carbon atom.[4] This evidence reinforces the findings of Linnett and Walker[10] that, in yeast (*S. cerevisiae* N.C.Y.C. 1062), the methylene group of glycine supplies C-2, and the amino nitrogen of glycine supplies the nitrogen atom of the thiazole nucleus of vitamin B_1. It is very likely therefore that an intact C–N unit, derived from glycine (**1**), enters the thiazole nucleus. Scheme I is consistent with these results.

The origin of the thiazole moiety of thiamin in bacteria differs from that in yeast in two respects. Firstly, the unit C-2,N is derived from tyrosine[29,30,32] and not from glycine. A simple modification[33] of the present biogenetic scheme can accommodate this difference. Secondly, the C_5 precursor is generated by condensation of 3-phosphoglyceraldehyde with a C_2 unit derived from C-3,C-2 of pyruvate[34] rather than from C-1,C-2 of fructose 6-phosphate. An acyloin condensation was proposed to account for the formation of the C_5 unit from these precursors. The distribution of label observed in the present work is not consistent with such a proposal.

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References and Notes

(1) Williams, R. R. *J. Am. Chem. Soc.* **1936,** *58*, 1063–1064.
(2) Grewe, R. *Hoppe-Seyler's Z. Physiol. Chem.* **1936,** *242*, 89–96.
(3) For a review of the conflicting evidence, see ref 4.
(4) White, R. L.; Spenser, I. D. *Biochem. J.* **1979,** *179*, 315–325.
(5) The thiazole phosphate (**4**) is the immediate precursor of the thiazole unit in the biosynthesis of thiamin.[6-8] The corresponding alcohol (**5**) is obtained on chemical degradation of thiamin.[9]
(6) Nose, Y.; Ueda, K.; Kawasaki, T. *Biochim. Biophys. Acta* **1959,** *34*, 277–279. Nose, Y.; Ueda, K.; Kawasaki, T.; Iwashima, A.; Fujita, T. *J. Vitaminol.* (*Kyoto*) **1961,** *7*, 98–114.
(7) Camiener, G. W.; Brown, G. M. *J. Am. Chem. Soc.* **1959,** *81*, 3800; *J. Biol. Chem.* **1960,** *235*, 2411–2417.
(8) Leder, I. G. *Biochem. Biophys. Res. Commun.* **1959,** *1*, 63–66; *J. Biol. Chem.* **1961,** *236*, 3066–3071.
(9) Williams, R. R.; Waterman, R. E.; Keresztesy, J. C; Buchman, E. R. *J. Am. Chem. Soc.* **1935,** *57*, 536–537.
(10) Linnett, P. E.; Walker, J. *J. Chem. Soc. C* **1967,** 796–799; *Biochem. J.* **1968,** *109*, 161–168; *Biochim. Biophys. Acta* **1969,** *184*, 381–385.
(11) Dickens, F. *Biochem. J.* **1938,** *32*, 1645–1653, and references quoted therein.
(12) D-[1-^{14}C]Glucose (nominal specific activity 56 mCi/mmol, nominal total activity 0.5 mCi, New England Nuclear), D-[2-^{14}C]glucose (nominal specific activity 53 mCi/mmol, nominal total activity 0.5 mCi, New England Nuclear), D-[6-^{14}C]glucose (nominal specific activity 53 mCi/mmol, nominal total activity 0.5 mCi, New England Nuclear), and D-[1-^{14}C]fructose (nominal specific activity 57 mCi/mmol, nominal total activity 0.5 mCi, Amersham-Searle) were each administered to a 2.8-L culture.
(13) Culture conditions were similar to those used in our earlier experiments,[4] except that ethanol rather than D-glucose was used as carbon source.
(14) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979,** 399–402.
