T] \leq 1.0 \times 10^{-4}$ M.[4]

Electronic Absorption Spectroscopy. The electronic absorption spectra of F in the absence and presence of Q provide direct evidence for ground-state association. As Q is added, the absorption intensities at the maxima in the original spectrum decrease with some band broadening toward the red. At and above $[Q_T] = 4 \times 10^{-5}$ M, new, red-shifted maxima appear with bands somewhat broader than in the original spectrum. Distinct isosbestic points are not observed, suggesting that these spectral changes are not due to a single, new absorbing species.

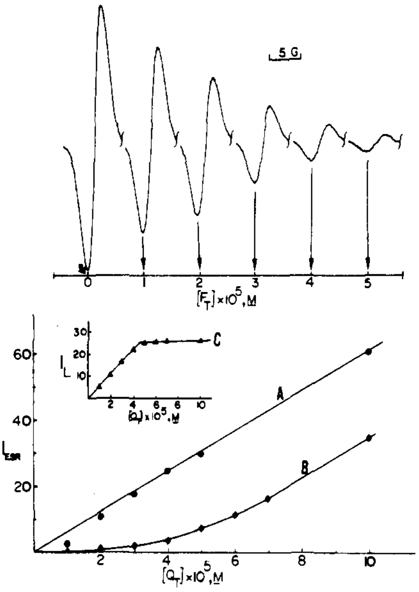

Figure 3. Upper: ESR spectrum of the low-field line of Q at $[Q_T] = 5.0 \times 10^{-5}$ M in the presence $[F_T]$ as indicated. Lower: spin intensity profiles for the low-field line in the absence (A) and presence (B) of $[F_T] = 3.0 \times 10^{-5}$ M. Inset: intensity loss profile as given by curve A − curve B. All were at pH 8.0.

Observations with Cetyltrimethylammonium Chloride (CTAC). Similar absorption spectral changes are observed when cetyltrimethylammonium chloride is added to an aqueous solution of $[F_T] = 3.0 \times 10^{-5}$ M (Figure 2, left). Emission studies on these *fluorescent* mixed aggregates reveal the characteristic pyrene excimer fluorescence[5] at [CTAC] $\geq 2 \times 10^{-5}$ M which correlates with the onset of changes in the absorption specta. The excimer fluorescence dominates the emission spectrum between 1×10^{-4} M $\leq$ [CTAC] $\leq 5 \times 10^{-3}$ M (Figure 2, right) and thereafter it rapidly decreases as F is dispersed among the increasing CTAC micelles (cmc = 4.0×10^{-3} M[6]). Transient measurements show that the pyrene monomer lifetime does not change with added CTAC up to the cmc (τ_f = 96 ns). Interestingly, while the pyrene excimer fluorescence shows no measurable rise time at [CTAC] < cmc, it shows a clear rise time of ~25 ns at and above the cmc (Figure 2, inset).

The $[F_T]$ in these experiments precludes diffusive encounter of F* and F so that excimer formation must be a static mechanism(s). At [CTAC] < cmc, we suggest that mixed aggregates of F and CTAC are formed with the monomer and excimer fluorescences arising from different aggregates of the general formulas $F[CTAC]_n$ and $F_m[CTAC]_n$ ($m \geq 2$), respectively. The negligible rise time of the excimer fluorescence may mean that the aggregates $F_m[CTAC]_n$ are small.[7] The development of a rise time (which means a decrease in the encounter frequency between F* and F) *after micellization* probably reflects the larger volume available to F* and F in the CTAC micelle compared with the premicellar aggregates.

The persistence of excimer fluorescence (and the aggregates $F_m[CTAC]_n$) below the cmc at high $[CTAC]/[F_T]$ is surprising since we expected that F would be dispersed and isolated through the aggregates $F[CTAC]_n$. However, the prevalence of the structures $F_m[CTAC]_n$ is consistent with the results of an ESR study on the aggregates F_mQ_n.

ESR Experiment. The three-line ($a_N = 16.9$ G, line width 1.7 G) ESR spectrum of Q[1] shows loss of signal intensity with added F as illustrated in Figure 3, upper.[10] We assign the "lost" intensity in the narrow line spectrum to mixed aggregates F_mQ_n ($n \geq 2$) in which the ESR spectrum of Q is severely broadened because of spin exchange.[11] The observed narrow line spin intensity, by this reasoning, arises only from monomeric Q (Q or F_mQ).

Figure 3, lower, is a spin intensity profile for Q in the absence (curve A[12]) and presence (curve B) of F (3.0×10^{-5} M). A replot of the data as a *spin intensity loss* profile (curve A − curve B = curve C) reveals that the intensity loss levels off near $[Q_T] = 4.5 \times 10^{-5}$ M. Eventually, the monomer spin intensity profiles (A and B) reach a plateau at $[Q_T]$ = cmc, coincident with the appearance of a severely broadened (~15 G) single-line ESR spectrum due to micellized Q.[1]

We assign the point of discontinuity in curve C as the saturation limit where all F (3.0×10^{-5} M) is aggregated as F_mQ_n ($n \geq 2$). This point in curve C ($[Q_T] = 4.5 \times 10^{-5}$ M) indicates that F and Q are incorporated into the mixed aggregates responsible for the ESR spin intensity loss in the ratio $F/Q \approx 0.7$ with several candidate structures being FQ_2, F_2Q_2, F_2Q_3, F_3Q_4, and F_3Q_5 as well as their multiples such as F_2Q_4.