(15) Harington, C. R.; Moggridge, R. C. G. *J. Chem. Soc.* **1939,** 443–446.
(16) Sawa, Y.; Ishida, T. *J. Pharm. Soc. Jpn.* **1956,** *76*, 337–338; *Chem. Abstr.* **1956,** *50*, 13875i.
(17) Mikhailov, B. M.; Bronovitskaya, V. P.; Platova, I. K. *Zh. Obshch. Khim.* **1956,** *26*, 3445–3449; *Chem. Abstr.* **1957,** *51*, 9588h.
(18) Murav'eva, K. M.; Shchukina, M. N. *Zh. Obshch. Khim.* **1963,** *33*, 3723–3725; *J. Gen. Chem. USSR* (*Engl. Trans.*) **1963,** *33*, 3655–3657.
(19) Kuhn, R.; Roth, H. *Ber. Dtsch. Chem. Ges. B* **1933,** *66*, 1274–1278.
(20) Leete, E.; Gregory, H.; Gros, E. G. *J. Am. Chem. Soc.* **1965,** *87*, 3475–3479.
(21) Nefkens, G. H. L.; Tesser, G. I.; Nivard, R. J. F. *Recl. Trav. Chim. Pays-Bas* **1960,** *79*, 688–698.
(22) Hill, R. E.; Rowell, F. J.; Gupta, R. N.; Spenser, I. D. *J. Biol. Chem.* **1972,** *247*, 1869–1882.
(23) Hill, R. E.; Horsewood, P.; Spenser, I. D.; Tani, Y. *J. Chem. Soc., Perkin Trans 1* **1975,** 1622–1627.
(24) Horecker, B. L. In "Carbohydrate Metabolism and its Disorders", Dickens, F., Randle, P. J., Whelan, W. J., Eds.; Academic Press: New York, 1968; Vol. 1, pp 139–167.
(25) The distribution of label from D-[1-^{14}C]-, D-[2-^{14}C]-, and D-[6-^{14}C]glucose within the ribitol moiety of vitamin B_2 has been interpreted in analogous fashion.[26]
(26) Plaut, G. W. E.; Broberg, P. L. *J. Biol. Chem.* **1956,** *219*, 131–138.
(27) Candy, D. J.; Blumson, N. L.; Baddiley, J. *Biochem. J.* **1964,** *91*, 31–35. Candy, D. J.; Baddiley, J. *Biochem. J.* **1965,** *96*, 526–529.
(28) Johnson, D. B.; Howells, D. J.; Goodwin, T. W. *Biochem. J.* **1966,** *98*, 30–37.
(29) Estramareix, B.; Therisod, M. *Biochim. Biophys. Acta* **1972,** *273*, 275–282.
(30) Bellion, E.; Kirkley, D. H.; Faust, J. R. *Biochim. Biophys. Acta* **1976,** *437*, 229–237.
(31) The amino nitrogen of tyrosine serves as the source of the nitrogen atom of the thiazole moiety in a bacterium.[32]
(32) White, R. H.; Rudolph, F. B. *Biochim. Biophys. Acta* **1978,** *542*, 340–347.
(33) Elimination of the *p*-hydroxybenzyl moiety from a Schiff base generated by condensation of tyrosine with the phosphoketopentose **2** leads to intermediates identical with those of Scheme I.
(34) White, R. H. *Biochemistry* **1978,** *17*, 3833–3840.

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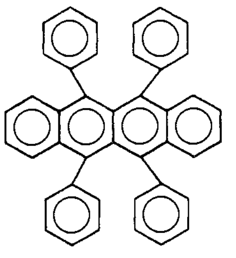
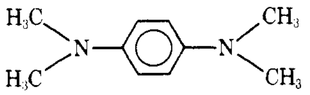
Quenching of the Fluorescent State of Rubrene Directly to the Ground State

Sir:

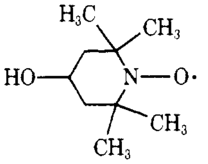
Until recently it was believed that exciplexes of aromatic hydrocarbons decay in a fashion similar to that of their parent aromatic monomers.[1] Here fluorescence and intersystem crossing account for most of the excitation energy; that is $\Phi_f + \Phi_{isc} \simeq 1.0$ is often a good rule. We report in this communication evidence in two systems where singlet quenching leads to internal conversion which is nearly quantitative. Quenching of fluorescence of rubrene (RUB) by *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) and by 4-hydroxy-2,2,6,6-tetramethylpiperidinoxy free radial (R·) has been studied.



RUB



TMPD



R·

The Stern–Volmer dependence of rubrene fluorescence quenching was determined from the steady-state spectral data. For TMPD and for R· the apparent rate constants in degassed benzene are $k_q = 6.0 \times 10^9$ and $k_q = 3.3 \times 10^9\ M^{-1}\ s^{-1}$, respectively. In neither case was a long-wavelength new emission observed. The absorption spectra of the solutions show no evidence of complexing in the ground state. To test the possibility that quenching resulted in rubrene triplet formation, a nanosecond laser flash experiment was performed. The method used has been described previously.[2] Benzene solutions with and without TMPD were examined in 1-cm cells. The quenched sample contained TMPD such that the rubrene fluorescence was reduced by 75%. Rubrene was selectively excited at 488 nm using a Coumarin 102 dye laser pumped by a 337-nm nitrogen laser. No transient absorption attributable to a short-lived exciplex or the TMPD cation radical was observed. Also, we observed no rubrene triplet even though triplet formation as small as ~5% could have been detected. For the unquenched sample, our result is consistent with the reported rubrene fluorescence yield in benzene of 1.0.[3] We did observe a broad, structureless absorption from 400 to 450 nm which decayed with a lifetime approximately equal to that of the rubrene fluorescence. This was assigned to an absorption of the lowest excited singlet state of rubrene.[4] The experiment was repeated

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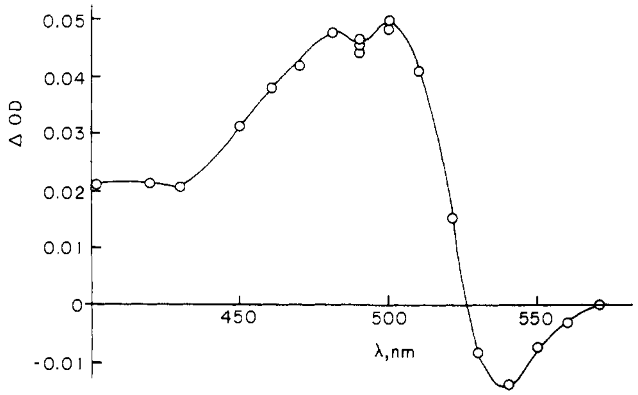

Figure 1. Transient absorption of rubrene triplets sensitized by anthracene. Absorption was monitored 50 μs after the flash. ΔOD is the difference spectrum uncorrected for ground state absorption.

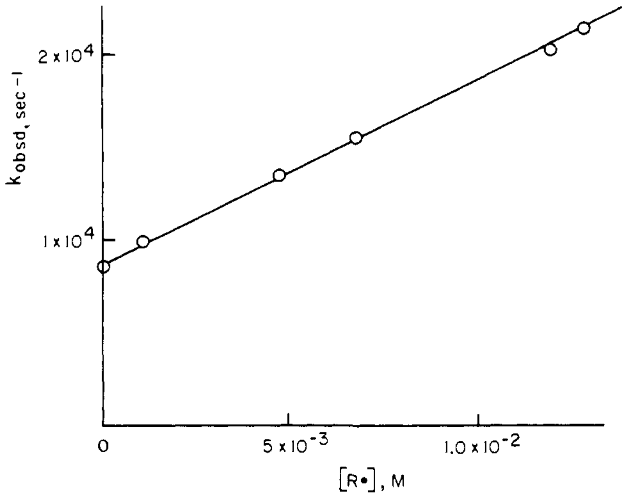

Figure 2. Rate constant for rubrene triplet decay vs. concentration of R· in toluene. See text for details.

with R· in degassed 1-propanol solution. Again, no new transients were observed.