The ESR spin loss data can be used to predict the fluorescence quenching data for the above candidate structures.[13] The concentrations of the uncomplexed and aggregated Q ([Q] and $[Q_a]$, respectively) can be calculated directly from the spin intensity data. In turn, [F] and $[F_a]$ are derived from $[Q_a]$, the composition of the candidate aggregate, and $[F_T]$. The values of $(\Phi_0/\Phi)_{pred}$ are shown in Table I.

Note that the calculated [F] and $[F_a]$ are very sensitive to the values of the percent spin loss for the higher $[Q_T]$s which accounts for the wide range in those $(\Phi_0/\Phi)_{pred}$. With this in mind, it seems that a reasonable correlation is obtained only for the composition F_2Q_3. Further, this composition ($F/Q = 0.66$) agrees exactly with the graphically observed saturation limit in curve C.

The following conclusions can be drawn. The spectroscopic observations on the above systems indicate that amphipathic compounds of opposite charge efficiently, and to some extent specifically, aggregate in very dilute aqueous solution. We suspect that the relative hydrophobic contents of the components may be important in determining the compositions of the dominant aggregates. Despite the analysis leading to F_2Q_3, we do not believe that only a single aggregate structure is formed in that system as noted by the absence of isosbestic points in the electronic absorption spectra. Rather, we believe that an ensemble of aggregates is formed in which F_2Q_3 is highly favored.[14] Finally, these results suggest experimental approaches for studying the association of amphipathic materials with possible application to biological systems[15] and the design of efficient systems for solar energy utilization.

References and Notes

(1) S. S. Atik, C. L. Kwan, and L. A. Singer, *J. Am. Chem. Soc.*, **101**, 5696 (1979).
(2) "Diffusional quenching" is by collisional or diffusion-controlled encounter of the excited fluorophor and quencher while "static quenching" involves fluorphors and quenchers positioned within effective interaction distance in the ground state. Fluorescence quenching by nitroxyl radicals has been shown to be diffusion limited with an interaction distance of $\sigma \approx 5$–6 Å.[3]
(3) J. A. Green, II, L. A. Singer, and J. H. Parks, *J. Chem. Phys.*, **58**, 2690 (1973).
(4) For diffusional quenching, the fluorescence lifetimes should follow the relationship $\tau_{obsd}^{-1} = \tau_f^{-1} + k_q[Q_T]$ where τ_{obsd} and τ_f are the lifetimes in the presence and absence of Q.
(5) J. B. Birks, "Photophysics of Aromatic Molecules," Wiley-Interscience, New York, 1979, p 301.
(6) C. L. Kwan, S. Atik, and L. A. Singer, *J. Am. Chem. Soc.*, **100**, 4783 (1978).
(7) A negligible rise time also would be predicted for structures of micelle size where $m \approx n$. However, we would expect that these mixed micelles would have decreasing *m* and increasing *n* as [CTAC] was increased leading to a gradual increase in excimer fluorescence rise time rather than a sudden onset at the cmc as is observed. We[8] and others[9] have observed a rise time for pyrene excimer fluorescence in ionic micelles.
(8) S. S. Atik and L. A. Singer, submitted to *Chem. Phys. Lett.*
(9) P. P. Infelta and M. Gratzel, *J. Chem. Phys.*, **70**, 179 (1979).
(10) Precipitation is not observed over the concentration range of this experiment so that the ESR spin intensity loss is due to changes involving solubilized materials.
(11) The ESR spectral features expected for randomly oriented nitroxyl radical moieties in F_mQ_n are different from those of rigid nitroxyl biradical systems. For example, see J. Michon and A. Rassat, *J. Am. Chem. Soc.*, **101**, 995 (1979), and references therein.
(12) We previously noted and commented on the less than theoretical intensity observed in the ESR spectra of very dilute ($<2.0 \times 10^{-5}$ M) aqueous solutions of Q.[1] Since the ESR data at $[Q_T] = 1.0 \times 10^{-5}$ M are not used in the following analyses, this observation does not jeopardize the conclusions below.
(13) The analysis that follows assumes that aggregates of the general composition F_mQ are relatively unimportant. Such structures would contribute to fluorescence quenching but not to the ESR spin intensity loss.
(14) The analyses leading to the results in Table I also indicate that for the overall equilibrium $2F + 3Q \rightleftharpoons F_2Q_3$, $K \approx 3 \times 10^{21}$ M^{-4} which illustrates the remarkable stability of these aggregates.
(15) A possible example of a highly specific aggregation of biological molecules is the association of lecithin and sodium cholate as described by D. G. Dervichian, *Adv. Chem. Ser.*, **No. 84** (1968).

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Farnesyl Pyrophosphate Synthetase. Mechanistic Studies of the 1′-4 Coupling Reaction in the Terpene Biosynthetic Pathway

Sir:

The 1′-4 condensation between isopentenyl pyrophosphate (isopentenyl-PP) and an allylic pyrophosphate is the key building reaction in the terpene biosynthetic pathway. The reaction was discovered in the late 1950s, and its mechanism has been the subject of considerable speculation.[1] An electrophilic condensation between C(1) of the allylic substrate and C(4) of isopentenyl-PP was proposed initially.[2] This mechanism was subsequently supplanted by another (Scheme I) thought to be more compatible with the stereochemistry of the reaction in which displacement of pyrophosphate and formation of the carbon-carbon bond were assisted by a nucleophilic group (X) located in the active site of the enzyme. The reaction was completed by elimination of the X group to generate a new C(2)-C(3) double bond.[3] Although a nucleophilic displacement was preferred for the condensation step, the "X-group" mechanism, when taken in the broadest context, is compatible with a nucleophilic or an electrophilic process. Recently we ruled out the former possibility using fluorinated