Next, these systems were investigated on a microsecond flash apparatus whose capability permits a more sensitive search for rubrene triplets. The flash had a duration of 1.5 μs and energy of ~160 J. Because nitroxyl radicals are known to quench triplet states of aromatic hydrocarbons,[5] we studied the quenching of rubrene triplet by R·. Figure 1 shows the absorption of rubrene triplets produced in toluene by energy transfer from anthracene triplets. The concentrations of anthracene and rubrene were 2.4×10^{-4} and 1.7×10^{-5} M, respectively. Excitation was by light of λ 300-390 nm and sample cells had a path length of 2 cm. The spectrum is essentially the same as that obtained by Yildiz, Kissinger, and Reilley who used biacetyl as a sensitizer.[6] Note that the emission seen around 540 nm has been assigned as triplet-triplet emission following the triplet-triplet absorption process. Our sensitivity would have allowed for detection of signals ~50 times weaker. Thus one would expect conversion to rubrene triplets as low as a few percent to be detectable.

Quenching of the rubrene triplet state was performed with concentrations of R· ranging from 0 to 2×10^{-2} M. Figure 2 shows the results of this study. Anthracene and rubrene concentrations were 2.7×10^{-4} and 3.3×10^{-5} M, respectively. All absorption intensities were monitored at 510 nm. The quenching constant obtained for this process is $k = 9.5 \times 10^5$ $M^{-1}\,s^{-1}$. The intercept of Figure 2 shows that the unquenched rubrene triplet lifetime is ~114 μs. This agrees well with the lifetime reported in benzene.[6]

It can be seen that the time resolution allows detection of rubrene triplet absorption for an R· concentration of 1.1×10^{-1} M. This concentration would result in triplet lifetimes of ~9 μs while the flash apparatus has a resolution of ~1 μs. Excitation of toluene solutions of rubrene (1×10^{-5} M) by 420-580-nm light in the absence and presence of R· (1×10^{-2} and 1.1×10^{-1} M) resulted in no detectable rubrene triplet absorption. Sample cells with 2- and 20-cm path lengths were used. These experiments were also done in benzene solution, where the triplet quenching constant is $k = 8 \times 10^5$ $M^{-1}\,s^{-1}$. Again, no triplet states of rubrene were observed. We also investigated the system of rubrene with 1-2 M TMPD in propanol and acetonitrile. Using excitation of $\lambda > 400$ nm, we observed no transient absorption that could be attributed to ion radicals ($TMPD^+$ and/or RUB^-). Thus, all of the results showed no measurable rubrene triplet formation nor electron-transfer processes.

We conclude from these findings that virtually all rubrene singlet quenching leads to formation of rubrene ground states. In the case of TMPD quenching of rubrene, electronic energy transfer is not a viable explanation for the quenching phenomena. While quenching of aromatic hydrocarbons by tertiary amines has commonly been shown to produce exciplex emission or enhanced triplet formation, these mechanisms are not seen to occur in the RUB-TMPD system. We thus conclude that TMPD effects rapid $S_1 \rightarrow S_0$ transitions in rubrene. Fluorescence quenching by R· could occur through an energy-transfer mechanism. This is not easily determined because R· does not fluoresce. However, when R· quenches the fluorescence of 9,10-diphenylanthracene, 9,10-dimethylanthracene, and perylene, all at diffusion controlled rates, large yields of triplets are produced, despite the fact that in those cases electronic excitation transfer would be energetically allowed.[7] Clearly the rate of enhanced intersystem crossing relative to the rate of some other process, be it enhanced internal conversion to the ground state or electronic energy transfer, is much lower with RUB than with the related hydrocarbons. We are inclined to the view that R·, like TMPD, causes crossing to the ground state but we cannot present a rigorous defense of that view. We do believe that failure of the excited singlet of RUB to decay by way of triplets, either spontaneously or under perturbation by quenchers, is noteworthy.

In a recent study of exciplex decay behavior, Watkins found that exciplexes of some substituted dimethylanilines with benzperylene gave high yields of internal conversion.[8] In analogy to the excited states of charge-transfer complexes, it was suggested that coupling of the exciplex with the ground state will govern rates of internal conversion while coupling with the monomer excited singlet governs emission. In the system RUB-TMPD studied here, an exciplex, if formed, would be expected to lie considerably below S_1 of rubrene even in nonpolar medium because of the good electron-donating character of the quencher. This large stabilization might lead to an increase in the internal conversion rate. It is, perhaps, surprising that acceleration of intersystem crossing is not observed because a large amount of charge transfer in the exciplex should enhance spin-orbit coupling.[9] A reduced singlet-triplet energy separation would also increase triplet formation according to the energy gap law, as suggested by Ting[10] and by Formosinho.[11] However, it is possible in this case that the lowest rubrene triplet lies above the exciplex state. The lack of electron transfer in polar solvent is further puzzling. In as ideal a situation as possible, we expect a quencher having low ionization potential combined with a solvent of high dielectric constant (acetonitrile, $\epsilon \simeq 37$) to produce a substantial yield of ions. Since these processes cannot be detected, it appears that the decay constant for an exciplex must be very large and accounted for almost entirely by internal conversion.

Our failure to observe absorption attributable to an exciplex probably indicates that any exciplex formed decays with a lifetime of less than nanoseconds. Ottolenghi[12] has found that the spectra of arene-amine exciplexes normally contain bands similar to those found in the spectra of the related ion radicals. In particular, the exciplex from TMPD and biphenyl shows transitions like those of TMPD$^+$ and the biphenyl anion radical.[13] A similar pattern is found in the exciplex spectrum of anthracene-*N*,*N*-diethylaniline.[14] In each of these cases the absorptions are relatively strong. We presume that any analogous exciplex from TMPD and RUB should have been observable had it lived long enough. The fact that no exciplex emission is observed also limits that possible exciplex lifetime. The radiative lifetime of RUB fluorescence is ~16 ns and the radiative lifetime of an exciplex should be of the same order of magnitude. We could have easily detected an emission quantum yield of 0.01; so the true lifetime of an exciplex would have to be less than a nanosecond to obliterate any detectable fluorescence. We conclude that either no exciplex is formed or that, if one is formed, it decays with a rate constant of 10^{10} s^{-1} or greater.

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References and Notes

(1) J. Birks, "Photophysics of Aromatic Molecules", Wiley-Interscience, New York, 1970
(2) E. Russell, A. Twarowski, D. Kliger, and E. Switkes, *Chem. Phys.*, **22**, 167 (1977)
(3) S. Strickler and R. Berg, *J. Chem. Phys.*, **37**, 814 (1962)
(4) A. Twarowski and D. Kliger, unpublished results
(5) V. Kuzmin, A. Tatikolov, and Y. Borisevich, *Chem. Phys. Lett.*, **53**, 52 (1978). R. Caldwell and R. Schwerzel, *J. Am. Chem. Soc.*, **94**, 1035 (1973); **95**, 1382 (1973)
(6) A. Yildiz, P. Kissinger, and C. Reilley, *J. Chem. Phys.*, **49**, 1403 (1968)
(7) V. Kuzmin and A. Tatikolov, *Chem. Phys. Lett.*, **51**, 45 (1977)
(8) A. Watkins, *Chem. Phys. Lett.*, **43**, 299 (1976)
(9) See ref 1; N. Christodouleas and S. McGlynn, *J. Chem. Phys.*, **40**, 166 (1964)
(10) C. Ting, *Chem. Phys. Lett.*, 1, 335 (1967)
(11) S. Formosinho, *Mol. Photochem.*, **7**, 41 (1976)
(12) M. Ottolenghi, *Acc. Chem. Res.*, **6**, 153 (1973)
(13) A. Alchalal, M. Tamir, and M. Ottolenghi, *J. Phys. Chem.*, **76**, 2229 (1972)
(14) R. Potashnik, C. Goldschmidt, M. Ottolenghi, and A. Weller, *J. Chem. Phys.*, **55**, 5344 (1971)

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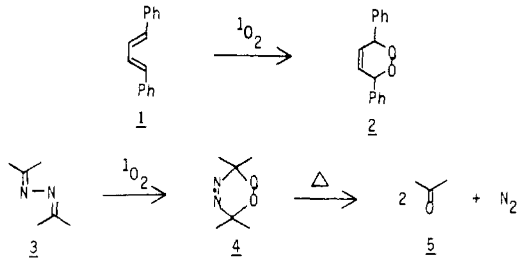
Photooxidation of Azines. Evidence for a Free-Radical Oxidation Initiated by Singlet Oxygen

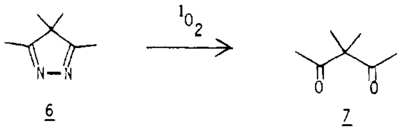
Sir:

The reaction of singlet oxygen with conjugated dienes (reaction with *trans,trans*-1,4-diphenyl-1,3-butadiene[1] (**1**) is shown below) is well known.[2] The simplest view of this reaction as a concerted [2 + 4] cycloaddition is widely accepted.[2] The photooxidation of acetone azine (**3**) to tetramethyl-1,2-dioxa-4,5-diazine (**4**) has recently been reported,[3] and the similarity of the course of this oxidation with that of dienes



prompted us to investigate the mechanism for the azine oxidation. We now report that the photooxidation of acetone azine proceeds via a free-radical pathway initiated by singlet oxygen.

Competitive photooxidation[4] of **3** vs. tetramethyl-4*H*-pyrazole (**6**) produces acetone (**5**) and diketone **7**, respectively,



as the sole products by VPC analysis. The relative rate of oxidation (Table I) of **3** is three times that of **6**, and this rate ratio is moderately independent of solvent and sensitizer. Since **3** is significantly distorted from the cisoid conformation[5] and since **6** is restricted to the cisoid form, the relative rate of oxidation of **6** is predicted to be much larger than that of **3** assuming a concerted [2 + 4] cycloaddition. The observed rate ratio is inconsistent with this view.

The results of the competitive photooxidation of **3** with **1** are provided in Table II. Both compounds are similary reactive (k^{rel} of **1** to **3**, 4.5 in $CDCl_3$). This relative rate of oxidation of **3** to **1** is moderately independent of sensitizer (Table II), and both reactions are inhibited by singlet oxygen quenchers, Dabco and β-carotene (Table III). A remarkable difference in their reactivity, however, is that the oxidation of acetone azine is completely quenched while that of the diene is moderately unaffected by the addition of 2,6-di-*tert*-butyl-*p*-cresol, a free-radical scavenger (Table III). These results are con-

Table I. Relative Rates of Photooxidation of Acetone Azine (**3**) and Tetramethyl-4*H*-pyrazole (**6**)

solvent[a]	sensitizer[b]	k^{rel} (**3**:**6**)[c]
CCl_4	10^{-4} M TPP	3.2
CCl_4	10^{-6} M TPP	3.8
CH_2Cl_2	10^{-4} M TPP	3.3
$CHCl_3$	10^{-4} M TPP	3.0
$CHCl_3$	10^{-4} M MB	3.8
$CDCl_3$	10^{-4} M TPP	3.2
$CFCl_3$	10^{-4} M TPP	3.1

[a] $[3]_0 = [6]_0 = 0.2$ M; $T = -20$ °C. [b] TPP, tetraphenylporphyrin; MB, methylene blue. [c] Relative rates were determined by monitoring product formation by VPC: ¼ in. × 10 ft 15% SE-30 on Chromosorb P at 50 °C.

Table II. Effect of Solvent and Sensitizer on the Relative Rates of Photooxidation of Acetone Azine (**3**) and *trans,trans*-1,4-Diphenyl-1,3-butadiene (**1**)

solvent	sensitizer[a]	k^{rel} (**1**:**3**)
$CDCl_3$[b]	TPP	4.5
$CDCl_3$[b]	MB	3.3
$CDCl_3$[b]	Ⓟ-RB	3.6
C_6F_6[c]	TPP	3.6
CCl_4[c]	TPP	3.5

[a] TPP (10^{-4} M), MB (10^{-5} M), Ⓟ-RB, polymer-anchored Rose Bengal[12] (100 mg/10 mL). [b] $[1]_0 = [3]_0 = 0.1$ M; $T = -20$ °C. [c] $[1]_0 = [3]_0 = 0.025$ M; $T = 0$ °C.

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